Korean J Physiol Pharmacol
Vol 9: 187-193, August, 2005

Protein Kinase C Activates ATP-sensitive Potassium Channels in Rabbit Ventricular Myocytes

Nari Kim, Jae Boum Youm, Hyun Joo, Hyungkyu Kim, Euiyong Kim, and Jin Han

Mitochondrial Signaling Laboratory, Department of Physiology and Biophysics, College of Medicine, Cardiovascular & Metabolic Disease Center, Biohealth Products Research Center, Inje University, Busan 614-735, Korea

Several signal transduction pathways have been implicated in ischemic preconditioning induced by the activation of ATP-sensitive K^+ (K_{ATP}) channels. We examined whether protein kinase C (PKC) modulated the activity of K_{ATP} channels by recording K_{ATP} channel currents in rabbit ventricular myocytes using patch-clamp technique and found that phorbol 12,13-didecanoate (PDD) enhanced pinacidil-induced K_{ATP} channel activity in the cell-attached configuration; and this effect was prevented by bisindolylmaleimide (BIM). K_{ATP} channel activity was not increased by 4 α-PDD. In excised inside-out patches, PKC stimulated K_{ATP} channels in the presence of 1 mM ATP, and this effect was abolished in the presence of BIM. Heat-inactivated PKC had no effect on channel activity. PKC-induced activation of K_{ATP} channels was reversed by PP2A, and this effect was not detected in the presence of okadaic acid. These results suggest that PKC activates K_{ATP} channels in rabbit ventricular myocytes.

Key Words: K_{ATP} channel, Patch-clamp electrophysiology, Protein kinase C, Phorbol 12,13-didecanoate, Bisindolylmaleimide

INTRODUCTION

Heart has the highest density of expression of ATP-sensitive K^+ (K_{ATP}) channels (Davis et al, 1991). These channels open when cytosolic ATP concentrations ($[ATP]_i$) fall below a particular threshold, 800 μM. The activation of K_{ATP} channels shortens the duration of action potentials, thereby decreasing contractility and conserving energy during ischemia (Faivre & Findlay, 1990; Venkatesh et al, 1991; Lebuffe et al, 2003; Otani et al, 2003).

K_{ATP} channels are also thought to play a role in the phenomenon of ischemic preconditioning. In several species, a short period of ischemic preconditioning protects the heart by reducing the size of infarcts that result from prolonged bouts of ischemia (Grover et al, 1992; Van Winkle et al, 1994; Walsh et al, 1994; Yao & Gross, 1994). The mechanism by which activation of K_{ATP} channels could provide a 'memory' of ischemic preconditioning is still a matter of debate (Parratt, 1994; Sanada & Kitakaze, 2004).

One possible basis for the prolonged protection following ischemic preconditioning might be long-term modulation of K_{ATP} channel activity by phosphorylation. Several signal transduction pathways have been implicated in ischemic preconditioning, including pathways that involve protein kinase C (PKC) (Okawa et al, 2003; Uchiyama et al, 2003; Wang et al, 2004). Moreover, there is evidence that the actions of PKC involve activation of K_{ATP} channels (Moon et al, 2004). However, it is not known whether PKC can activate single cardiac K_{ATP} channel in the presence of physiological concentrations of ATP. Therefore, the purpose of the study was to determine whether PKC modulated the activity of K_{ATP} channels in rabbit ventricular myocytes.

METHODS

Cell isolation

Individual ventricular myocytes were isolated from rabbit hearts by enzymatic dissociation according to the established method (Han et al, 1993). Initially, each heart was cannulated and was perfused retrogradely for 5 min with Tyrode's solution, while gently squeezing the heart to remove all blood. The heart was then perfused with nominally Ca^{2+}-free Tyrode's solution for 5 min, followed by perfusion for 15~25 min with Ca^{2+}-free Tyrode's solution that contained 0.01% collagenase (5 mg/50 cc; Yakult, Japan). Thereafter, the heart was perfused with Kraftbrühe (KB) solution for 5 min. Finally, the cannula was removed, the atria were discarded, and the ventricular walls and septum were cut vertically into four to six pieces. Individual cells were dissociated from these pieces of tissue by agitating the tissue samples gently in a small beaker that contained KB solution. Isolated ventricular cells were stored in KB solution at 4°C and were used within 12 h.

Corresponding to: Jin Han, Mitochondrial Signaling Laboratory, Department of Physiology and Biophysics, College of Medicine, Inje University, 633-165 Gaegeum-dong, Busanjin-gu, Busan 614-735, Korea. (Tel) 82-51-890-6714, (FAX) 82-51-894-5714, (E-mail) phyhanj@ijnc.inje.ac.kr

ABBREVIATIONS: PKC, protein kinase C; K_{ATP} channel, ATP sensitive potassium channel; PDD, phorbol 12,13-didecanoate; BIM, bisindolylmaleimide; PP2A, protein phosphatase; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; OA, okadaic acid.

Langendorff columns were kept at 37°C during all steps.

