Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1766–1770, March 1994
Physiology

K^+ currents expressed from the guinea pig cardiac I_{sK} protein are enhanced by activators of protein kinase C

(potassium channel/*min*K channel/delayed rectifier)

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Communicated by Leslie L. Iversen, October 28, 1993 (received for review July 26, 1993)

ABSTRACT **We have isolated cardiac cDNA and genomic clones encoding the guinea pig I_{sK} protein. The deduced amino acid sequence is ≈78% identical to the rat, mouse, and human variants of this channel, and the structure of the gene encoding the protein is also similar to that in other species. For example, the gene is present only once in the haploid genome, the protein-coding sequence is present on a single uninterrupted exon, an intron exists in the 5′ untranslated domain, and multiple alternative polyadenylylation sites are used in processing the transcript. Expression of the guinea pig protein in *Xenopus* oocytes results in a slowly activating, voltage-dependent K^+ current, I_{sK}, similar to those expressed previously from the rat, mouse, and human genes. However, in sharp contrast to the rat and mouse currents, activation of protein kinase C with phorbol esters increases the amplitude of the guinea pig I_{sK} current, analogous to its effects on the endogenous I_{Ks} current in guinea pig cardiac myocytes. Mutagenesis of the guinea pig cDNA to alter four cytoplasmic amino acid residues alters the phenotype of the current response to protein kinase C from enhancement to inhibition, mimicking that of rat and mouse I_{sK} currents. This mutation is consistent with reports that phosphorylation of Ser-102 by protein kinase C decreases the current amplitude. These data explain previously reported differences in the regulatory properties between recombinant rat or mouse I_{sK} channels and native guinea pig I_{Ks} channels and provide further evidence that the I_{sK} protein forms the channels that underlie the I_{Ks} current in the heart.**

In cardiac myocytes, the resting membrane potential and rate of repolarization during an action potential are largely controlled by K^+ currents. Although a large number of cardiac K^+ currents have been well characterized by voltage clamp techniques (1), the molecular nature of the channels underlying these currents has proven more difficult to determine. In recent years, cDNAs encoding many K^+-channel subunits have been cloned from cardiac tissues, and most are related to the channels originally isolated from the Shaker locus of *Drosophila* (2). When expressed heterologously, these proteins assemble as either homo- or heterotetramers to form functional K^+ channels (3–8). The currents resulting from the expression of these cloned proteins have been compared directly to endogenous cardiac currents in attempts to determine the physiological functions of these proteins in the heart. Such correlations, however, have been difficult to make due, perhaps, to the formation of heterotetramers *in vivo* from a number of distinct gene products (9–11).

The I_{sK} protein represents an exception to this general difficulty in correlating cloned channels with endogenous currents. This protein, which is found in the heart (12–16), is structurally unrelated to other known K^+ channels. Its expression in *Xenopus* oocytes (17) or a human embryonic kidney cell line (HEK293; ref. 15) does, however, result in the appearance of a slowly activating K^+ current that closely resembles the slow component (I_{Ks}) of the delayed rectifier recorded from guinea pig (18) and canine (19) ventricular myocytes and human atrium (20). Although the I_{sK} and I_{Ks} currents are very similar biophysically and pharmacologically, one major discrepancy is that protein kinase C (PKC) has been reported to have different effects on the two currents. The I_{sK} current, recorded after expression of the rat or mouse protein in oocytes, is inhibited by activators of PKC (14, 21), whereas the endogenous I_{Ks} current in guinea pig cardiac myocytes is enhanced (in a temperature-dependent manner; ref. 22). The significance of these differences, however, has been obscured by the possible effects of species variability. The effects of PKC on the I_{sK} current have been recorded from oocytes expressing either the rat or mouse protein, whereas the effects on I_{Ks} have been carefully studied only in myocytes isolated from other species—most often the guinea pig. We sought to determine if the discrepancy in the responses of the currents to PKC is due simply to sequence variability between the species variants of the I_{sK} protein or whether it might reflect true differences in the structural composition of the expressed I_{sK} and endogenous I_{Ks} channels.

