Protein kinase C inhibition of cloned inward rectifier ($HRK1/K_{IR}2.3$) K^+ channels expressed in *Xenopus* oocytes

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1. The effect of protein kinase activators on cloned inward rectifier channels expressed in *Xenopus* oocytes was examined using a two-electrode voltage clamp. PKA activators caused no change in $K_{IR}1.1$, $K_{IR}2.1$, or $K_{IR}2.3$ current. The PKC activators phorbol 12-myristate 14-acetate (PMA) and phorbol 12,13-dibutyrate (PDBu) inhibited $K_{IR}2.3$ currents, but not $K_{IR}2.1$ or $K_{IR}1.1$ current. This inhibition was blocked by staurosporine. An inactive phorbol ester, 4α-phorbol 12,13-didecanoate (4α-PDD), had no effect on $K_{IR}2.3$.

2. Upon changing solution from 2 to 98 mM K^+, $K_{IR}2.3$ but not $K_{IR}1.1$ or $K_{IR}2.1$ currents typically 'ran down' over 5 min to 60–80% of maximum amplitude. Rundown occurred even if PMA was applied before changing to high $[K^+]$ solution, indicating that rundown was independent of PKC activity. Rundown was evoked by substituting NMG^+ for Na^+, showing that it results from low $[Na^+]$ and not from high $[K^+]$.

3. These results suggest that $K_{IR}2.3$, but not $K_{IR}1.1$ or $K_{IR}2.1$, is subject to regulation, both by PKC activation and as a consequence of low $[Na^+]_o$. The difference in secondary regulation may account for specific responses to PKC stimulation of tissues expressing otherwise nearly identical K_{IR} channels.

In 1993, cloning of three major types of inwardly rectifying K^+ channels (K_{IR} channels) was reported. These were ROMK1 ($K_{IR}1.1$), a mild inward rectifier with rectification properties similar to the ATP-sensitive K^+ channels (Ho *et al.* 1993), IRK1 ($K_{IR}2.1$), a classic, strongly inwardly rectifying K^+ channel (Kubo, Baldwin, Jan & Jan, 1993*a*), and GIRK1 ($K_{IR}3.1$), a G-protein-activated strongly inwardly rectifying K^+ channel (Kubo, Reuveny, Slesinger, Jan & Jan, 1993*b*). These channels were distinct from previously cloned voltage-gated K^+ channels in that they contained only two apparent transmembrane domains (Kubo *et al.* 1993*a*). Since that time, several other K_{IR} channels have been cloned so that at present six families of K_{IR} channels have been identified, most containing several subtypes of channel (Doupnik, Davidson & Lester, 1995).

Inward rectifier channels are found in numerous cell types including ventricular myocytes, neurons, glial cells, skeletal muscle fibres, vascular smooth muscle cells and endothelial cells (see Doupnik *et al.* 1995 for review). In these different cell types the channels serve several functions, including determination and maintenance of a stable resting potential, modulation of cellular excitability, and possibly regulation of extracellular K^+ concentration. Furthermore, it has been suggested that these channels might be involved in the genesis of electrophysiological disorders such as cardiac arrhythmias (Beaumont, Michaels, Delmar, Davidenko & Jalife, 1995).

The aim of this study was to assess the effects of protein kinase activation on three different cloned K_{IR} channels expressed in *Xenopus* oocytes: $K_{IR}1.1$ (Ho *et al.* 1993), $K_{IR}2.1$ (Kubo *et al.* 1993*a*) and $K_{IR}2.3$ (Makhina, Kelly, Lopatin, Mercer & Nichols, 1994; Perier, Radeke & Vandenberg, 1994). All three channels possess consensus sites for phosphorylation by both protein kinases A and C. Since $K_{IR}2.1$ and $K_{IR}2.3$ have been shown to have a similar tissue distribution (Kubo *et al.* 1993*a*; Perier *et al.* 1994), a differential sensitivity to protein kinase activation could implicate one channel as a potential target for physiological channel modulation. The results demonstrate that alone among these channels, $K_{IR}2.3$ is downregulated by stimulation of PKC in *Xenopus* oocytes.

A preliminary report of some of the findings of this study has been given to the Biophysical Society (Henry, Pearson, Melton, Makhina & Nichols, 1995).

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METHODS

Expression of cloned K_{IR} channels in oocytes

cDNAs were propagated in the transcription-competent vectors pBluescript SK(−) (Stratagene, La Jolla, CA, USA) or pSport1 (BRL, Gaithersburg, MD, USA) in *E. coli* XL-1 blue. cRNA was transcribed *in vitro* using T7 or T3 RNA polymerase (Ambion, Austin, TX, USA) and capping from linearized cDNA. Stage V–VI *Xenopus* oocytes were isolated by partial ovariectomy following anaesthesia by immersion in 0·1% tricaine, and then defolliculated by treatment with 1 mg ml^{-1} collagenase (Type 1A, Sigma) in 0 mM Ca^{2+} ND96 (below) for 1 h. From 2 to 24 h after defolliculation, oocytes were pressure injected with ~50 nl cRNA (1–100 ng ml^{-1}). Oocytes were maintained at room temperature in ND96 containing 2 mM Ca^{2+} and supplemented with penicillin (100 u ml^{-1}) and streptomycin (100 mg ml^{-1}) for 1–7 days before recording.