Electrophysiology

Single-channel currents were measured in the cell-attached, inside-out, and outside-out configurations (Hamill et al, 1981). Channel activity was measured using a patch-clamp amplifier (EPC-7; List, Darmstadt, Germany; Axopatch-1D, Axon Instruments, Foster City, CA). Patch pipettes (5~10 MΩ resistance) were pulled from borosilicate glass capillaries (Clark Electrochemical, Pangbourne, England) using a vertical puller (PP-83; Narishige, Japan). The tips of the pipettes were coated with Sylgard and fire-polished. Membrane currents were digitized at a sampling rate of 48 kHz and were stored on digital audio tapes using a digital recorder (DTR-1200; Biologic, Grenoble, France). To analyze single-channel activity, data were transferred to a computer (80486 DX2-66; IBM, USA) with pCLAMP ver. 6.0 software (Axon Instruments, Burlingame, CA, USA) through an analogue-to-digital converter (Digidata-1200; Axon Instruments).

Data analysis

The threshold for the open state was set at half the single-channel amplitude (Colquhoun et al, 1983). A histogram of open times was plotted using data from continuous recordings of at least 60 s. The open probability (Po) was calculated using the formula:

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

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 recording period in the absence of ATP by the mean unitary current amplitude. Po was calculated from 30-s data records.

Rundown of K_{ATP} channels

The activity of K_{ATP} channels in rabbit ventricular myocyte membrane patches excised into ATP-free solution decreases slowly with time; this phenomenon is known as 'rundown'. In the present study, upon excision, patches were exposed continuously to ATP (0.1 or 1.0 mM), except for a brief exposure to ATP-free solution at the beginning and end of experiments to estimate the number of channels in a patch (see above) and the degree of rundown. Data from patches that exhibited >50% rundown were discarded. In experiments designed to test the effects of PKC on K_{ATP} channel activity, patches were exposed continuously to 1 mM ATP unless stated otherwise. This concentration of ATP was used to approximate a near-physiological concentration of ATP, while simultaneously producing a Po that would allow single-channel events to be observed.

Solutions and drugs

Normal Tyrode's solution contained (in mM): 143.0 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 glucose, and 5.0 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). pH was adjusted to 7.4 with NaOH. The outside of the cell membrane was exposed to a solution that contained (in mM): 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES. pH was adjusted to 7.4 with KOH. The inside of the cell membrane was exposed to a solution that 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, and 10 HEPES. pH was adjusted 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, and 10 HEPES. pH was adjusted to 7.4 with KOH.

Adenosine (20 μM), ATP (0.1 or 1.0 mM), glibenclamide (50 μM), guanosine 5'-triphosphate (GTP; 100 μM), bisindolylmaleimide (BIM, 30 nM) and guanosine 5'-*O*-(3-thiotriphosphate) (GTPγS; 50 μM) were added to either extracellular or intracellular solutions according to the experimental protocols described in the text. After addition of drugs to the test solution, the pH was readjusted to 7.4 with KOH. Pertussis toxin (1~5 μg/ml), cholera toxin (10 μg/ml), albumin (3 mg/ml), and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Research Biochemicals, Natick, MA, USA) were dissolved in KB solution in which myocytes were incubated for more than 7 h. Unless stated otherwise, reagents were obtained from Sigma (St. Louis, MO, USA), pinacidil was obtained from Research Biochemicals, okadaic acid (OA) was from RBI (Natick, USA), and protein phosphatase 2A (PP2A) was from UBI (Lake Placid, NY, USA). Experiments were performed at room temperature (25±2°C).

Solution exchange system

In most experiments, the DAD-12 superfusion system was used to switch bath solutions. This system is designed to simplify the application of various concentrations of drugs and solutions to cells, and takes advantage of what many refer to as the 'sewer-pipe effect'. If the cell remains within the stream of the solution when the outlet is pointed at the cell to be studied, the cell is essentially immersed completely in the solution that is applied. The DAD-12 enables the user to rapidly stop and start the flow and to change up to 12 parameters of the flow.

Statistics

Data are presented as means±SEM. Student's unpaired *t*-test was used to calculate statistical significance ($p < 0.05$).

RESULTS

Effects of PKC on pinacidil-induced single-channel activity

Fig. 1 illustrates the effects of phorbol 12,13-didecanoate (PDD) on pinacidil-induced single-channel currents recorded from K_{ATP} channels in isolated rabbit ventricular myocytes in the cell-attached configuration. Administration of pinacidil to the bath solution activated single-channel currents (Fig. 1A), and this effect was enhanced by the application of 0.1 μM PDD (Fig. 1B) to the bath. The addition of glibenclamide (10 μM) reversed the effects of PDD

