Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11528–11532, December 1993
Physiology

The min K channel underlies the cardiac potassium current I_{Ks} and mediates species-specific responses to protein kinase C

MICHAEL D. VARNUM*, ANDREAS E. BUSCH†‡, CHRIS T. BOND*, JAMES MAYLIE†, AND JOHN P. ADELMAN*

*Vollum Institute for Advanced Biomedical Research, and †Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR 97201

Communicated by Bertil Hille, September 3, 1993

ABSTRACT **A clone encoding the guinea pig (gp) min K potassium channel was isolated and expressed in *Xenopus* oocytes. The currents, gpI_{sK}, exhibit many of the electrophysiological and pharmacological properties characteristic of gpI_{Ks}, the slow component of the delayed rectifier potassium conductance in guinea pig cardiac myocytes. Depolarizing commands evoke outward potassium currents that activate slowly, with time constants on the order of seconds. The currents are blocked by the class III antiarrhythmic compound clofilium but not by the sotalol derivative E4031 or low concentrations of lanthanum. Like I_{Ks} in guinea pig myocytes, gpI_{sK} is modulated by stimulation of protein kinase A and protein kinase C (PKC). In contrast to rat and mouse I_{sK}, which are decreased upon stimulation of PKC, myocyte I_K and gpI_{sK} in oocytes are increased after PKC stimulation. Substitution of an asparagine residue at position 102 by serine (N102S), the residue found in the analogous position of the mouse and rat min K proteins, results in decreased gpI_{sK} in response to PKC stimulation. These results support the hypothesis that the min K protein underlies the slow component of the delayed rectifier potassium current in ventricular myocytes and account for the species-specific responses to stimulation of PKC.**

The delayed rectifier K^+ current, I_K, is vitally important for initiation of repolarization of cardiac action potentials (1). I_K and its relationship to cardiac function have been extensively studied in guinea pig myocytes (2–5). This current exhibits complex kinetics with very slow activation rates and does not inactivate (3, 6). Drugs that inhibit this outward potassium current also extend action potential duration (APD) and are effective class III antiarrhythmic agents (7). Noble and Tsien (8) proposed that in sheep Purkinje fibers I_K consists of more than one component. This hypothesis was later substantiated in guinea pig myocytes by use of the class III antiarrhythmic sotalol derivative E4031, which demonstrated that I_K consists of a fast activating, inwardly rectifying component, I_{Kr}, and a slowly activating component, I_{Ks} (9). I_{Ks} comprises the major component of I_K and, due to its slow kinetics of deactivation, represents the predominant repolarizing current during increased heart rate. Until recently, the most potent class III compounds have been specific for I_{Kr}, with the exception of clofilium, which appears to block both I_{Kr} and I_{Ks} (7). However, under conditions such as tachycardia, class III agents that only block I_{Kr} are significantly less effective (10). Recently, a novel class III agent (NE10064) has been described (11); this compound possesses potent antiarrhythmic activity, prolongs APD, and specifically blocks I_{Ks} (12). In addition, I_{Ks} is modulated by the second messengers protein kinase C (PKC), protein kinase A (PKA), and relative levels of intracellular calcium (13–16). PKC has species-specific effects, decreasing mouse I_K but increasing guinea pig I_K (14, 17).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Takumi *et al.* (18) used expression cloning to isolate a cDNA clone from rat kidney that encodes a 130-amino acid peptide; the sequence predicts a single transmembrane domain and lacks significant homology to other cloned potassium channels. However, when *in vitro* synthesized mRNA derived from this clone is injected into *Xenopus* oocytes, depolarizing commands give rise to outward potassium currents (I_{sK}) with properties similar to I_{Ks} in cardiac myocytes. The mRNA encoding the min K channel has been detected in several tissues, including neonatal rat and mouse heart and human heart (17, 19, 20).

We now report the cloning and expression in *Xenopus* oocytes of the guinea pig (gp) min K protein. The mRNA encoding gpmin K is present in heart, and the expressed channels exhibit electrophysiological, pharmacological, and regulatory properties similar to I_{Ks} recorded in guinea pig ventricular myocytes. The results strongly suggest that the cloned guinea pig min K protein is responsible for the slowly activating cardiac potassium current I_{Ks} and account for the species-specific responses to PKC stimulation.