Mutagenesis

Oligonucleotide-directed mutagenesis of cDNAs in Bluescript was performed using established methods of oligonucleotide-directed mutagenesis *in vitro*. Single strand DNA was rescued from M13mp18 vector (Stratagene) and mutations introduced by priming second strand synthesis by oligonucleotides harbouring the desired mutation. Selection against non-mutant DNA was accomplished as follows: uracil-containing template was used in mutagenic reactions and the resulting heteroduplex transformed into F′ *E. coli*. Preferential degradation of wild-type strand resulted in high frequency of mutant transformants (Kunkel, 1985). Transformants obtained in the mutagenesis reaction were manually sequenced to confirm mutation.

Electrophysiology

K_{IR} currents were measured using the two-electrode voltage clamp technique. Oocytes were placed in a small chamber (200 ml) mounted on the stage of a binocular microscope (SMZ-1, Nikon Instruments). The chamber was connected through agar bridges to the current-sensing headstage of the voltage clamp amplifier (OC-725 Oocyte Clamp, Warner Instruments Corp., Hamden, CT, USA), and constantly perfused with a laminar flow of bathing solution supplied by one of five reservoirs connected to a manifold at the inlet to the chamber. The location of this manifold assured rapid entry (<5 s) to the chamber of new solution when a new reservoir was selected.

Experiments were performed at room temperature (19–22 °C). Intracellular electrodes were filled with 3 M KCl and had initial tip resistances from 0·5 to 5 MΩ. Experiments were controlled using pCLAMP software to control a Digidata 1200 D/A interface (Axon Instruments) running on an 80486-based microcomputer (486/33C, Gateway 2000). Data were recorded by digitizing on-line and storing on the computer's hard disk, and/or by digitizing at 22 kHz onto videotape (Neuro-corder DR-890, Neuro-Data Instruments) for later playback onto a chart recorder (Gould Instruments) or computer capture with Axotape software (Axon Instruments). In studying inward rectifier currents, oocytes were typically impaled while bathed in ND96 solution. Only cells with very negative membrane potentials and small leakage and outward currents were used in these experiments.

Solutions and chemicals

Recordings were performed in solutions based on ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Na-Hepes, pH 7·5) or KD98 (98 mM KCl, 1 mM $MgCl_2$, 5 mM K-Hepes, pH 7·5). In some experiments investigating the effect of low $[Na^+]$ on channel activity, K^+ was elevated to 5 mM and all Na^+ was replaced with the organic cation *N*-methyl-D-glucamine (NMG). All phorbol ester analogues (PMA: phorbol 12-myristate 14-acetate; PDBu: phorbol 12,13-dibutyrate; 4α-PDD: 4α-phorbol 12,13-didecanoate) and the protein kinase inhibitor staurosporine were obtained from Sigma and dissolved in dimethyl sulphoxide (DMSO) for a stock concentration of 10 mM. We observed that DMSO could cause a non-specific 'rundown' of all three channels, if it had been exposed to air or plastics for any considerable period of time. We routinely used fresh DMSO, which was without effect at the concentrations used in these experiments. For most experiments, phorbol esters were typically diluted to 1 μM or 100 nM, so that the DMSO concentration did not exceed 0·01%. Application of DMSO at this concentration did not appear to alter K_{IR} channel function (data not shown). Forskolin (50 μM), 3-isobutyl-1-methylxanthine (IBMX, 100 μM), and 8-(4-chlorophenylthio)-cAMP (pCPT-cAMP, 100 μM) were obtained from Sigma and were dissolved first in water and then subsequently diluted to the stated concentrations in the experimental saline.

RESULTS

Oocytes that had been injected with cRNA for K_{IR} channels and maintained in low $[K^+]$ solution (ND96) exhibited highly negative membrane potentials (−50 to −95 mV) within 1 day after injection. Current–voltage relationships measured with voltage ramps, or voltage steps, between −140 and 100 mV showed that the oocytes expressed inwardly rectifying K^+ currents with the properties reported previously for each cloned channel (i.e. $K_{IR}1.1$ exhibited weak rectification at depolarized potentials, while $K_{IR}2.1$ and $K_{IR}2.3$ exhibited strong rectification at potentials above the K^+ equilibrium potential, E_K). Upon changing to KD98 solution, the inward current amplitude increased rapidly due to increase in K^+ driving force and relief of rectification (Hagiwara, Miyazaki & Rosenthal, 1976), and the reversal potential shifted from ~−90 mV to 0 mV. With $K_{IR}2.1$ and $K_{IR}1.1$, the current amplitude increased to near maximum amplitude in ~1 min. In some cases there was a further small, slow increase of current (Fig. 1), although the nature of this phenomenon was not apparent and we did not investigate it further. In contrast, $K_{IR}2.3$ current also increased to a maximum amplitude within ~1 min. However, in many cells, the current then slowly decreased over ~5 min to a steady-state value of 60–80% maximum current (Fig. 1). This 'rundown' phenomenon was observed in most but not all cells expressing $K_{IR}2.3$, but was not observed with $K_{IR}2.1$ or $K_{IR}1.1$ current, even when the same batch of oocytes produced rundown when injected with $K_{IR}2.3$.