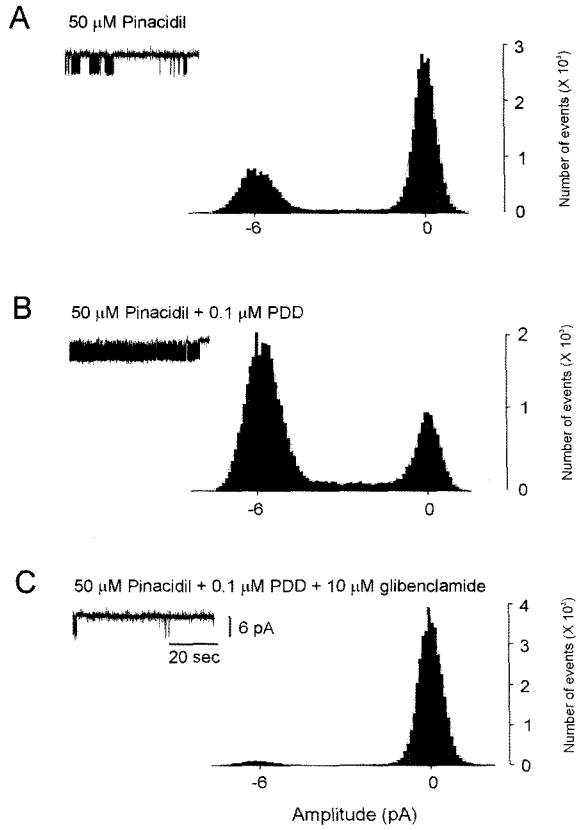

Fig. 1. Effects of phorbol 12,13-didecanoate (PDD) on pinacidil-induced activity of single K_{ATP} channels in isolated rabbit ventricular myocytes. Single-channel current traces and corresponding histograms obtained from recordings in the cell-attached configuration are shown during (A) control conditions (50 μM pinacidil), (B) activation by 0.1 μM PDD in the presence of 50 μM pinacidil, and (C) exposure to 10 μM glibenclamide in the presence of 0.1 μM PDD and 50 μM pinacidil. The membrane potential was held at −80 mV. Single-channel currents are downward deflections in the trace. Zero-current levels are indicated by broken lines. Data in the histograms were obtained from 45-s current recordings.

on the pinacidil-induced opening of K_{ATP} channels (Fig. 1C).

To examine how PKC would affect the PDD-induced enhancement of pinacidil-induced K_{ATP} channel activation, we performed the experiments illustrated in Fig. 2. We first evaluated the effects of the highly selective PKC inhibitor, bisindolylmaleimide (BIM), on the actions of PDD. BIM (30 nM) was applied extracellularly to pinacidil-activated K_{ATP} channels prior to and during the addition of 0.1 μM PDD. BIM had no effect on pinacidil-induced K_{ATP} channel activation, but prevented the enhancement of channel activation by pinacidil (Fig. 2B).

To verify that PKC was essential for the PDD-induced enhancement of pinacidil-induced K_{ATP} channel activation, we examined the effects of 4 α-PDD, an inactive congener of PDD, on the actions of PDD. As shown in Fig. 2C and D, 1 μM 4 α-PDD failed to alter the effects of PDD on pinacidil-induced K_{ATP} channel activation.

PKC activates K_{ATP} channels in the presence of ATP

To investigate the direct actions of PKC on K_{ATP} channels,

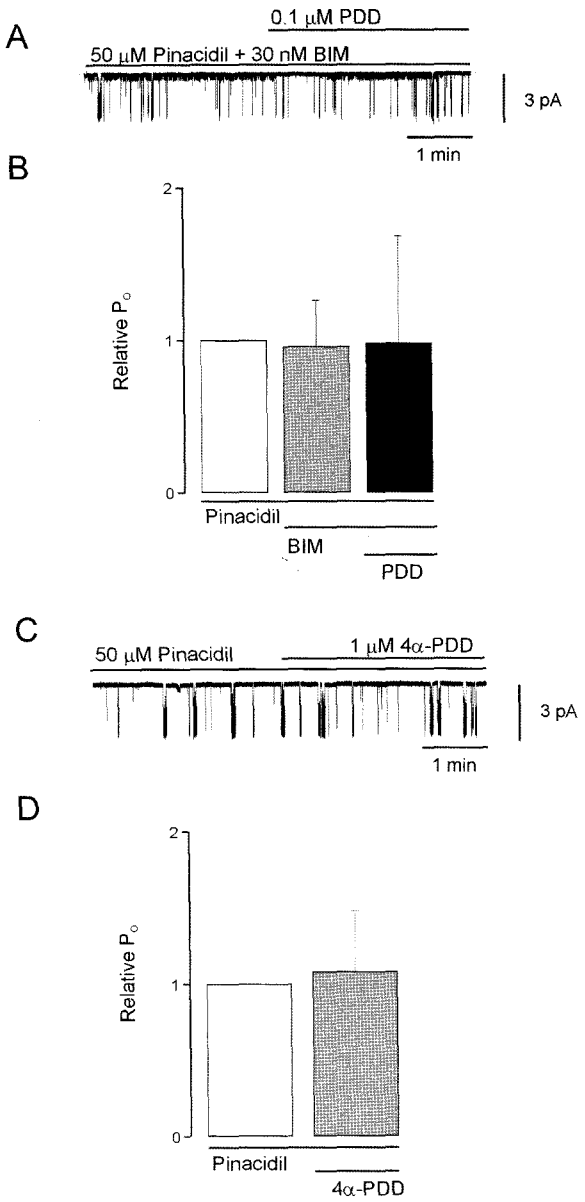

Fig. 2. Effect of PDD on pinacidil-induced opening of K_{ATP} channels in the presence of bisindolylmaleimide (BIM), a highly selective inhibitor of protein kinase C (PKC). (A) Single-channel current trace (cell-attached configuration) obtained from an isolated ventricular myocyte held at −40 mV. Pinacidil (50 μM) caused an increase in channel activity. Extracellular application of PDD (0.1 μM) in the presence of BIM (30 nM) was ineffective. Data were sampled at 400 Hz and filtered at 1 kHz. The broken line indicates the zero-current level. (B) Change in channel activity in response to PDD in the presence of BIM. The histogram shows the pooled Po data for the following conditions: pinacidil alone; pinacidil+BIM; and additional application of PDD. (C) Single-channel current trace (cell-attached configuration at −40 mV) illustrating that pinacidil enhanced K_{ATP} channel activity. Extracellular application of 4 α-PDD (1 μM) was ineffective in the presence of pinacidil. Data were sampled at 400 Hz and filtered at 1 kHz. The broken line indicates the zero-current level. (D) Change in channel activity in response to 4 α-PDD. The histogram shows the pooled Po data for the following conditions: pinacidil alone and additional application of 4 α-PDD.