In this report, we present the cloning and expression of the guinea pig cardiac I_{sK} protein and demonstrate that the reported differences in the effects of PKC can be explained by natural sequence variations between the rat and guinea pig proteins. In addition, mutagenesis of the guinea pig I_{sK} protein at four cytoplasmic amino acid residues transforms the effect of PKC on the current from enhancement to inhibition. These results provide further evidence that the I_{sK} protein does, in fact, form the channels that underlie the cardiac I_{Ks} current and, therefore, functions in repolarization of the cardiac action potential.

METHODS

Materials and General Methods. Phorbol 12,13-didecanoate (PDD) was from Calbiochem and guinea pig genomic DNA was from Clontech. Poly(A)$^+$ mRNA was isolated from guinea pig cardiac atria or ventricles by standard techniques (23, 24). Northern blot analysis (25) and standard molecular biological procedures were carried out as described (26, 27). The cloning of the rat I_{sK} protein has been described (12).

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Abbreviations: PDD, phorbol 12,13-didecanoate, PKC, protein kinase C; *I*, current.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L20462).

Cloning of cDNAs Encoding the Guinea Pig Cardiac I_{sK} Protein. cDNAs encoding the guinea pig cardiac I_{sK} protein were cloned using PCR. Degenerate oligonucleotide primers, encoding peptide sequences that are conserved between the mouse, rat, and human I_{sK} proteins [DAWQEK (oligonucleotide 1), KLEALY (oligonucleotide 2), and GFFTLG (oligonucleotide 3)] were synthesized. Oligonucleotide 1 was used to prime first-strand cDNA synthesis from guinea pig cardiac poly(A)$^+$ mRNA as described (28). The region of the I_{sK} mRNA flanked by primers 1 and 2 was then amplified by PCR using the first-strand cDNA as the template, oligonucleotide 1 as the 3′ primer, and oligonucleotide 2 as the 5′ primer. [α-^{32}P]dCTP was added to the PCR reaction (100 μCi/ml; 1 Ci = 37 GBq) to label the products. PCR was performed for 25 cycles of 1 min at 94°C, 2 min at 37°C, and 3 min at 72°C. The reaction product was fractionated on a DNA sequencing gel, the region of the gel around the expected size of the product [$\approx$170 $\pm$ 10 bp (12, 14, 29)] was excised, and the DNA was eluted into 0.5 ml of water. This product was reamplified by PCR (50 cycles without radioactive dCTP) using oligonucleotides 1 and 3 as the primers. *Hin*dIII and *Eco*RI sites were incorporated into the 5′ ends of upstream and downstream primers, respectively, to aid in cloning. The reamplified fragment was digested with *Eco*RI and *Hin*dIII, isolated by preparative gel electrophoresis, and subcloned into pKSII+. The nucleotide sequence of the cDNA was determined using the dideoxynucleotide chain termination method.

The regions of the cDNA flanking the fragment described above were cloned using the 5′ and 3′ rapid amplification of cDNA ends (RACE) variations of the PCR (30). To obtain a full-length cDNA, and to verify the nucleotide sequence, oligonucleotides derived from the cDNAs encoding the 5′ and 3′ untranslated domains of the I_{sK} mRNA were synthesized and used to amplify a fragment encoding the entire open reading frame from guinea pig cardiac poly(A)$^+$ mRNA. Three independently amplified cDNA clones from each of three independently synthesized first-strand cDNA pools were isolated, and the nucleotide sequences of both strands were determined for each.

Isolation of Genomic DNA Clones Encoding the 5′ Flanking Region of the I_{sK} Gene. To amplify the 5′ flanking sequence from genomic DNA, inverted PCR was performed. Guinea pig genomic DNA was digested with *Hin*P1 I for 2 hr and religated at a low concentration (2 μg/ml) to promote intramolecular ligation. This circularized DNA was used as the template for the amplification of the flanking sequences by inverted PCR as described (31). Nested oligonucleotides derived from the 5′ and 3′ coding sequences of the I_{sK} gene were used as primers for two sequential amplifications. The final PCR product was isolated, cloned, and sequenced by standard techniques.