After current amplitude stabilized in KD98 solution, transient application (~3 min) of a cocktail of PKA activators (forskolin, IBMX, pCPT-cAMP) did not cause any substantial change in $K_{IR}1.1$, $K_{IR}2.1$ or $K_{IR}2.3$ current (data not shown). However, transient application of the PKC activator PMA (1 μM) produced a marked inhibition of $K_{IR}2.3$ current, but not $K_{IR}2.1$ or $K_{IR}1.1$ current (Fig. 2*A* and *B*). Onset of current inhibition occurred ~1 min after exposure of cells to PMA, and the inhibition of current

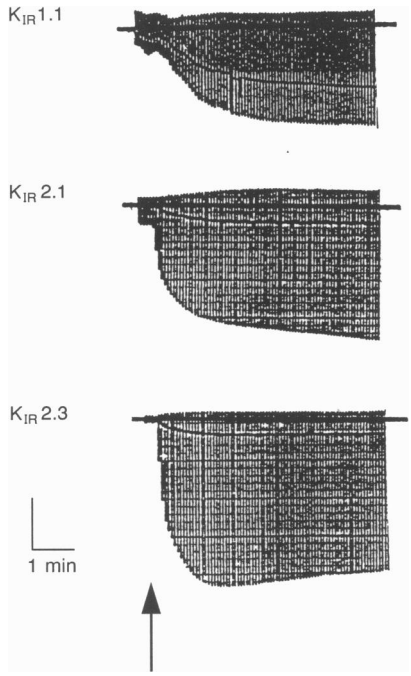

Figure 1. 'Rundown' of K_{IR}2.3 current following solution changes

Current traces from voltage ramps between −140 and 40 mV, applied every 4 s; holding potential, −20 mV. For K_{IR}1.1, K_{IR}2.1 and K_{IR}2.3, changing the bathing solution from ND96 to KD98 resulted in a rapid increase in inward current amplitude due to the increase of K^+ driving force and relief of Na^+ block of the channel. K_{IR}2.3 current reached a maximum in ~1 min and then slowly decreased for several minutes (i.e. 'rundown'). K_{IR}1.1 and K_{IR}2.1 currents did not decrease over time. Vertical scale bar: 15 μA for K_{IR}2.1 and K_{IR}2.3, 7·5 μA for K_{IR}1.1.

continued to develop for 5–8 min until current was decreased to roughly half the amplitude before PMA addition. PMA-induced inhibition of K_{IR}2.3 current appeared to reverse only slowly or not at all, as no recovery was seen for ~30 min after PMA application.

We performed several experiments to confirm that PMA was decreasing K_{IR}2.3 current by stimulating protein kinase C. First, 0·01% DMSO in KD98 was applied with no PMA and found to have no effect on K_{IR}2.3 current (data not shown). We then tested the ability of other phorbol