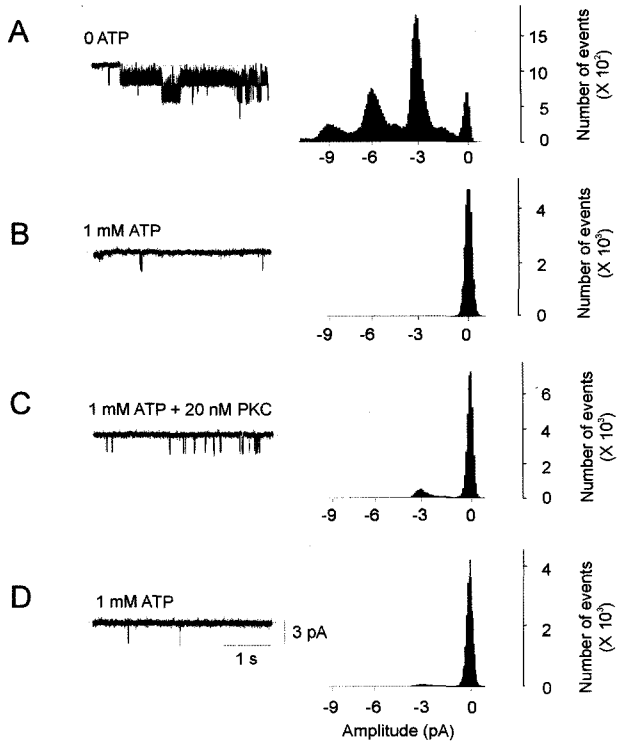

Fig. 3. Effects of PKC on K_{ATP} channel activity in the presence of ATP. Single-channel current traces and corresponding histograms for recordings obtained from excised inside-out patches (A) in control conditions (ATP-free bath solution), (B) in the presence of 1 mM ATP, (C) in the presence of 1 mM ATP and 20 nM PKC, and (D) after washout of PKC. The membrane potential was held at −80 mV. Single-channel currents are downward deflections in the trace. Zero-current levels are indicated by broken lines. Data in the histograms were obtained from 45-s current recordings.

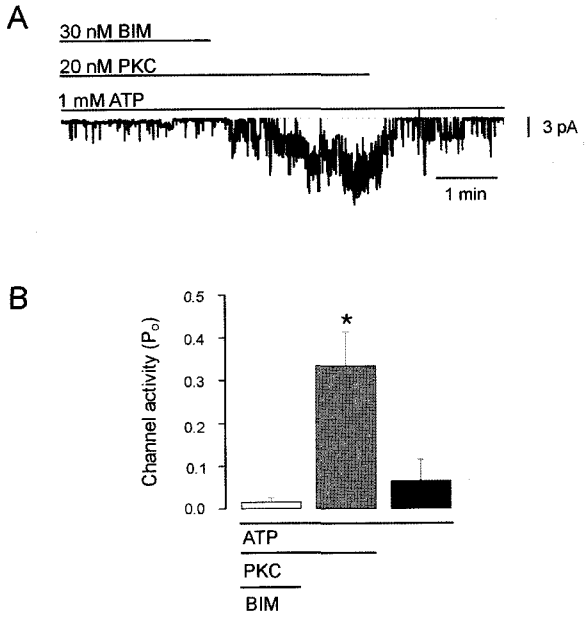

Fig. 4. Effect of BIM on K_{ATP} channel activity stimulated by PKC in the presence of ATP. (A) Current recording from an inside-out patch recorded at −40 mV. PKC (20 nM) had no effect on the K_{ATP} channel activity. Subsequent removal of PKC caused channel activity to return to near-control levels. Data were sampled at 400 Hz and filtered at 1 kHz. Broken line indicates the zero-current level. (B) Changes in channel activity in response to PKC in the presence of BIM. Histogram showing the pooled Po data for the following conditions: PKC+BIM; PKC; repeat control (1 mM ATP) after washout of PKC. All experiments were performed in the presence of 1 mM ATP. *Significantly different ($p<0.05$) vs. the control value (in the presence of BIM).

we tested the effect of PKC on the activity of single K_{ATP} channels in the presence of 1 mM ATP using excised inside-out patches. Fig. 3 shows that, after excision of the patch (Fig. 3A), 1 mM ATP inhibited spontaneous K_{ATP} channel opening (Po reduced from 0.380 to 0.016; Fig. 3B). In the continuous presence of ATP, exposure of the intracellular surface of the excised membrane to 20 nM PKC caused an enhancement of K_{ATP} channel activity (Po=0.120; Fig. 3C). After washout of PKC, channel activity returned to the control level (Po=0.035; Fig. 3D). Such an enhancement of channel activity by PKC in the presence of ATP was observed in 11 replications of the aforementioned experiment.