Mutagenesis. The N102S mutant was constructed by restriction fragment replacement. The I_{sK} cDNA was digested with *Sma* I and *Pst* I and replaced with a synthetic oligonucleotide duplex containing a point mutation to convert the codon encoding Asn-102 to Ser. The region of the cDNA replaced by the synthetic duplex was sequenced on both strands.

A quadruple mutant (N102S;C103F;S105A;C107Y) was constructed by PCR. An upstream primer that contained the four mutations was used to amplify the region flanked by the *Sma* I and *Eco*RI sites from the cDNA. This mutagenized PCR product was then digested with *Sma* I and *Eco*RI and ligated into the guinea pig I_{sK} cDNA. The entire region of the cDNA replaced by this PCR product was sequenced on both strands to confirm the DNA sequence.

To increase translational efficiency, $\approx$50 bp of the 5′ untranslated region was deleted. The cDNA was digested with *Sac* I and *Hin*dIII, blunt-ended with the Klenow fragment of DNA polymerase, and recircularized with T4 DNA ligase.

Expression and Assay of the I_{sK} Protein. cDNAs encoding the open reading frame of the I_{sK} protein were cloned into pGEM-A (32), and complementary RNA (cRNA) was synthesized *in vitro* by transcription with T7 RNA polymerase as described (28). Oocytes were isolated from *Xenopus laevis* and injected with 5 ng of I_{sK} cRNA. Cells were cultured at 18°C in ND96 (12) supplemented with gentamicin at 100 μg/ml. Currents were measured 1–5 days after injection using standard two microelectrode voltage clamp techniques as described (12, 17, 32). Oocytes were clamped to a holding potential of −80 mV, and the I_{sK} current was activated with step depolarizations to 0 mV at 90-sec intervals. Current amplitude was measured as peak time-dependent current during 20-sec test pulses.

RESULTS

Cloning and Gene Structure. Using PCR techniques, cDNAs encoding the guinea pig cardiac I_{sK} protein were isolated. The longest cDNA was $\approx$750 bp in length; it contained a 375-bp protein-coding region (Fig. 1). Restriction enzyme digests of guinea pig genomic DNA, probed with this entire cDNA, resulted in a pattern of hybridizing fragments expected from a single-copy gene. For example, digests with *Eco*RI, *Bgl* II, or *Xba* I (enzymes that do not cut within the cDNA probe) each yielded a single hybridizing fragment, whereas two fragments that hybridized to the probe were detected in digests with *Pst* I, which does cut once within the cDNA (data not shown). Thus, the gene encoding the protein was present once in the haploid guinea pig genome.

The coding sequence was present in genomic DNA on a single uninterrupted exon (data not shown), but an intron was found within the 5′ noncoding region of the gene. The coding and 5′ untranslated regions were cloned from genomic DNA, and their sequences were compared to that of the cDNA. The nucleotide sequences of the coding region and the 46 bp immediately preceding it in the 5′ untranslated domain were

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CGGAGCCAGACCCCGCTTGCCCGGGACGTTCACTCTCCCACCGCGGAAGCTTGCAGCCCA   -3
GGATGATCTTGCCTAATTCCACAGCTGTGATGCCCTTCCTGACCACCGTGTGGCAGGGGA   58
  M  I  L  P  N  S  T  A  V  M  P  F  L  T  T  V  W  Q  G  T   20

CAGTTCAACCCAGTAGCAATGCATCTGGCCTGGCCCGGAGGTCCCCTCTCCGCGATGACG  118
  V  Q  P  S  S  N  A  S  G  L  A  R  R  S  P  L  R  D  D  G   40

GCAAGCTGGAGGCACTCTACATCCTCATGGTGCTGGGCTTCTTTGGTTTCTTCACTCTGG  178
  K  L  E  A  L  Y  I  L  M  V  L  G  F  F  G  F  F  T  L  G   60