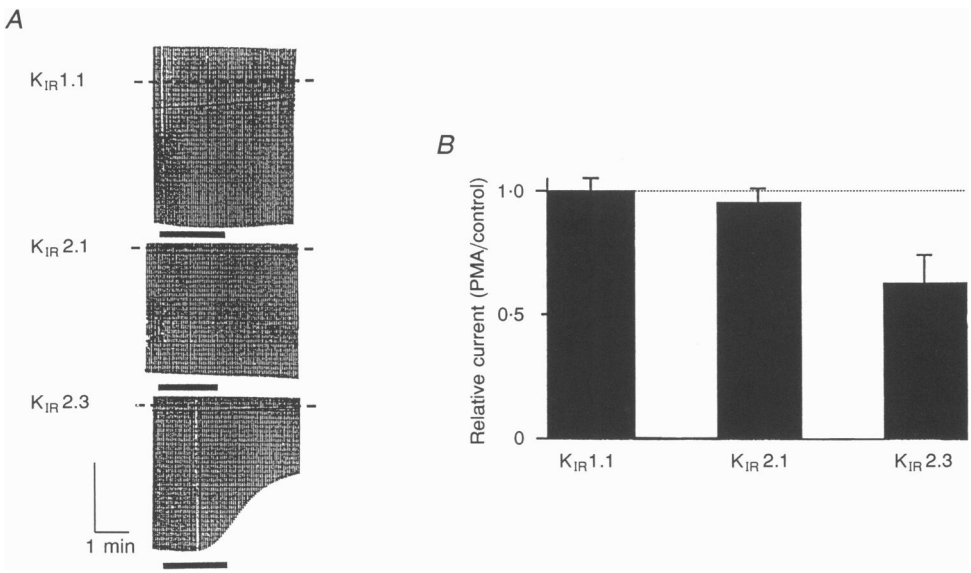

Figure 2. Sensitivity of K_{IR} channels to PKC activation

A, representative current records from oocytes expressing K_{IR} channels in response to voltage ramps between −140 and 40 mV; holding potential, −20 mV. Horizontal bars (2 min) indicate the period of PMA (1 mM) application. K_{IR}1.1 and K_{IR}2.1 currents were insensitive to PMA application, while K_{IR}2.3 current was inhibited by PMA application. Inhibition of K_{IR}2.3 current occurred with a delayed onset and developed slowly over several minutes until the current was reduced ~50%. Zero current indicated by dashed line; vertical scale bar is 10 μA for K_{IR}1.1 and K_{IR}2.3, 20 μA for K_{IR}2.1. *B*, histogram shows relative current inhibition by 1 μM PMA. PMA significantly reduced K_{IR}2.3 current amplitude to 63 ± 11% ($n = 4$) of pre-control ($P < 0·05$, Student's paired t test). Current was measured at −100 mV, 5 min after PMA application. The dashed line in this and subsequent histograms represents the control current level (normalized to 1).

esters to decrease $K_{IR}2.3$ current. Application of 1 μM PDBu, another phorbol ester known to activate PKC, caused a similar decrease in $K_{IR}2.3$ current ($n = 5$), whereas 4α-PDD, an inactive phorbol ester, caused no decrease in current ($n = 3$; Fig. 3*A* and *B*). PMA (100 nM) was found to inhibit $K_{IR}2.3$ to the same extent as 1 μM PMA, and 10 μM PMA also produced no further inhibition of the current (data not shown). These results are consistent with the known mechanism of phorbol ester stimulation of PKC, in which a phorbol ester such as PMA activates PKC completely and irreversibly (Miyake, Tanaka, Tsuda, Yamanishi, Kikkawa & Nishizuka, 1983). To verify further that PMA was inhibiting $K_{IR}2.3$ current via PKC activation, we tested the ability of staurosporine, an inhibitor of PKC