In the presence of the highly selective PKC inhibitor, BIM (30 nM), PKC (20 nM) had no effect on K_{ATP} channel activity. However, upon washout of BIM, PKC caused an increase in channel activity. Subsequent washout of PKC caused channel activity to return to the control level (Fig. 4).

The application of heat-inactivated PKC had no effect on K_{ATP} channel activity. Fig. 5A shows a representative result obtained, when the intracellular surface of an inside-out patch was exposed to 1 mM ATP. In the continuous presence of ATP (Po=0.072), application of 20 nM heat- inactivated PKC to the intracellular surface failed to enhance channel activity (Po=0.070). The same result was observed in four separate experiments (Fig. 5B).

PKC-induced activation of K_{ATP} channels is reversed by protein phosphatase

The data presented in Figs. 1~5 demonstrated that PKC activated single K_{ATP} channels in the presence of 1 mM ATP. This suggested that the phosphorylation of K_{ATP} channels by PKC was essential for the activation of these channels. To investigate whether PKC-mediated phosphorylation of K_{ATP} channels might be found in the PKC-induced activation of these channels, we investigated the effects of OA (Fig. 6), which (used at a low concentration of 5 nM) blocks the activity of PP2A in excised inside-out patches. In 6/6 patches tested, application of PKC in the presence of ATP caused a significant increase of K_{ATP} channel activity. After washing out PKC, OA was applied to the patches, and K_{ATP} channel activity was found to be maintained significantly above the control level ($p<0.05$). Upon removal of OA, K_{ATP} channel activity returned to the control level. These effects of OA are illustrated in Fig. 6B.

We also examined whether intracellular application of PP2A in the presence of PKC would lead to the inhibition of PKC-induced activation of K_{ATP} channels. Fig. 7A illustrates the effects of applying PP2A (1 U/ml) to the intracellular membrane surface of an inside-out patch in the presence of 20 nM PKC. The increase of K_{ATP} channel activity due to PKC was reversed by the application of

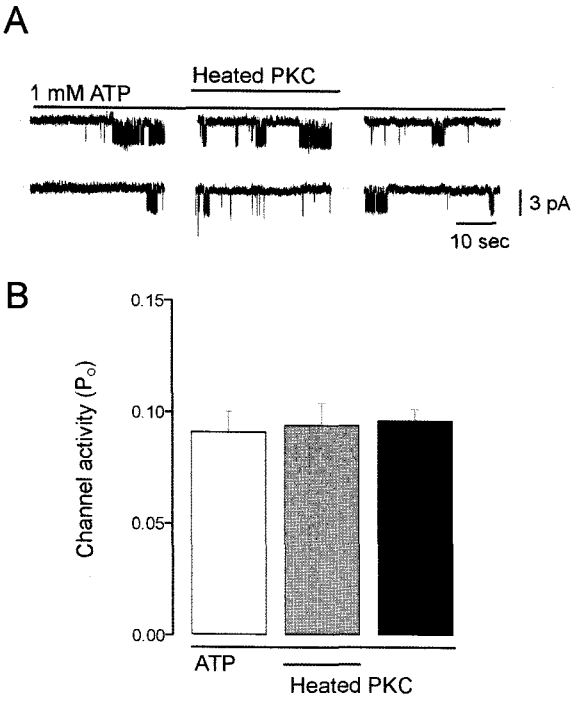

Fig. 5. Effect of heat-inactivated PKC on K_{ATP} channel activity in the presence of ATP. (A) Current recording from an inside-out patch at −40mV. Heat-inactivated PKC (20 nM) had no effect on the activity of the K_{ATP} channel in the presence of 1 mM ATP. Data were sampled at 400 Hz and filtered at 1 kHz. The broken line indicates the zero-current level. (B) Changes in channel activity in response to heat-inactivated PKC in the inside-out configuration. Histogram showing the pooled Po data for the following conditions: ATP alone; heat-inactivated PKC+ATP; repeat ATP alone after washout of heat-inactivated PKC.

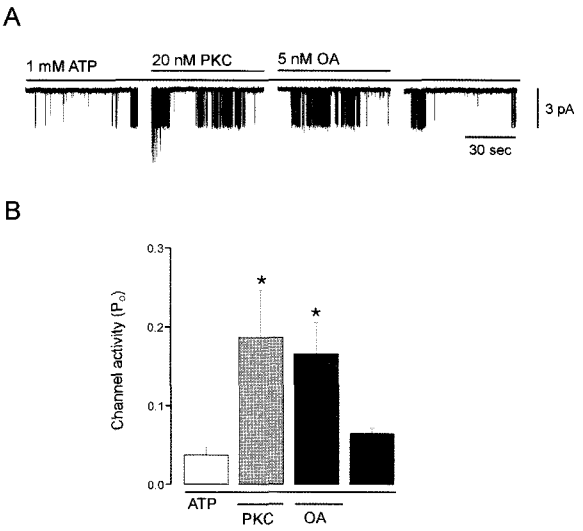

Fig. 6. Effect of okadaic acid (OA), a potent inhibitor of protein phosphatase 2A (PP2A), on the spontaneous reversal of PKC-induced activation of K_{ATP} channels. (A) Current recording from an inside-out patch at −40 mV. Addition of 1 mM ATP to the internal side of the patch reduced K_{ATP} channel activity. Enhanced K_{ATP} channel activity persisted upon removal of PKC and exposure to 5 nM OA. Removal of OA caused K_{ATP} channel activity to return to control levels. Data from six patches treated as described above were sampled at 400 Hz and filtered at 1 kHz. (B) Histogram showing the pooled Po data for the following conditions: control (1 mM ATP); PKC; OA; and repeat control after washout of OA. All experiments were performed in the presence of 1 mM ATP. *Significantly different ($p<0.05$) vs. the control value (in presence of 1 mM ATP).