MATERIALS AND METHODS

Isolation of gpmin K Coding Sequence, PCR, and RNA Extraction. A guinea pig genomic DNA library constructed in λEMBL3 was purchased from Clontech. A PCR fragment encompassing the entire rat min K coding sequence (18) was radiolabeled by random priming and used as probe to screen 750,000 guinea pig genomic DNA clones (Colony/Plaque Screen; NEN). Hybridization was in 1 M NaCl/1% SDS/50% formamide/100 μg of yeast tRNA per ml at 37°C; filters were washed in 0.2× SSC/0.1% SDS (1× SSC = 0.15 M NaCl/15 mM sodium citrate) at 42°C and exposed to Kodak x-ray film. Positively hybridizing phage were purified by repeated screenings at reduced density. Restriction analysis revealed an ≈650-bp hybridizing *Hin*dIII–*Eco*RI restriction fragment, which was subcloned into M13 phage, and the nucleotide sequence of the insert was determined as described (17). This same fragment was subcloned into pS^- and used as substrate for *in vitro* mRNA synthesis. Site-directed mutagenesis was performed using the altered sites method (Promega). Oligonucleotides were synthesized on an Applied Biosystems 391 DNA synthesizer; PCRs were performed with AmpliTaq DNA polymerase on a Perkin–Elmer 9600 thermocycler (Perkin–Elmer/Cetus). RNA was isolated as described (21).

Oocyte Expression and Electrophysiology. *In vitro* synthesis of mRNA and oocyte injection and handling have been described (22). Two-electrode voltage clamp recordings were made from oocytes 2–5 days after RNA injection with a TEV-200 or CA-1 amplifier (Dagan Instruments, Minneapo-

Abbreviations: PKC, protein kinase C; PKA, protein kinase A; *I*, current; *V*, voltage; PDD, phorbol 12,13-didecanoate; RT, reverse transcription; CPT-cAMP, 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate.

‡Present address: Eberhard-Karls-Universität Tubingen, Physiologisches Institut I, Gmelinstrasse 5, 72076 Tubingen, F.R.G.

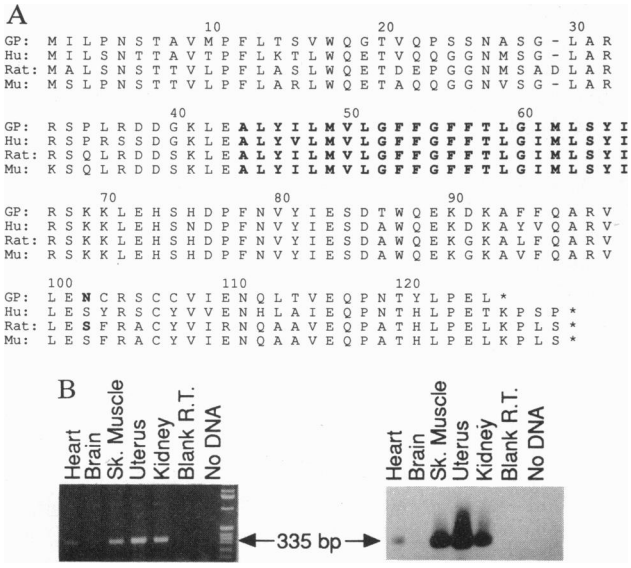

FIG. 1. Primary structure and expression pattern of gpmin K. (*A*) Amino acid sequences of cloned min K proteins. The predicted transmembrane domains are in bold type, as are residues in the guinea pig and rat sequences that mediate species-specific responses to PKC (see text). GP, guinea pig; Hu, human; Mu, mouse. (*B*) Tissue distribution of guinea pig min K mRNA. Reverse transcribed RNA from the indicated tissues was used in the PCR with oligonucleotides specific for sequences within the coding region of the min K mRNA. Reaction products were separated through an agarose gel (*Left*), prepared as a Southern blot and probed with a radiolabeled oligonucleotide directed to an internal sequence (*Right*). A weak signal was consistently detected from brain. From left: heart, brain, skeletal muscle, uterus, kidney. Control lanes show PCRs that used a mock RT reaction, without added RNA, as substrate (Blank R.T.), and in which no substrate was added (No DNA).

lis) interfaced to an LSI 1173 computer. Oocytes were continuously superfused with a solution containing (mM) NaCl, 96; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 1; Hepes, 5; pH 7.6 at room temperature (21–23°C). All experiments were performed with the oocyte membrane held at −80 mV. The voltage dependence of I_{sK} was determined from measurements of tail currents following repolarization to −60 mV. The baseline for the tail currents was obtained from a 1-s prepulse to −60 mV preceding each test pulse. Activation curves were fitted by a Boltzmann relation with a Levenberg–Marquardt algorithm to minimize the sum of squares. Values from experiments with multiple data points are presented as mean ± SEM. The following chemicals were used. phorbol 12,13-didecanoate (PDD), 4-α-phorbol 12,13-didecanoate, staurosporine, and 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) (Sigma); isoproterenol (Winthrop Pharmaceuticals, New York); clofilium (Research Biochemicals, Natick, MA); E-4031 (Eisae Co., Tsukuba Research Laboratories, Ibaraki, Japan); *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H89) and cherlerythrine (LC Laboratories, Woburn, MA).