GCATCATGCTGAGCTATATTCGATCCAAGAAACTGGAGCACTCGCACGACCCGTTCAACG  238
  I  M  L  S  Y  I  R  S  K  K  L  E  H  S  H  D  P  F  N  V   80

TGTACATCGAGTCAGACACCTGGCAGGAGAAGGACAAAGCATTCTTCCAGGCCCGGGTTC  298
  Y  I  E  S  D  T  W  Q  E  K  D  K  A  F  F  Q  A  R  V  L   100

TGGAGAACTGCAGATCGTGTTGCGTCATTGAAAACCAGCTGACCGTAGAGCAGCCCAACA  358
  E  N  C  R  S  C  C  V  I  E  N  Q  L  T  V  E  Q  P  N  T   120

CATACCTGCCCGAGCTTTAGTCTTCCTTCTGAGTGCCACGGCTGGTGAAGGGTGGCCAAG  418
  Y  L  P  E  L                                                125

                                  ▼                       ▼
CTGCACGTGATAATGTGGTATTTCCAATCACATGCTTCTCAGATTCATTATTGTATCCAT  478

GCCTCTGGGTTTCTTTTGTCACTTTTGGGGCTTTGTGGAAGAGGTTTAATGATACTATTT  538
    ▼ ▼
CCTAAAATCACATTCCCTCTACAGCAGACTGTTGATTATTCCCAAATATCTGTCCTTGCC  598
                                                          ▼
TTGTTATATAGAGCAGAATTCCTGACCATGGCCTCCAAATATATGGGCTGCAAGCTGGAA  658

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA                                688
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FIG. 1. cDNA and deduced protein sequences of the guinea pig I_{sK} protein. The nucleotide sequence of the longest cDNA isolated is shown (accession no. L20462). Five alternative polyadenylylation sites were identified in the 11 cDNA clones sequenced, and their positions are indicated by the arrowheads (▼). The five sites (from 5′ to 3′) were represented 2, 3, 1, 2, and 3 times, respectively, in the 11 clones. Transition mutations (C → T) at positions −13 and −41 in the 5′ untranslated domain of the mRNA were found in two of the other cDNAs.

identical in the cDNA and genomic DNA clones (Fig. 2). The sequences, however, diverged completely upstream (5′) from this point. Furthermore, the 10 bp immediately upstream of the point of divergence conformed well to the consensus sequence for an intron/exon splice acceptor site (5′-Y_6NCAG-3′; ref. 33). These data demonstrated the presence of an intron within the I_{sK} gene, located 47 bp upstream from the translation initiation codon.

Heterogeneity was observed in the 3′ untranslated domain of the cDNAs, resulting from polyadenylylation of the mRNA at five distinct sites (Fig. 1). This heterogeneity in the lengths of the RNAs was also reflected in Northern blots. The full-length cDNA probe hybridized to a diffuse class of ventricular poly(A)$^+$ mRNAs, ranging from ≈0.6 to ≈1.2 kb in length. Similar results were obtained with mRNA isolated from guinea pig atria (data not shown).

Deduced Amino Acid Sequence of the Guinea Pig I_{sK} Protein. The open reading frame of the cDNAs predicted a protein of 125 amino acids with a molecular mass of 14.3 kDa. The deduced sequence was ≈78% identical to the rat, mouse, and human variants of the I_{sK} protein and, like these other proteins, contained a single hydrophobic (putative transmembrane) domain. The protein contained two consensus N-glycosylation sites (Asn-5 and Asn-26) and potential sites for phosphorylation by PKC at Ser-68 and Ser-105 and by casein kinase II at Thr-86. There were three Cys residues within the cytoplasmic, carboxyl-terminal domain (at positions 103, 106, and 107). The Ser residue at position 103 in the rat protein, which is known to be phosphorylated by PKC, has diverged to an Asn residue in the guinea pig protein (Asn-102; Fig. 1).