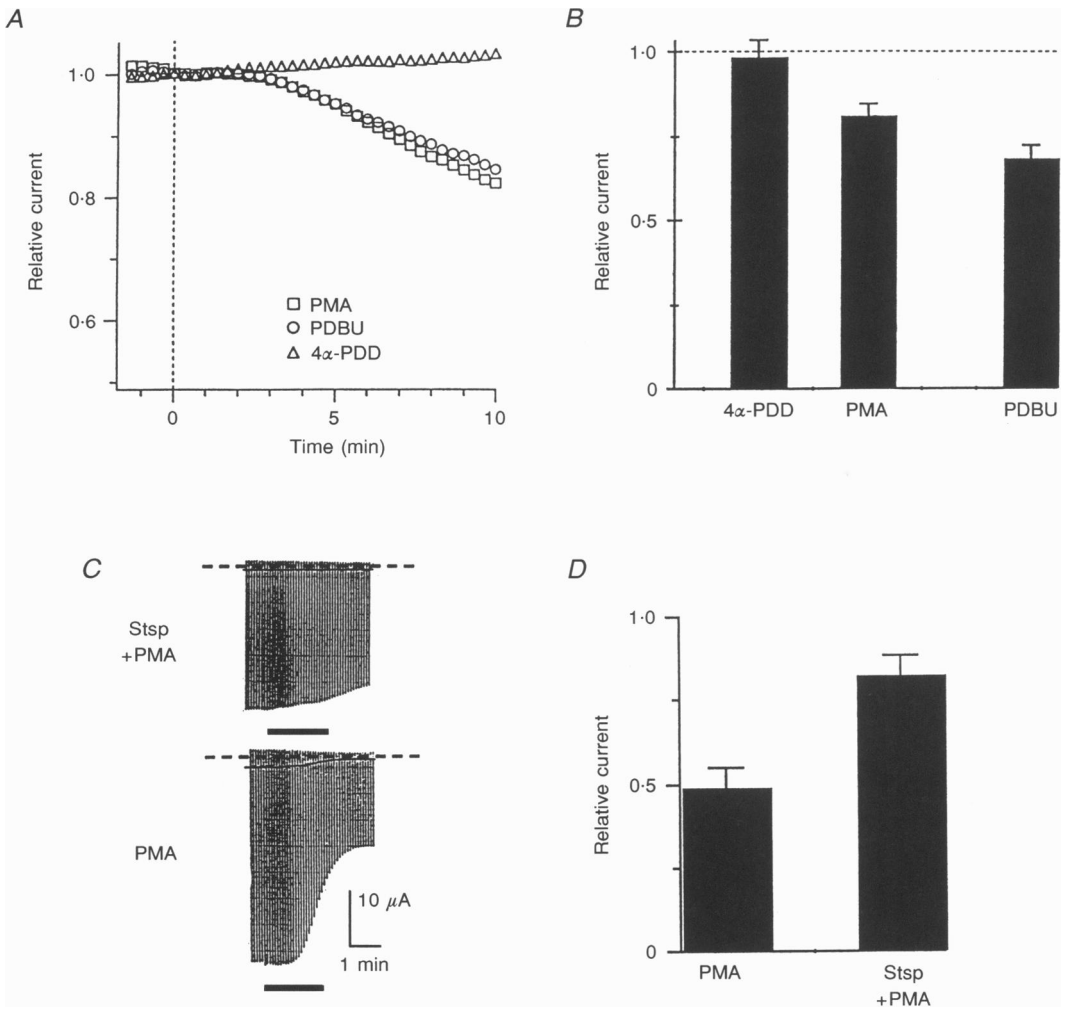

Figure 3. Specificity of phorbol ester-evoked inhibition of $K_{IR}2.3$ current

A, time course of relative current at −140 mV recorded from oocytes expressing $K_{IR}2.3$ current in response to 1 μM phorbol ester application. Cells were held at −20 mV and ramped between −140 and 40 mV (900 ms) every 20 s. *I–V* relationships measured in this way were indistinguishable from *I–V* relationships measured at the end of 200 ms voltage pulses. Data presented for PMA were obtained from cells to which 4α-PDD had previously been applied and had elicited no response. PMA was applied to these cells ~15 min after 4α-PDD removal. Phorbol ester analogue (1 μM) was applied for ~2 min at the time indicated by the dashed line. *B*, summary of the effect of phorbol ester application on $K_{IR}2.3$ current at −140 mV. Histogram indicates proportion of current remaining 10 min after phorbol ester application. 4α-PDD did not inhibit $K_{IR}2.3$ current, while subsequent application of PMA or PDBu was still effective at reducing current (PMA, $P < 0{\cdot}05$, $n = 3$; PDBu, $P < 0{\cdot}01$, $n = 5$). *C*, representative current traces recorded during voltage ramps between 40 and −140 mV show the effect of 100 nM PMA application in the presence or absence of the protein kinase inhibitor staurosporine (Stsp; 1 μM). Phorbol esters were applied for the times indicated by the horizontal bars. The dashed line indicates zero current. *D*, summary histogram shows that staurosporine decreased the effect of PMA application on $K_{IR}2.3$ current at −140 mV. In the presence of staurosporine, PMA reduced the current to 82 ± 4% of control ($n = 3$), while in oocytes from the same batch not treated with staurosporine, PMA reduced current to 49 ± 6% of control ($n = 4$). This difference was significant ($P < 0{\cdot}01$, unpaired *t* test).

(Tamaoki, Nomoto, Takahashi, Kato, Morimoto & Tomita, 1986), to block PMA-induced inhibition of $K_{IR}2.3$. When oocytes were preincubated with 1 μM staurosporine, PMA application in the presence of staurosporine greatly reduced the effect of PMA application (Fig. 3*C* and *D*). In oocytes from the same batch, 100 nM PMA reduced $K_{IR}2.3$ current to 49 ± 6% (mean ± S.E.M.) of the original level in 7 min ($n = 4$), while 100 nM PMA in the presence of 1 μM staurosporine reduced current to only 82 ± 4% of the original level ($n = 3$). In the presence of higher concentrations of staurosporine (10 μM, $n = 2$), PMA application failed to inhibit $K_{IR}2.3$ current. Taken together, these data indicate that activation of PKC by phorbol esters inhibits $K_{IR}2.3$ current expressed in *Xenopus* oocytes.

The steep voltage dependence of inward rectifiers results from pore blockage by intracellular Mg^{2+} and polyamine ions (Vandenberg, 1987; Matsuda, Saigusa & Irisawa, 1987; Lopatin, Makhina & Nichols, 1994; Ficker, Taglialatela, Wible, Henley & Brown, 1994). Current through $K_{IR}2.3$ channels could be reduced by increasing the degree of rectification (i.e. the potency or voltage dependence of channel block). To determine if current inhibition resulted from changes in voltage dependence, the effect of PMA was examined over a broad range of potentials by applying voltage ramps to the cells, or by stepping the cells to a series of test potentials. Representative current traces and current–voltage relations are shown in Fig. 4. The steady-state voltage dependence was unaffected by PMA application, as were the kinetics of rectification as seen in current traces recorded from voltage steps to negative potentials, indicating that current reduction was not closely related to the mechanism of rectification.