RESULTS

A guinea pig genomic DNA clone encoding the min K protein was isolated using the rat min K coding sequence as probe. Although the predicted protein is highly homologous to min K proteins from other species, the open reading frame is five residues shorter than rat min K and four shorter than mouse or human min K. To determine whether this C-terminal truncation reflects the sequence encoded in the guinea pig min K mRNA or resulted from the presence of an intron in the genomic DNA, mRNA was isolated from guinea pig heart tissue and converted to single-stranded cDNA by reverse transcription (RT). This cDNA was used as substrate for PCRs using oligonucleotides that flank the predicted stop codon. The nucleotide and predicted amino acid sequences derived from the reaction products confirmed the presence of a stop codon at the position indicated in Fig. 1*A*. The tissue distribution of guinea pig min K mRNA was determined using RT-PCR. The results shown in Fig. 1*B* demonstrate expression in heart, skeletal muscle, uterus, and kidney; a weak signal was consistently detected from brain.

Expression of Guinea Pig I_{sK}. In oocytes expressing the cloned gpmin K, depolarizations to potentials positive to −50 mV evoked a slowly activating outward current, after an initial delay in onset, which failed to reach steady state during 30-s steps (Fig. 2*A*). The kinetics of activation following the initial delay were described by a sum of two exponentials plus a constant, a fast component that decreased from 3.4 ± 1.2 s at −20 mV to 1.6 ± 0.4 s at 40 mV and a slow component that decreased from 32 ± 8 s at −20 mV to 12.8 ± 1.3 s at 40 mV ($n = 3$). The relative amplitude of the fast component was 0.25 at −20 mV and 0.31 at 40 mV. Applying a third exponential to I_{sK}, to account for the delay in onset, yields a time constant that decreases from ≈1.5 s at −20 mV to 0.5 s

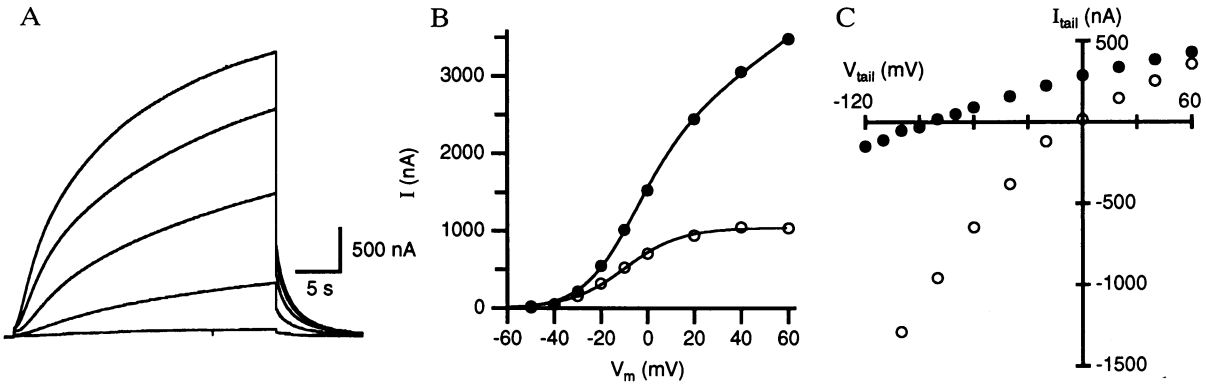

FIG. 2. Expression of gpmin K clone in *Xenopus* oocytes. (*A*) Currents elicited by 30-s depolarizing pulses from −40 to 40 mV in 20-mV steps. (*B*) Outward current–voltage (*I–V*) relation of I_{sK} (●) measured as the difference between the final and initial current during 30-s depolarizing pulses. The continuous curve was drawn according to the product of a linear *I–V* relation and a Boltzmann function representing $g_{max}(V_m - E_{rev})/(1 + e^{-(V_m - V_{1/2})/k})$; $g_{max} = 21.7$ μS, $E_{rev} = -101$ mV, $V_{1/2} = -9.8$ mV, $k = 11.6$ mV. Voltage dependence of activation of I_{sK} (○) was determined from measurements of tail currents as described in the text. The continuous curve was drawn according to a Boltzmann function, $I_{max}/(1 + e^{-(V_m - V_{1/2})/k})$; $I_{max} = 1037$ nA, $V_{1/2} = -9.5$ mV, $k = 11.8$ mV. (*C*) The open channel *I–V* relation of guinea pig min K in 2 (●) and 100 mM (○) external K^+ (substituted for Na^+). "Instantaneous" tail currents were determined after the capacity transient following repolarization to potentials between −120 and 60 mV from a fixed test potential of 20 mV.