Functional Expression and Regulation by PKC. Injection of cRNA encoding the guinea pig I_{sK} protein into *Xenopus* oocytes resulted in voltage-gated outward K$^+$ currents that displayed delayed rectification and continued to activate slowly with a sigmoidal time course throughout long (20 sec) depolarizations (Fig. 3*A*). Upon repolarization, the current deactivated slowly, requiring >6 sec for full deactivation. These kinetic properties were similar to those of I_{sK} currents previously expressed from other species variants (e.g., rat, Fig. 3*D*) (17).

In control experiments, the amplitude of the guinea pig I_{sK} current increased an average of 25.3% during repeated 20-sec depolarizing steps from −80 to 0 mV over 40 min (Table 1). Similar time-dependent run-up of the current has been reported for other species variants (17). Continuous exposure to 50 nM PDD, to activate PKC, significantly (P = 0.0003) increased the guinea pig I_{sK} currents by an average of 48.6%

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Genomic:   GTGTGTGTGTGTAATTTAGCCAATGTGCACTTCTGATTTCAGGC  44

cDNA:      .........................................

           ┌──────────┐
TGCATCTTTCTTTTCGGCAGCTTGCCCGGGACGTTCACTCTCCCACCGCGGAAGC  99
     |     | ||||||||||||||||||||||||||||||||||||||||||
.....CGGAGCCAGACCCCGCTTGCCCGGGACGTTCACTCTCCCACCGCGGAAGC -13

TTGCAGCCCAGGATGATCTTGCCTAATTCCACAGCTGTGATGCCCTTC····    147
|||||||||||||||||||||||||||||||||||||||||||||||||
TTGCAGCCCAGGATGATCTTGCCTAATTCCACAGCTGTGATGCCCTTC····     43
          MetIleLeuProAsnSerThrAlaValMetProPhe····     14
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FIG. 2. The guinea pig I_{sK} gene contains an intron at position −47 in the 5′ untranslated domain. The 5′ untranslated and part of the coding sequences of the I_{sK} cDNA and genomic DNA clones have been aligned. Identical residues at any position are indicated by a vertical line between them. The cDNA and genomic DNA sequences are identical from position −46 in the 5′ untranslated domain through the coding sequence. Upstream from position −46, however, the two sequences diverge completely, demonstrating the presence of an intron in the gene at position −47. The overline indicates the sequence of a putative intron/exon splice acceptor site.

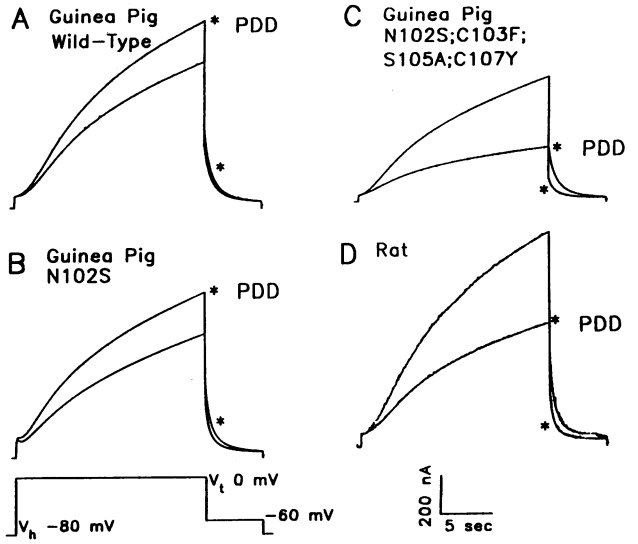

FIG. 3. Effects of 50 nM PDD on I_{sK} currents elicited during depolarizing voltage clamp steps from −80 to 0 mV. Currents were measured in *Xenopus* oocytes after the injection of cRNAs encoding the wild-type guinea pig I_{sK} protein (*A*), a single point mutant (N102S) of the guinea pig protein (*B*), a quadruple mutant (N102S;C103F;S105A;C107Y) of the guinea pig protein (*C*), or the rat I_{sK} protein (*D*). Average currents before and ≈20 min after the application of PDD (*) are superimposed in each panel.

over the same time period (Table 1 and Fig. 3*A*). This PDD-induced increase of the I_{sK} current had a slow onset, with a delay of several minutes, and continued to increase throughout the entire time course (Fig. 4). As reported previously, exposure to 50 nM PDD significantly ($P < 0.0001$) inhibited the rat I_{sK} current (Table 1 and Fig. 3*D* and 4).