PP2A. Data from four patches are summarized in Fig. 7B. The inhibition of the stimulatory effects of PKC on K_{ATP} channels by PP2A was blocked by 5 nM OA (Fig. 8).

DISCUSSION

We showed that PDD, an ester that activates PKC, could enhance the activity of single K_{ATP} channels in rabbit ventricular myocytes. This effect was mediated by the activation of PKC, because a structural analogue of PDD, 4 α-PDD, that does not stimulate PKC activityfailed to affect K_{ATP} channel activity and BIM, an inhibitor of PKC, inhibited the activation of channels induced by PKC. These results indicate that PKC-induced phosphorylation can activate K_{ATP} channels. Light et al. (1995) suggested that, at physiological (millimolar) concentrations of ATP, PKC-mediated phosphorylation of K_{ATP} channels should increase channel activity. Our data support this prediction, because PKC activated single K_{ATP} channels in the presence of millimolar concentrations of ATP.

Hu et al. (1996) demonstrated that PKC can activate K_{ATP} channels in intact cells (Hu et al, 1996). However, the same authors reported that the PKC activator PDD did not activate K_{ATP} channels at ATP concentrations >1 mM. It is possible that the two- to four-fold increase in K_{ATP} channel activity expected in the presence of millimolar concentrations of ATP may not have been detected in their whole-cell recordings because the expected change in the magnitude of the whole-cell current (~100 pA) is similar to the standard error of their data. Therefore, the data of (Hu et al, 1996) are not necessarily in conflict with our data. In another study (Liu et al, 1991), demonstrated at the whole-cell level that PKC can activate K_{ATP} channels. The results of both of the aforementioned studies concur with our conclusion that PKC can activate K_{ATP} channels.

In the present study, OA (5 nM) prevented the spontaneous reversal of PKC-induced activation of K_{ATP} channels, and application of exogenous PP2A in the presence of PKC reversed the PKC-induced activation of K_{ATP} channels. These observations suggest that endogenous membrane-associated PP2A is responsible for the reversal of PKC-induced activation of K_{ATP} channels. This concurs with our previous findings and implies that, in the presence of physiological concentrations of ATP, the activity of ventricular K_{ATP} channels is modulated by phosphorylation and dephosphorylation by PKC and PP2A, respectively. This process of phosphorylation and dephosphorylation could dynamically regulate the activity of K_{ATP} channels in the myocardium, which might be a mechanism by which K_{ATP} channel activity reversibly controls cellular excitability in the heart.

It has been reported that PKC can either inhibit or activate K_{ATP} channels in insulin-secreting cell lines, depending on the time course of experiments (Dunne et al, 1994). It has also been demonstrated that PKC activates

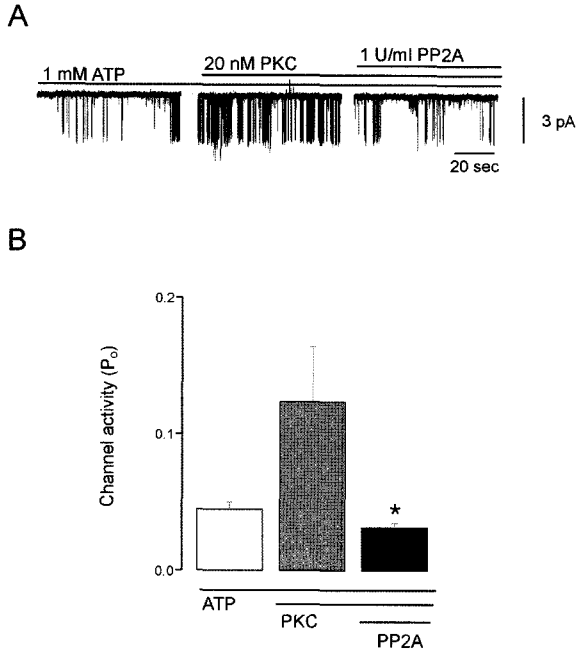

Fig. 7. Effect of exogenous PP2A on K_{ATP} channel activity stimulated by PKC. (A) Current recording from an inside-out patch at −40 mV. In the presence of 1 mM ATP, PKC (20 nM) caused an increase in channel activity. In the same patch, PP2A (1 U/ml) reversed the stimulatory effects of PKC on K_{ATP} channel activity. Data were sampled at 400 Hz and filtered at 1 kHz. The broken line indicates the zero-current level. (B) Histogram showing the pooled Po data for the following conditions: ATP alone; ATP + PKC; and additional application of PP2A in the presence of ATP + PKC. *Significantly different ($p < 0.05$) vs. the control value (before application of PP2A).