Comparison of the amino acid sequences of these channels indicates that although there are multiple potential sites for PKC-dependent phosphorylation in all channels, $K_{IR}2.3$ possesses only three consensus sequences that are not also present in $K_{IR}2.1$ or $K_{IR}1.1$. We have mutated these sites to examine the possibility that PKC acting at these sites is responsible for the above effects. A truncation mutant (E418stop) which removed the unique C-terminal consensus site resulted in expressed channels that were still inhibited by PMA application. A double mutant (S36C,S39A) which

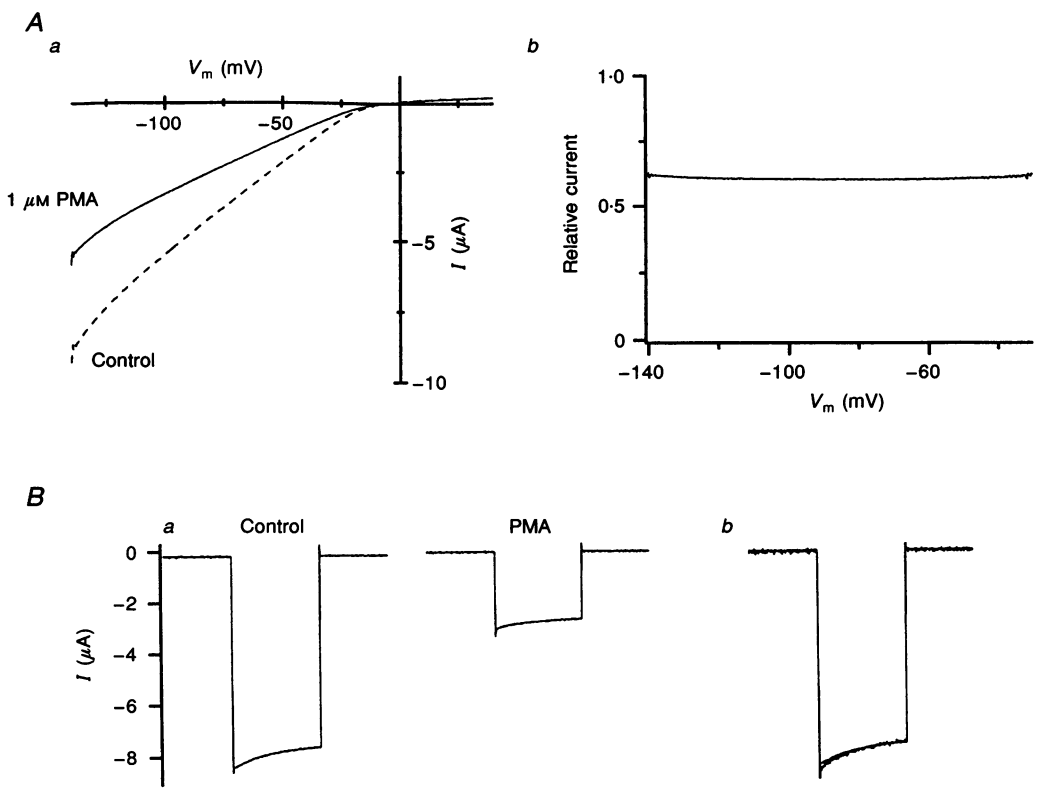

Figure 4. PMA effect is voltage independent

A a, representative current records of $K_{IR}2.3$ current recorded in KD98 solution in response to voltage ramps between 40 and −140 mV. Dashed current trace indicates current recorded before 1 mM PMA application; continuous line indicates current recorded after inhibition by PMA. *A b*, relative current after PMA (as a fraction of the control current) *versus* membrane potential, for the currents in *A a* (for −140 to −15 mV). *Ba*, current traces recorded during (200 ms) voltage jumps from −20 to −140 mV, before (Control) and after PMA-induced inhibition. Recordings were made in KD98 solution. *Bb*, when the same current traces are scaled to the same steady-state amplitude and superimposed, no differences are apparent in the 'inactivation' kinetics of $K_{IR}2.3$ current.

abolished the two unique N-terminal sites did not produce functional channels (data not shown). More extensive mutagenesis will be necessary to examine the possibility that a conserved site can be PKC phosphorylated in $K_{IR}2.3$, but not in other channels, due perhaps to alternative secondary structure in different channels.

Most experiments studying PMA-induced inhibition of $K_{IR}2.3$ were performed in KD98 solution, so that PMA was applied after the 'rundown' of $K_{IR}2.3$ (see Fig. 1). To determine if 'rundown' was required to confer PKC sensitivity to $K_{IR}2.3$ current, PMA was applied to oocytes expressing $K_{IR}2.3$ current bathed in ND96 solution (or an altered solution containing 90 mM Na^+ and 5 mM K^+, rather than 2 mM K^+, in order to enhance K_{IR} current) before application of KD98 and thus before current rundown. PMA (1 μM) effectively inhibited $K_{IR}2.3$ current in ND96 solution, reducing current to $40 \pm 5\%$ of the amplitude present before PMA application ($n = 4$; Fig. 5*A*), or in similar solutions with low K^+, high Na^+ concentrations. After maximal PMA-induced inhibition in ND96, subsequently changing to KD98 solution still produced rundown (to $76 \pm 6\%$ of maximum current, $n = 4$). Because PKC-induced inhibition did not require previous rundown of the current, and because rundown could be elicited after maximal current inhibition by PKC, it seems likely that these two processes are mediated by distinct intracellular mechanisms.