The guinea pig cDNA was mutated in attempts to regenerate the PKC phosphorylation site (Ser-103) that is responsible for the diminution of the rat I_{sK} current induced by phorbol esters (34). We made a single amino acid replacement of Asn-102 in the guinea pig I_{sK} protein with a Ser residue (N102S). The N102S current was still significantly ($P < 0.0001$) enhanced after application of 50 nM PDD (Table 1 and Figs. 3*B* and 4). The amino acid sequence of the guinea pig I_{sK} protein, however, differs from the rat sequence in this region not only at N102 but also at three other adjacent residues (C103, S105, and C107). Mutagenesis at all four of these positions to replace each with the corresponding amino acid of the rat protein (N102S; C103F; S105A; C107Y) remarkably altered the response of the guinea pig I_{sK} current to PDD. The currents from this quadruple guinea pig mutant were significantly (P = 0.0041) inhibited by 50 nM PDD (Table 1 and Figs. 3*C* and 4). The extent of the inhibition and its time course closely paralleled that of the wild-type rat I_{sK} current (Fig. 4).

DISCUSSION

To our knowledge, this is the first report describing the cloning and expression of cDNAs encoding the guinea pig cardiac I_{sK} protein. The general structure of the guinea pig protein is comparable to other species variants, and its expression in *Xenopus* oocytes results in voltage-gated K$^+$ currents that are similar to those expressed from the proteins of the other species. However, sequence differences do exist within the carboxyl-terminal, cytoplasmic domains of the different variants, and they result in differential regulation of the expressed currents by PKC. In this study, we show that the guinea pig I_{sK} current is enhanced by activation of PKC with phorbol esters, whereas the rat and mouse currents are

Table 1. Time dependence of the peak I_{sK} currents expressed in *Xenopus* oocytes during repetitive voltage clamp pulses to 0 mV in the absence or presence of PDD

	− PDD				+ 50 nM PDD				
I_{sK} cRNA	*n*	$I_{t=0}$, μA	$I_{t=30}$, μA	% change	*n*	$I_{t=0}$, μA	$I_{t=30}$, μA	% change	*P*
Guinea pig									
Wild type	11	0.694 ± 0.063	0.859 ± 0.065	+25.3 ± 3.9	10	0.554 ± 0.054	0.822 ± 0.083	+48.6 ± 3.3	0.0003
N102S	10	0.734 ± 0.046	0.913 ± 0.059	+24.5 ± 2.2	7	0.408 ± 0.050	0.657 ± 0.068	+64.1 ± 5.3	<0.0001
N102S;C103F; S105A;C107Y	5	0.380 ± 0.027	0.446 ± 0.028	+17.8 ± 5.0	5	0.540 ± 0.045	0.363 ± 0.075	−33.1 ± 11.8	0.0041
Rat	7	1.166 ± 0.125	1.427 ± 0.125	+23.8 ± 2.6	5	1.041 ± 0.108	0.628 ± 0.118	−40.2 ± 8.8	<0.0001

The data demonstrate both the normal run-up of the I_{sK} currents observed during repetitive depolarizations (−PDD) as well as the effects of 50 nM PDD on the currents. Data are mean ± SEM. Cells were depolarized from −80 mV to 0 mV for 20 sec every 1.5 min. The mean baseline currents ($I_{t=0}$) were averaged during a 10-min period prior to normal application of 50 nM PDD. Currents were also averaged from 22 to 30 min after normal exposure to 50 nM PDD ($I_{t=30}$). The same time course was followed for both the time controls (−PDD) and PDD-treated oocytes. The level of significance (*P*) was determined using an unpaired *t* test, comparing the mean time-dependent percent changes in the magnitude of I_{sK} during the pulse in the absence and presence of PDD.

diminished (14, 21). These contrasting effects of PKC on the guinea pig and mouse I_{sK} currents are consistent with its effects on the endogenous I_{Ks} currents in cardiac myocytes isolated from these two species (14, 22). Mutagenesis of the guinea pig protein at selected cytoplasmic residues transforms the phenotype of the PKC response from enhancement to inhibition.