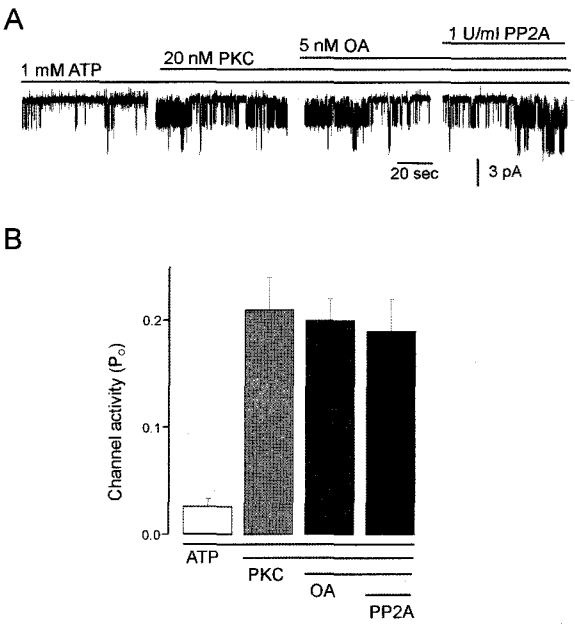

Fig. 8. Effect of exogenous PP2A on K_{ATP} channel activity stimulated by PKC in the presence of OA. (A) Current recording from an inside-out patch at −40 mV. In the presence of 1 mM ATP, PKC (20 nM) caused an increase in channel activity. In the presence of ATP, PKC, and 5 nM OA, the enhanced K_{ATP} channel activity persisted. Note that subsequent application of PP2A had no effect on the activity of K_{ATP} channels when OA was present. Data were sampled at 400 Hz and filtered at 1 kHz. The broken line indicates the zero-current level. (B) Changes in channel activity in response to PP2A in the presence of OA. Histogram showing the pooled Po data for the following conditions: ATP alone; ATP + PKC; ATP + PKC + OA; and additional application of PP2A in the presence of ATP + PKC + OA.

K_{ATP} channels in insulin-secreting cell lines via the stimulation of G-protein-coupled somatostatin receptors (Ribalet & Eddlestone, 1995). In follicle-enclosed oocytes (Honore & Lazdunski, 1991), smooth muscle (Bonev & Nelson, 1993), and kidney (Wang & Giebisch, 1991), activation of PKC by acetylcholine (Honore & Lazdunski, 1991; Bonev & Nelson, 1993) or bradykinin (Wang & Giebisch, 1991) leads to an inhibition of K_{ATP} channel activity. The results of the present study demonstrated that PKC can activate ventricular K_{ATP} channels at near-physiological concentrations of ATP. Therefore, it would appear that the effects of PKC on K_{ATP} channel function are tissue-specific and depend on the signaling pathway(s) to which PKC is linked.

Previous studies have associated activation of K_{ATP} channels (Parratt, 1994; Parratt & Kane, 1994) and activation of PKC (Liu et al, 1994; Ytrehus et al, 1994; Speechly-Dick, 1995) with the process of ischemic preconditioning. More specifically, several investigators suggested that K_{ATP} channels may be a link in the signaling pathway through which activation of PKC triggers ischemic preconditioning (Jenkins et al, 1995; Van Winkle et al, 1995), including human heart (Speechly-Dick et al, 1995). Most studies on this issue have been performed at the whole-heart level. Our results indicate that PKC can directly activate single cardiac K_{ATP} channels. Therefore, our findings are consistent with the hypothesis that PKC-catalyzed phosphorylation of K_{ATP} channels (or an associated membrane protein) leads to ischemic preconditioning.

In conclusion, K_{ATP} channels may be regulated by several intracellular signaling pathways that act via PKC-dependent phosphorylation to provide a link to one or more of the signaling pathways that trigger ischemic preconditioning.

ACKNOWLEDGEMENT

This work was supported by Grants R05-2003-000-00413-0 and R05-2004-000-00905-0, and by the *BioHealth Products Research Center* of the Korea Science and Engineering Foundation.

REFERENCES

Bonev AD, Nelson MT. Muscarinic inhibition of ATP-sensitive K^+ channels by protein kinase C in urinary bladder smooth muscle. *Am J Physiol* 265: C1723−C1728, 1993

Colquhoun D, Sigworth FJ. Fitting and statistical analysis of single channel records. In: Sakmann B, Neher E ed, *Single-Channel Recording*. 1st ed. New York, Plenum, p 191−263, 1983

Davis NW, Standen NB, Stanfield PR. ATP-dependent potassium channels of muscle cells: their properties, regulation, and possible functions. *J Bioenerg Biomembr* 23: 509−535, 1991

Dunne MJ. Phorbol myristate acetate and ATP-sensitive potassium channels in insulin-secreting cells. *Am J Physiol* 267: C501–506, 1994

Faivre JF, Findlay I. Action potential duration and activation of the ATP-sensitive potassium current in isolated guinea pig ventricular myocytes. *Biochem Biophys Acta* 1029: 167–172, 1990

Grover GJ, Sleph PG, Dzwonczyk S. Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A_1-receptors. *Circulation* 86: 1310–1316, 1992

Hamill OP, Marty A, Nether E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cell-free membrane patches. *Pflügers Arch* 391: 85–100, 1981

Han J, Kim E, So I, Earm YE. ATP-sensitive potassium channels are modulated by intracellular lactate in rabbit ventricular myocytes. *Pflügers Arch* 425: 546–548, 1993