Rundown results from low [Na^+]

Because of the parallel presence of rundown and PKC-induced inhibition only in $K_{IR}2.3$ currents, the rundown phenomenon was investigated in greater detail, with the possibility in mind that rundown reflects a convergent modulatory mechanism.

To determine if rundown resulted from the increase in [K^+] or from the decrease in [Na^+], [Na^+] was reduced by substitution of NMG^+ rather than with K^+. NMG^+ substitution also produced substantial rundown ($61 \pm 8\%$ maximum current, $n = 7$). After rundown was complete in NMG^+ solutions, subsequent change to KD98 solution produced no further rundown (10% increase over initial maximum current, $n = 2$; Fig. 5*B*). Additional experiments indicated that rundown would occur if [Na^+] in the bathing solution was decreased to less than 50 mM (data not shown). Thus, rundown appears to result not from a high [K^+] but from a low [Na^+]. Examining current–voltage relationships of the current reduced by rundown indicated that the rundown process inhibited current in a voltage-independent fashion (not shown).

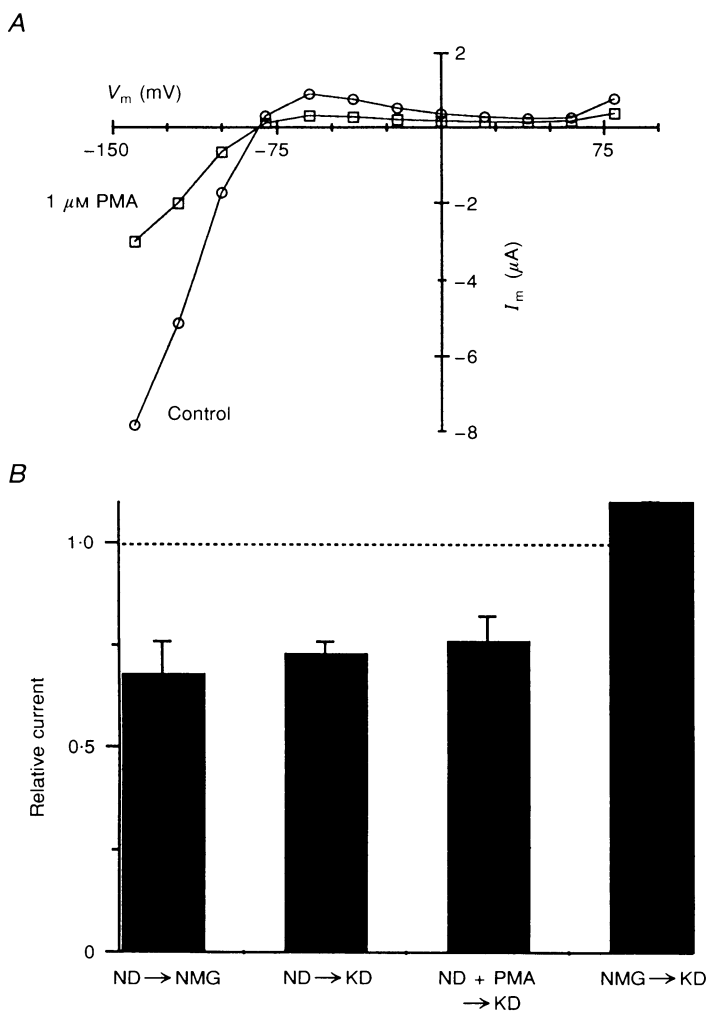

Figure 5. PMA inhibits $K_{IR}2.3$ current in low [K^+] solutions

A, current–voltage relationship from a representative oocyte in ND96 solution. The cell was held at −20 mV and stepped to potentials between −140 and 80 mV (duration 200 ms). Application of 1 μM PMA decreased $K_{IR}2.3$ current measured in ND96 solution in the same manner as current measured in KD98 solution. *B*, relative current at −140 mV following solution change (relative to maximal current immediately after the solution change), for the changes indicated. ND → NMG, change from 90 mM Na^+, 5 mM K^+ to 90 mM NMG^+, 2 mM K^+; ND → KD, change from ND96 (2 mM K^+) to KD98 (98 mM K^+); ND + PMA → KD, change from ND96 to KD98 after PMA-induced inhibition of $K_{IR}2.3$ had reached steady state; NMG → KD, change from 90 mM NMG^+, 5 mM K^+ to KD98 after rundown in NMG solution had reached steady state.