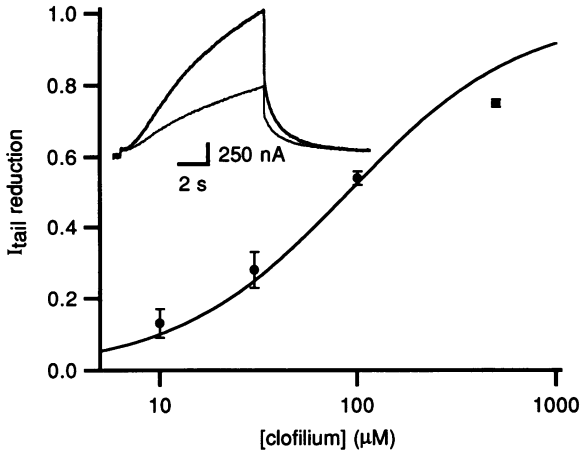

FIG. 3. Dose–response relationship for inhibition of gpmin K tail currents by clofilium. Data represent mean ± SEM, $n = 4$. The continuous curve was drawn according to the Michaelis–Menten equation ($K_i = 92.7$ μM). (*Inset*) Current traces elicited by 10-s steps to +20 mV before (upper trace) and after (lower trace) application of 100 μM clofilium.

at 40 mV. The delay in onset is more prominent at lower test pulse potentials and is exaggerated if the prepulse duration is made shorter or the prepulse potential is more negative. This dependence on previous potential (23, 24) suggests that the delay is due to a shift in equilibrium between multiple closed states toward the open state.

The voltage dependence of I_{sK} can be approximated by the product of a linear *I–V* relation and a Boltzmann function representing the voltage dependence of channel activation (Fig. 2*B*). However, since the open channel conductance may rectify, measurement of tail currents yields a better estimate of the voltage dependence of activation of I_{sK}. Because currents through min K channels fail to reach steady state even with long depolarizing commands, analysis of tail currents provides a "quasi" steady-state voltage dependence profile. Fig. 2*B* shows tail current amplitudes with a fitted Boltzmann function. The voltage for half-maximal activation, $V_{1/2}$, and maximally activated tail current, I_{max}, varied between different batches of oocytes; the average values for $V_{1/2}$ and I_{max} were −4.3 ± 1.4 mV and 1327 ± 129 nA ($n = 16$), respectively. The effect of stimulation of PKA and PKC on $V_{1/2}$ and I_{max} are therefore expressed as changes relative to control values in the same oocyte. The slope factor was less variable and was 11.2 ± 0.1 mV ($n = 16$).

Consistent with min K channels cloned from other species, gpmin K is selective for potassium ions. The reversal potential of I_{sK} in 2, 20, or 100 mM external K^+ (substituted for sodium) followed a slope of 58.4 mV per decade change in K^+, consistent with a channel selective for K^+ over Na^+ and Cl^- ($n = 4$, data not shown). In guinea pig myocytes, I_{Ks} exhibits only slight rectification in comparison to the strong rectification of I_{Kr} (9). Measurement of instantaneous tail currents at various potentials following a fixed test pulse revealed a small degree of rectification that increased slightly when extracellular K^+ was increased from 2 to 100 mM (Fig. 2*C*).

The sensitivity of guinea pig I_{sK} to compounds known to block I_{Kr}, I_{Ks}, and I_K in guinea pig myocytes was tested. The class III antiarrhythmic E4031, which blocks I_{Kr} but not I_{Ks} in myocytes ($IC_{50} = 400$ nM; ref. 6), had no effect on gpI_{sK} at concentrations as high as 5 μM (102.8% ± 2.6% of control, $n = 6$). Lanthanum, which at low concentrations (1 μM) also blocks I_{Kr} (25), did not reduce gpI_{sK} (1 μM La^{3+}, 102.9% ± 1.7% of control, $n = 4$). Higher concentrations of La^{3+} (100 μM) did induce a slight reduction (not shown), consistent with results reported for myocytes (25). The class III antiarrhythmic clofilium, which blocks both components of myocyte I_K (7, 26), inhibited gpI_{sK} with a K_i of 92.7 μM (Fig. 3). This is comparable to the reduction of myocyte I_K by clofilium, in which 100 μM blocked 56.7% (26).

Regulation by PKA and PKC. Guinea pig ventricular I_K is increased following (*i*) stimulation of β-adrenergic receptors by isoproterenol, (*ii*) addition of cAMP analogs to permeabilized guinea pig myocytes, or (*iii*) application of PKA catalytic subunit to the intracellular face of excised membrane patches (13, 27–29). In oocytes expressing gpmin K, the membrane-permeable cAMP analog CPT-cAMP increased gp I_{sK} 32.3% ± 3.0% ($n = 6$) (Fig. 4 *A* and *B*). CPT-cAMP treatment slightly shifted the voltage dependence of activation to more negative potentials and increased its voltage sensitivity (Fig. 4*C*; Table 1). Comparable effects were seen when endogenous oocyte β-adrenergic receptors (30) were stimulated by 2 μM isoproterenol (Table 1). Oocyte membrane capacitance was unaffected by either CPT-cAMP or isoproterenol (−0.1% ± 0.5%, $n = 6$; −3.2% ± 1.2%, $n = 5$, respectively). The selective PKA inhibitor H89 (31) decreased I_{sK} when applied alone and attenuated the effect of concomitantly applied CPT-cAMP ($n = 3$) (Fig. 4*A*).