Honore E, Lazdunski M. Hormone-regulated K^+ channels in follicle enclosed oocytes are activated by vasorelaxung K^+ channel openers and blocked by antidiabetic sulphonylureas. *Proc Natl Acad Sci USA* 88: 5438–5442, 1991

Hu K, Duan D, Li GR, Nattel S. Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. *Circ Res* 78: 492–498, 1996

Jenkins DP, Kerac M, Yellon DM. Preconditioning: protein kinase C and the K_{ATP} channel in isolated rat heart. *J Mol Cell Cardiol* 27: A142 Abstract, 1995

Lebuffe G, Schumacker PT, Shao ZH, Anderson T, Iwase H, Vanden Hoek TL. ROS and NO trigger early preconditioning: relationship to mitochondrial K_{ATP} channel. *Am J Physiol Heart Circ Physiol* 284: H299–H308, 2004

Light PE, Allen BG, Walsh MP, French RJ. Regulation of adenosine triphosphate-sensitive potassium channels from rabbit ventricular myocytes by protein kinase C and type 2A protein phosphatase. *Biochemistry* 34: 7252–7257, 1995

Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded preconditioning is mediated by A_1 adenosine receptors in rat heart. *Circulation* 84: 350–356, 1991

Liu Y, Ytrehus K, Downey JM. Evidence that translocation of protein kinase C is a key event during ischemic preconditioning of rabbit myocardium. *J Mol Cell Cardiol* 26: 661–668, 1994

Moon CH, Kim MY, Kim MJ, Lee S, Yi KY, Yoo SE, Lee DH, Lim H, Kim HS, Lee SH, Baik EJ, Jung YS. KR-31378, a novel benzopyran analog, attenuates hypoxia-induced cell death via mitochondrial K_{ATP} channel and protein kinase C-epsilon in heart-derived H9c2 cells. *Eur J Pharmacol* 506: 27–35, 2004

Okawa H, Horimoto H, Mieno S, Nomura Y, Yoshida M, Shinjiro S. Preischemic infusion of alpha-human atrial natriuretic peptide elicits myoprotective effects against ischemia reperfusion in isolated rat hearts. *Mol Cell Biochem* 248: 171–177, 2003

Otani H, Okada T, Fujiwara H, Uchiyama T, Sumida T, Kido M, Imamura H. Combined pharmacological preconditioning with a G-protein-coupled receptor agonist, a mitochondrial K_{ATP} channel opener and a nitric oxide donor mimics ischemic preconditioning. *Clin Exp Pharmacol Physiol* 30: 684–693, 2003

Parratt JR. Protection of the heart by ischemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 15: 19–25, 1994

Parratt JR, Kane KA. K_{ATP} channels and ischemic preconditioning. *Cardiovasc Res* 28: 783–787, 1994

Ribalet B, Eddlestone GT. Characterization of the G protein coupling of a somatostatin receptor in the K_{ATP} channel in insulin-secreting mammalian cell lines. *J Physiol (Lond)* 485: 73–86, 1995

Sanada S, Kitakaze M. Ischemic preconditioning: emerging evidence, controversy, and translation trials. *Int J Cardiol* 97: 263–276, 2004

Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and ATP-dependent K^+ channel?: studies of contractile function after stimulated ischemia in an atrial in vitro model. *Circ Res* 77: 1030–1035, 1995

Uchiyama Y, Otani H, Wakeno M, Okada T, Uchiyama T, Sumida T, Kido M, Imamura H, Nakao S, Shingu K. Role of mitochondrial K_{ATP} channels and protein kinase C in ischemic preconditioning. *Clin Exp Pharmacol Physiol* 30: 426–436, 2003

Van Winkle DM, Chien GL, Wolff RA, Soifer BE, Kuzume K, Davis RF. Cardioprotection provided by adenosine receptor activation is abolished by blockade of the K_{ATP} channel. *Am J Physiol* 266: H829–839, 1994

Van Winkle DM, Kuzume K, Dote K, Wolff RA. Infarct limitation by protein kinase C (PKC) is attenuated by blockade of ATP-sensitive potassium (K_{ATP}) channels. *J Mol Cell Cardiol* 27: A142 Abstract, 1995

Venkatesh N, Lamp ST, Weiss JN. Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia and metabolic inhibition in mammalian ventricle. *Circ Res* 69: 623–637, 1991

Walsh RS, Tsuchida A, Daly JJF, Thornton JD, Cohen MV, Downey JM. Ketamine-xylazine anaesthesia permits a K_{ATP} channel antagonist to attenuate preconditioning in rabbit myocardium. *Cardiovasc Res* 28: 1337–1341, 1994

Wang N, Minatoguchi S, Chen XH, Uno Y, Lu C, Misao Y, Nagai H, Takemura G, Fujiwara H. Benidipine reduces myocardial infarct size involving reduction of hydroxyl radicals and production of protein kinase C-dependent nitric oxide in rabbits. *J Cardiovasc Pharmacol* 43: 747–757, 2004

Wang W, Giebisch G. Dual modulation of renal ATP-sensitive K^+ channel by protein kinase A and C. *Proc Natl Acad Sci USA* 88: 9722–9725, 1991

Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs: efficacy, time course, and role of K_{ATP} channels. *Circulation* 89: 1229–1236, 1994

Ytehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 266: H1145–H1152, 1994