The structure of the gene encoding the I_{sK} protein has been well conserved among different species (29, 35, 36). In addition to the similarities in the organization of the gene, the structure of the I_{sK} protein itself is also highly conserved. For example, a comparison of the deduced amino acid sequences of the four species variants demonstrates a high degree of sequence identity, especially within the hydrophobic (putative pore-forming) domain. The sequence of the guinea pig protein, does, however, differ from the other species variants in three major ways. First, it is shorter than the rodent or human isotypes, resulting from a deletion of the four carboxyl-terminal amino acids present in the other proteins. Second, it contains three cytoplasmic Cys residues in contrast to the single conserved Cys found in the other three species. Finally, it lacks the Ser residue found in other variants (Ser-103 in the rat protein) that, when phosphorylated by PKC, results in a decrease in the current amplitude (34).

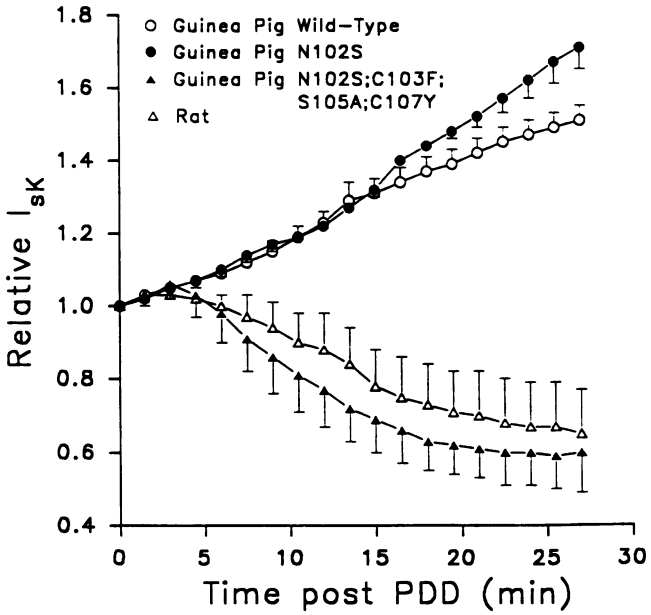

FIG. 4. Time course of the effects of PDD on the amplitudes of I_{sK} currents elicited by depolarizing voltage clamp steps from −80 to 0 mV. The relative amplitude of each of the four I_{sK} currents (defined as the amplitude of the current elicited by a voltage clamp pulse at any given time divided by the amplitude of the current at time $t = 0$) is plotted as a function of time. Fifty nanomolar PDD was applied at time $t = 0$ and was present thereafter.