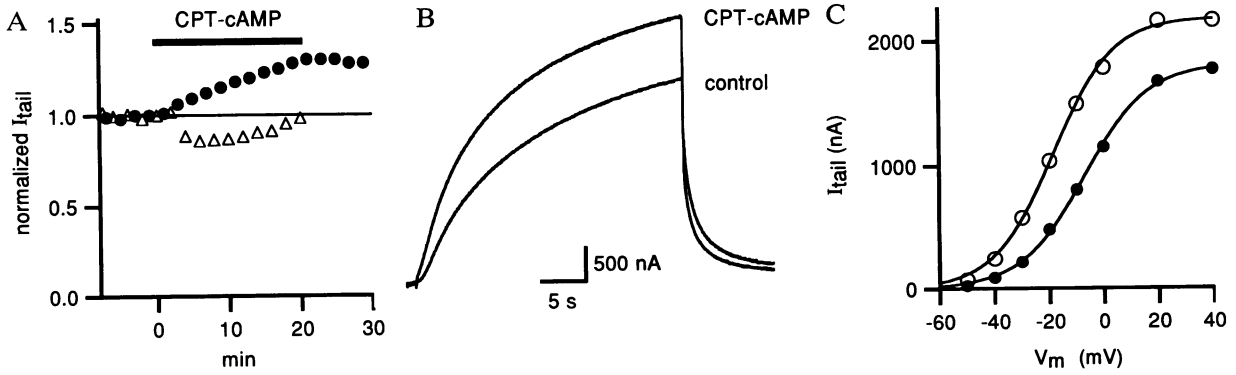

FIG. 4. PKA regulation of gpmin K. (*A*) Time course of CPT-cAMP effect (1 mM applied for 20 min) with (△) and without (●) prior and concomitant application of H89 (30 μM). Ten-second steps to +20 mV were made every 2 min; tail currents were measured at −60 mV and normalized to control values (before CPT-cAMP application). (*B*) Leak-subtracted current traces elicited by 30-s steps to +20 mV before and after application of CPT-cAMP. (*C*) Effects of PKA stimulation on activation of I_{sK}, determined from tail current measurements at −60 mV, following 30-s depolarizations to test potentials shown, before (●) and after (○) application of CPT-cAMP. Continuous curves were drawn according to a Boltzmann function (as in Fig. 2*B*). Control: $I_{max} = 1813$ nA, $V_{1/2} = -7.2$ mV, $k = 11.7$ mV. Treated: $I_{max} = 2181$ nA, $V_{1/2} = -18.5$ mV, $k = 11.0$ mV.

Table 1. Regulation of gpmin K by PKA and PKC

Group	I_{tail}, % control	*n*	Boltzmann parameter			
			I_{max}, % control	$\Delta V_{1/2}$, mV	*k*, mV	*n*
Wild type						
Control	100		100	—	11.2 ± 0.1	16
CPT-cAMP	132 ± 3*	6	110 ± 2*	−4.1 ± 1.9*	10.3 ± 0.3*	6
ISO	147 ± 7*	7	113 ± 6*	−6.1 ± 1.9*	10.5 ± 0.3*	5
PDD	133 ± 4*	11	118 ± 5*	−0.7 ± 1.1	11.2 ± 0.2	5
N102S						
Control	100		100	—	10.2 ± 0.3**	7
PDD	72 ± 6*	8	86 ± 4*	9.9 ± 0.9*	12.8 ± 0.3*	5

Values represent mean ± SEM. I_{tail}, tail current at −60 mV following 10-s depolarization to 20 mV; *n*, number of oocytes; ISO, isproterenol. Boltzmann parameters were determined from tail currents following a 30-s test pulse. *, $P < 0.05$ for respective paired control values determined by a *t* test; **, $P < 0.05$ for N102S control compared to wild-type control determined by an unpaired *t* test.

Stimulation of PKC increases I_K in guinea pig myocytes (14, 28). Application of PDD to oocytes expressing gpmin K induced an increase of the current (Fig. 5*A* and *B Inset*). After phorbol application, the tail current was increased by 33.2% ± 3.7% ($n = 11$). The inactive enantiomer α-PDD had no effect (Fig. 6). The increase of I_{sK} by PDD was blocked in oocytes treated with the PKC inhibitor cherlerythrine (ref. 32; Figs. 5*A* and 6); inhibitor alone had no effect. Similar effects were seen with the less selective inhibitor staurosporine (data not shown). The effects of PKC on the voltage dependence of gpmin K (Fig. 5*B*) differ from those seen following PKA stimulation. Although PKC stimulation increased I_{max}, the $V_{1/2}$ and the slope factor, *k*, were unchanged (Table 1).