DISCUSSION

These experiments indicate that $K_{IR}2.3$ channels expressed in *Xenopus* oocytes are sensitive to inhibition by protein kinase C, while $K_{IR}2.1$ and $K_{IR}1.1$ channels are unaffected by PKC stimulation. While a direct effect of phorbol esters on $K_{IR}2.3$ channels was not excluded, control experiments with other phorbol esters and with the kinase inhibitor staurosporine indicated that phorbol esters are likely to have inhibited $K_{IR}2.3$ by activating protein kinase C. Furthermore, since $K_{IR}2.1$ and $K_{IR}1.1$ currents were not inhibited by phorbol esters, it seems likely that inhibition of $K_{IR}2.3$ current results from a specific interaction with the channel (and does not result from a non-specific inactivation or removal of membrane proteins). Although preliminary mutagenic analysis does not implicate an involvement of the three unique consensus sites for PKC-dependent phosphorylation in $K_{IR}2.3$, further experiments will be necessary to define the site of action of PKC.

Another property unique to $K_{IR}2.3$ channels was the tendency of these channels to exhibit 'rundown' in solutions with low external $[Na^+]$. The mechanism of rundown appeared to be distinct from the mechanism of inhibition from PKC stimulation, since PKC-induced inhibition did not abolish rundown, and rundown did not abolish PKC-induced inhibition. We have not investigated the mechanism of channel rundown in detail although, based on the results presented, it seems likely to be a consequence of altering transmembrane Na^+ flux. Previous studies have demonstrated the presence of Na^+–Ca^{2+} and Na^+–H^+ exchangers in *Xenopus* oocytes (Cartaud, Boyer & Ozon, 1984; Burckhardt, Kroll & Fromter, 1992). Changes in $[Na^+]$ could thus conceivably be affecting $K_{IR}2.3$ current through changes in intracellular $[Ca^{2+}]$ or $[H^+]$.

The lack of effect on the voltage dependence, or kinetics, of rectification indicates that PKC stimulation does not affect the mechanism of rectification, and is unlikely to result from direct effects within the permeation pathway. Since the inhibition by PKC was not complete, it seems unlikely that PKC causes a removal of channels from the membrane. We thus expect an effect on voltage-independent gating, but the small unit conductance of this channel (Makhina *et al.* 1994) prevented us from examining the effect of PKC stimulation on single channel activity.

Modulation of $K_{IR}2.3$ but not $K_{IR}2.1$ by PKC provides a novel mechanism for regulation of excitability in the nervous system. Recent reports have shown that neurotensin and substance-P act through PKC to decrease K_{IR} currents in dopaminergic neurons of the substantia nigra (Wu & Wang, 1995) and neurons of the nucleus basalis (Takano, Stanfield, Nakajima & Nakajima, 1995), respectively. An inwardly rectifying K^+ channel in T84 epithelial cells has also been shown to be downregulated by PKC (Tabcharani, Boucher, Eng & Hanrahan, 1994). While at least three subfamilies of constitutively active inward rectifier channels have been reported to be present in the brain, it is not clear which channels correspond to currents expressed in these cells. Our findings indicate that $K_{IR}2.1$ is not modulated by PKC in the *Xenopus* oocyte expression system, and is therefore not likely to be modulated *in vivo*. Since $K_{IR}2.3$ channels were modulated by PKC stimulation in a manner similar to that reported in the CNS, and since $K_{IR}2.3$ is present in the CNS, $K_{IR}2.3$ is a good candidate for the channel protein. PKC is stimulated in the heart during episodes of ischaemia and may be necessary for the induction of ischaemic preconditioning (Armstrong, Downey & Ganote, 1994; Speechly-Dick, Mocanu & Yellon, 1994). During myocardial ischaemia, there may be an increase in weakly rectifying K^+ currents mediated by activation of K_{ATP} channels (Billman, 1994). Despite the fact that Northern blot analysis has shown RNA for both $K_{IR}2.1$ and $K_{IR}2.3$ in the heart (Perier *et al.* 1994), no reports have shown that cardiac inward rectifier current is decreased by PKC stimulation. It seems likely that $K_{IR}2.3$ channels play a limited role in cardiac function.

Our results conflict somewhat with the report of Fakler, Brandle, Glowatzki, Zenner & Ruppersberg (1994). This group demonstrated stimulation by PKA of $K_{IR}2.1$ channels that had 'run down' in excised membrane patches. They also demonstrated a downregulation of $K_{IR}2.1$ channels by *N*-heptyl-5-chloro-1-naphthalenesulphonamide (a non-phorbol stimulator of protein kinase C). We did not observed any effect of phorbol esters on $K_{IR}2.1$ channels in intact cells, and it seems possible that the change in environment after patch excision might account for the difference in results. However, Fakler *et al.* (1994) also reported a decrease in $K_{IR}2.1$ currents in intact oocytes exposed to 12-*O*-tetradecanoylphorbol 13-acetate (TPA), and these results are not reconcilable with ours in any simple way. Since we used different phorbol esters, it is conceivable that TPA would have inhibited $K_{IR}2.1$ currents in our experiments. However, the positive effect of PMA and PDBu on $K_{IR}2.3$ does exclude the possibility that the negative effect on $K_{IR}2.1$ was artifactual.

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