The I_{sK} protein is expressed in a variety of tissues and, in some cases, is thought to be localized to the apical membranes of epithelial cells (37). Although the physiological function of the protein in most tissues remains unknown, evidence has accumulated that, in the heart, it forms the channels that underlie the slow component of the delayed rectifier current, I_{Ks} (reviewed in ref. 38). Data supporting this conclusion stems from several lines of investigation. First, the I_{sK} gene and the protein are expressed in the heart (12–16). Second, heterologous expression of the I_{sK} protein in oocytes or HEK293 cells results in the appearance of a K^+ current that resembles the cardiac I_{Ks} current in many of its biophysical and pharmacological properties (15, 17). Finally, all of the I_{Ks}-like current that can be expressed from cardiac mRNA can be attributed to transcription of the I_{sK} gene (12). Thus, the two currents are similar in many ways. One major discrepancy has, however, persisted in equating the two currents. Activators of PKC, such as phorbol esters or diacylglycerols, enhance the amplitude of the endogenous cardiac I_{Ks} current [measured in guinea pig myocytes (22)] but have been reported to decrease the I_{sK} currents expressed in *Xenopus* oocytes from the rat (21) or mouse (14) proteins. We demonstrate here, however, that whereas the amplitude of the I_{sK} current elicited by the expression of the rat protein is indeed diminished by PDD, the guinea pig I_{sK} current (like the endogenous I_{Ks} current in guinea pig cardiac myocytes) is enhanced by phorbol esters. Thus, the apparent discrepancy of the effects of activators of PKC on the expressed I_{sK} and endogenous cardiac I_{Ks} currents can be explained simply as the result of amino acid sequence variability between the different species variants of the I_{sK} protein. A minor difference still exists in the temperature dependence of the PKC effects on the two currents. In the guinea pig cardiac myocyte, activation of PKC enhances the amplitude of the I_{Ks} current when measured at 32°C but has little effect at 22°C (22). In contrast, the effects of this kinase are apparent on the I_{sK} current expressed in the oocyte at room temperature (≈24°C). This difference may, however, simply be a reflection of the temperature at which these two different types of cells normally function (the myocyte at ≈37°C and the oocyte at ≈18°C). Additional support for equating the two currents comes from preliminary electrophysiological investigations of the K^+ currents in cultured ventricular myocytes isolated from newborn mouse heart. These cells exhibit an I_{Ks}-like delayed rectifier current that is inhibited by activation of PKC with phorbol 12-myristate-13-acetate in the same way that the expressed mouse I_{sK} current is inhibited (14).

The decrease in the amplitude of the rat I_{sK} current after activation of PKC has been demonstrated, by site-directed mutagenesis, to be due to the phosphorylation of Ser-103 (34). The data presented here on the guinea pig I_{sK} current are consistent with that result. A mutated form of the guinea pig channel, in which this phosphorylation site has been regenerated, expresses currents that are diminished by activation of this kinase. However, simple replacement of Asn-102 with a Ser residue is, in itself, insufficient to regenerate the PKC effects. At least one of the other three residues that differ between the rat and guinea pig channels in this region (Cys-103, Ser-105, Cys-107) must also be involved in its regulation by this kinase. The necessity for changing at least one of these other residues may be explained in two ways. In the context of the guinea pig sequence, Ser-102 may simply not be recognized as a substrate for PKC. Alternatively, phosphorylation at an additional site within the protein may also explain the results. Activation of PKC in oocytes expressing the wild-type guinea pig I_{sK} protein results in an increase of the current amplitude (Fig. 3), and this increase may be due to phosphorylation of another cytoplasmic serine residue (e.g., Ser-105). If the effects of this additional phosphorylation were dominant, the N102S mutation would display the same phenotype as the wild-type protein—i.e., an increase in current amplitude by activators of PKC. Additional mutagenesis to remove this other phosphorylation site would, therefore, be required before the inhibitory effects of the N102S mutation could be observed.

Activation of mammalian cardiac α_1-adrenergic receptors stimulates the hydrolysis of inositol phospholipids resulting in the formation of inositol 1,4,5-trisphosphate and 1,2-diacylglycerols (39). The latter activate PKC. Marked species differences exist in the electrophysiological responses of cardiac myocytes to α_1-adrenoceptor stimulation. In the guinea pig, atrial and ventricular action potential durations are decreased by α_1 agonists or by direct activation of PKC with phorbol esters (40, 41). This decrease in action potential duration results primarily from an increase in the delayed rectifier K^+ current (I_K). This current is enhanced by both α_1 agonists and direct activation of PKC with phorbol esters, and both effects are blocked by H-7, a PKC inhibitor (22, 42, 43). Furthermore, guinea pig I_K is also increased by intracellular perfusion with purified PKC (44). Thus, a clear cascade of events has been established for α_1-adrenoceptor modulation of the guinea pig action potential duration, and this study provides further insight into the final molecular pathway. The results presented here also provide further evidence to support the idea that the I_{sK} protein forms the channels that underlie the I_{Ks} current in the heart. One of the physiological functions of the I_{sK} protein is, therefore, in diastolic repolarization of cardiac myocytes, and this role in the heart makes the I_{sK} protein an attractive target for the development of therapeutic agents that alter the duration of the cardiac action potential.

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