The increase in gpI_{sK} following stimulation of PKC is similar to the effects of PKC stimulation in guinea pig myocytes but contrasts with results in mouse myocytes (17) and in oocytes expressing cloned mouse or rat I_{sK} (17, 33), where the currents are decreased after PKC stimulation. In the latter case, we have previously demonstrated that substitution of the serine residue at position 103 by alanine (S103A) eliminated the current decrease by PKC stimulation (34). The analogous residue in gpmin K is an asparagine (N102; see Fig. 1*A*). To determine whether this difference underlies the species-specific response to PKC, N102 was altered by site-directed mutagenesis to serine (N102S). Expression of gpminK (N102S) in oocytes resulted in voltage-dependent potassium channels indistinguishable from wild type, except in the response to PKC stimulation. Application of phorbol ester induced a significant decrease in the current amplitude (at 40 min, −28.0% ± 6.3%; $n = 8$) (Fig. 5 *A* and *C Inset*), comparable to that seen with rat I_{sK} expressed in *Xenopus* oocytes (34). The voltage dependence of activation of gpmin K(N102S) (Fig. 5*C*) was positively shifted and its voltage sensitivity reduced by PDD (Table 1).

DISCUSSION

Several pieces of evidence support the hypothesis that the min K potassium channel underlies the slow component of the delayed rectifier potassium conductance in cardiac myocytes. (*i*) I_{Ks} and I_{sK} demonstrate similar kinetic characteristics, distinct from other potassium currents. Both show a prolonged lag following membrane depolarization and slow activation with time constants on the order of seconds. Neither inactivates, and both are increased in amplitude with trains of pulses (9, 14, 18, 33). (*ii*) I_{Ks} and I_{sK} share pharmacological profiles, being blocked with similar potency by clofilium but not by the sotalol derivative E4031. Low concentrations of La^{3+}, which block I_{Kr}, had no effect. In addition, I_{Ks} and I_{sK} are blocked with equal potency by NE10064 (12). (*iii*) I_{Ks} and I_{sK} show similar responses to changes in $[Ca^{2+}]_i$ (15, 16, 33) and stimulation of PKA and PKC (13, 14). Indeed, the species-specific responses to PKC are now understood at the structural level, being due to single amino acid differences between min K proteins (34). (*iv*) The mRNA encoding the min K protein is expressed in heart.

Although application of PKA catalytic subunit to excised membrane patches increases myocyte I_K (13), reports of the effects of PKA stimulation on cloned min K channels expressed in oocytes have differed. Honoré *et al.* (17) reported that 1 mM 8-bromoadenosine 3′,5′-cyclic monophosphate had no effect on mouse I_{sK} in oocytes. In contrast, although Blumenthal and Kaczmarek (35) reported that elevated levels

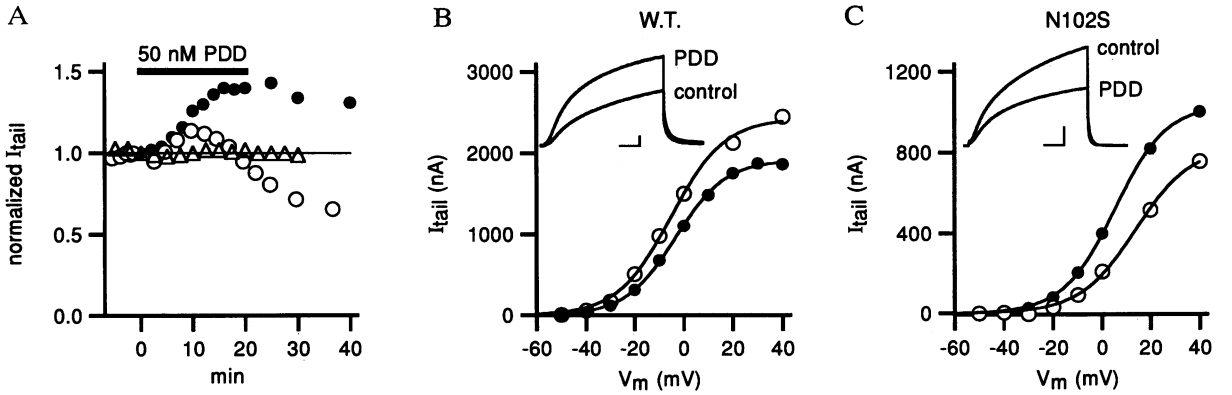

FIG. 5. PKC regulation of gpmin K. (*A*) Time course of PDD effect (50 nM, 20 min) on tail currents (see legend to Fig. 4*A*) for oocytes expressing wild-type gpmin K (●), wild-type gpmin K with prior and concomitant application of 5 μM cherlerythrine (△), and gpmin K N102S (○). (*B*) Voltage dependence of activation of wild-type I_{sK}, before (●) and after (○) 20-min application of 50 nM PDD. Continuous curves were drawn according to a Boltzmann function (as in Fig. 2*B*). Control: $I_{max} = 1917$ nA, $V_{1/2} = -3.3$ mV, $k = 10.1$ mV. Treated: $I_{max} = 2429$ nA, $V_{1/2} = -5.1$ mV, $k = 10.9$ mV. (*Inset*) Current traces elicited by 30-s steps to +20 mV (scale bars: 5 s, 500 nA). (*C*) gpmin K N102S. Control: $I_{max} = 1036$ nA, $V_{1/2} = 5.3$ mV, $k = 10.6$ mV. Treated: $I_{max} = 900$ nA, $V_{1/2} = 16.5$ mV, $k = 12.6$ mV.

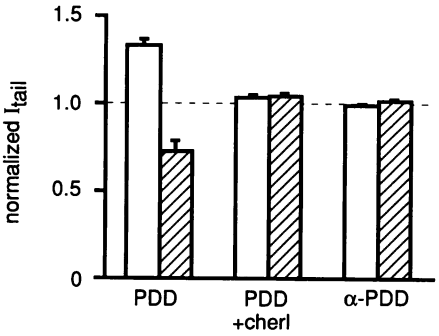

FIG. 6. Effect of PKC stimulation on wild-type (open bars) and mutant N102S (shaded bars) gpmin K. Conditions were as follows: 50 nM PDD, 20 min (n = 11, 8); 50 nM PDD in the presence of 5 μM cherlerythrine (n = 3, 3); 100 nM α-PDD, 20 min (n = 4, 4). Values represent maximum changes in tail currents observed during and 20 min after PDD application. Tail currents were measured at −60 mV after 10-s depolarizations to +20 mV. Cherlerythrine (cherl) prevented the PDD-mediated increase of wild-type gpmin K currents (P < 0.005) and the decrease of N102S gpmin K currents (P < 0.02).

of cAMP increased rat I_{sK} in oocytes, the increased current amplitudes correlated with increased membrane capacitance, suggesting that regulation involves selective insertion and deletion of channels from the plasma membrane. In addition, these authors reported that increased I_{sK} was not due to changes in voltage dependence or kinetics. We found no change in membrane capacitance as a result of either CPT-cAMP or isoproterenol application. Furthermore, these agents increased current amplitude, shifted the $V_{1/2}$ to more negative potentials, and steepened the response to voltage, consistent with the effects of PKA stimulation on I_K and I_{Ks} in guinea pig ventricular myocytes (28, 36, 37).

Stimulation of PKC also increased gpI_{sK}; however, voltage-dependent parameters were not changed. This is consistent with results obtained for PKC stimulation of guinea pig myocyte I_{Ks} (14, 28) and indicate that PKA and PKC affect min K channels through different mechanisms. Site-directed mutagenesis has identified residues responsible for the species-specific effects of PKC stimulation on I_{sK}. It is possible that the min K channel activates in response to voltage by subunit aggregation (ref. 38; M.D.V., J.M., and J.P.A., unpublished data). PKC-mediated phosphorylation of S103 in rat min K might present an electrostatic hinderance to subunit interactions, effectively limiting the number of available channels. However, in the absence of a serine residue at the analogous position, PKC stimulation increases guinea pig I_{sK}. In this case, PKC may affect an intermediary protein, which, in turn, acts to modulate the channel. We have previously shown that rat I_{sK} is increased by elevation of $[Ca^{2+}]_i$ and decreased by cytochalasin D, presumably through inhibiting changes in the cytoskeletal actin network (33, 39). Either of these processes may be affected by PKC-mediated phosphorylation.

Min K subunits have a molecular architecture so far unique among potassium channels. It is interesting that a protein with an architecture similar to min K, also expressed in cardiac cells, has been shown to function as a chloride channel (40). This 72-amino acid protein, phospholemman, was originally characterized for its propensity to serve as a substrate for phosphorylation (41). Indeed, currents through phospholemman channels share the distinct slow activation and sigmoidal delay properties with I_{Ks} and I_{sK}; they are also increased by trains of pulses (40). Thus, it appears that cardiac cells express at least two members of a structurally and functionally distinct class of voltage-dependent ion channels that operate on a relatively slow time scale and are modulated by a variety of intracellular second messengers.

We thank Yan-na Wu for patience and expert oocyte preparation and injections. We also acknowledge the expert technical support of Mark Doyle. This work was supported by Grants HL48286 (J.M.) and NS28504 (J.P.A.) from the National Institutes of Health. M.D.V. was supported in part by a Tartar Fellowship Award.

1. Carmeliet, E. (1977) *J. Physiol. (Paris)* **73**, 903–923.
2. Hume, J. R. & Uehara, A. (1985) *J. Physiol. (London)* **368**, 525–544.
3. Matsuura, H., Ehara, T. & Imoto, Y. (1987) *Pflügers Arch.* **410**, 596–603.
4. Doer, T., Denge, R. & Trautwein, W. (1990) *Pflügers Arch.* **416**, 230–237.
5. Wettwer, E., Scholtysik, G., Schaad, A., Himmel, H. & Ravens, U. (1991) *J. Cardiovasc. Pharmacol.* **17**, 480–487.
6. Bennett, P. B., McKinney, L. C., Kass, R. S. & Begenisich, T. (1985) *Biophys. J.* **48**, 553–567.
7. Colatsky, T. J., Folmer, C. H. & Starmer, C. F. (1990) *Circulation* **82**, 2235–2242.
8. Noble, D. & Tsien, R. W. (1969) *J. Physiol. (London)* **200**, 205–231.
9. Sanguinetti, M. C. & Jerkiewicz, N. K. (1990) *J. Gen. Physiol.* **96**, 195–215.
10. Jurkiewicz, N. K. & Sanguinetti, M. C. (1993) *Circ. Res.* **72**, 75–83.
11. Tatla, D. S., David, B. C., Malloy, K. J. & Moorehead, T. J. (1993) *FASEB J.* **7**, A107 (abstr.).
12. Busch, A. E., Malloy, K. J., Varnum, M. D., Adelman, J. P., North, R. A. & Maylie, J. (1993) *Circulation*, in press.
13. Walsh, K. B., Arena, J. P., Kwok, W.-M., Freeman, L. & Kass, R. S. (1991) *Am. J. Physiol.* **260**, H1390–H1393.
14. Tohse, N., Kameyama, M. & Irisawa, H. (1987) *Am. J. Physiol.* **253**, H1321–H1324.
15. Tohse, N. (1990) *Am. J. Physiol.* **258**, H1200–H1207.
16. Busch, A. E. & Maylie, J. (1993) *Cell. Physiol. Biochem.* **3**, 245–252.
17. Honoré, E., Attali, B., Romey, G., Heurteaux, C., Ricard, P., Lesage, F., Lazdunski, M. & Barhanin, J. (1991) *EMBO J.* **10**, 2805–2811.
18. Takumi, T., Ohkubo, H. & Nakanishi, S. (1988) *Science* **242**, 1042–1045.
19. Folander, K., Smith, J. S., Antanavage, J., Bennett, C., Stein, R. B. & Swanson, R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2975–2979.
20. Krafte, D. S., Dugrenier, N., Dillon, K. & Volberg, W. A. (1992) *Biophys. J.* **61**, A387.
21. Bond, C. T., Hayflick, J. S., Seeburg, P. H. & Adelman, J. P. (1989) *Mol. Endocrinol.* **3**, 1257–1261.
22. Christie, M. J., Adelman, J. P., Douglass, J. & North, R. A. (1989) *Science* **244**, 221–224.
23. Boyle, M. B., Azhderian, E. M., MacLusky, N. J., Naftolin, F. & Kaczmarek, L. K. (1987) *Science* **235**, 1221–1224.
24. Hausdorff, S. F., Goldstein, S. A. N., Rushin, E. E. & Miller, C. (1991) *Biochemistry* **30**, 3341–3346.
25. Sanguinetti, M. C. & Jurkiewicz, N. K. (1990) *Am. J. Physiol.* **259**, H1881–H1889.
26. Arena, J. P. & Kass, R. S. (1988) *Mol. Pharmacol.* **34**, 60–68.
27. Yazawa, K. & Kameyama, M. (1990) *J. Physiol. (London)* **421**, 135–150.
28. Walsh, K. B. & Kass, R. S. (1988) *Science* **242**, 67–69.
29. Walsh, K. B., Begenisich, T. B. & Kass, R. S. (1989) *J. Gen. Physiol.* **93**, 841–854.
30. Kusano, K., Miledi, R. & Stinnakre, J. (1982) *J. Physiol. (London)* **328**, 143–170.
31. Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K. & Hidaka, H. (1990) *J. Biol. Chem.* **265**, 5267–5272.
32. Herbert, J. M., Augereau, J. M., Gleye, J. & Maffrand, J. P. (1990) *Biochem. Biophys. Res. Commun.* **172**, 993–999.
33. Busch, A. E., Kavanaugh, M. P., Varnum, M. D., Adelman, J. P. & North, R. A. (1992) *J. Physiol. (London)* **450**, 491–502.
34. Busch, A. E., Varnum, M. D., North, R. A. & Adelman, J. P. (1992) *Science* **255**, 1705–1707.
35. Blumenthal, E. M. & Kaczmarek, L. K. (1992) *J. Neurosci.* **12**, 290–296.
36. Sanguinetti, M. C., Jurkiewicz, N. K., Scott, A. & Siegl, P. K. S. (1991) *Circ. Res.* **68**, 77–84.
37. Hartzell, H. C. (1988) *Prog. Biophys. Mol. Biol.* **52**, 165–247.
38. Varnum, M. D., Busch, A. E., Maylie, J. & Adelman, J. P. (1993) *Biophys. J.* **64**, A198 (abstr.).
39. Cooper, J. A. (1987) *J. Cell Biol.* **105**, 1473–1478.
40. Moorman, J. R., Palmer, C. J., John, J. E., Durieux, M. E. & Jones, L. R. (1992) *J. Biol. Chem.* **267**, 14551–14554.
41. Palmer, C. J., Scott, B. T. & Jones, L. R. (1991) *J. Biol. Chem.* **266**, 11126–11130.