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A Novel Long N-terminal Isoform of Human L-type Ca^{2+} Channel Is Up-regulated by Protein Kinase C*

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Human L-type voltage-dependent Ca^{2+} channels (α_{1C}, or $Ca_v1.2$) are up-regulated by protein kinase C (PKC) in native tissues, but in heterologous systems this modulation is absent. In rat and rabbit, α_{1C} has two N-terminal (NT) isoforms, long and short, with variable initial segments of 46 and 16 amino acids, respectively. The initial 46 amino acids of the long-NT α_{1C} are crucial for PKC regulation. However, only a short-NT human α_{1C} is known. We assumed that a long-NT isoform of human α_{1C} may exist. By homology screening of human genomic DNA, we identified a stretch (termed exon 1a) highly homologous to rabbit long-NT, separated from the next known exon of α_{1C} (exon 1b, which encodes the alternative, short-NT) by an ~80 kb-long intron. The predicted 46-amino acid protein sequence is highly homologous to rabbit long-NT. Reverse transcriptase PCR showed the presence of exon 1a transcript in human cardiac RNA. Expression of human long-NT α_{1C} in *Xenopus* oocytes produced Ca^{2+} channel enhanced by a PKC activator, whereas the short-NT α_{1C} was inhibited. The long-NT isoform may be the Ca^{2+} channel enhanced by PKC-activating transmitters in human tissues.

Voltage-dependent L-type Ca^{2+} channels are crucial for cardiac and smooth muscle contraction and hormone secretion, and they regulate gene expression in the brain (1–3). Their function is highly regulated by hormones and neurotransmitters, largely via activation of protein kinases (3, 4). Regulation by PKC[1] is believed to be of substantial physiological importance, mediating all or part of the effects of several hormones and intracellular messengers (4). PKC enhances L-type Ca^{2+} currents in diverse human tissues and cell lines: heart, neuroblastoma, T-cells, and endocrine cells (5–10). Dual modulation by PKC is often observed with activation followed by, or concomitant with, inhibition (5, 10). Similar enhancement by PKC, sometimes followed by inhibition, has been described in other mammals (11, 12) and was reproduced in *Xenopus* oocytes expressing the cloned rabbit cardiac L-type Ca^{2+} channels (13, 14). However, expression of human L-type channels, encoded by all cDNA cloned to date, yielded Ca^{2+} channels that were only inhibited by PKC; the enhancement could not be reconstituted (15). The reason for the inability to reproduce the PKC modulation of human L-type channels remained unknown.

The main, pore-forming subunit of cardiac/smooth muscle L-type channel (α_{1C} or $Ca_v1.2$), also present in the brain, is the product of the α_{1C} gene, *CACNA1C* (16). Several splice variants of *CACNA1C* are known (17, 18). The resulting isoforms of human α_{1C} protein show differential distribution in human tissues, and in failing *versus* normal myocardium. They play important roles in Ca^{2+}-dependent inactivation, oxygen sensing, and drug sensitivity (18–22). However, the genomic structure of the beginning of N-terminal region of human α_{1C} is not entirely clear. In the two best studied mammalian species, rat and rabbit, two N-terminal isoforms of α_{1C} cDNA are known, which most probably represent variable splicing products of the same gene (23). These splice variants encode long- and short-NT α_{1C} proteins, with variable initial segments of 46 and 16 aa, respectively (23–26) (the total length of the cytosolic part of the NT region of α_{1C} is ~154 aa in the long-NT α_{1C}). Traditionally, the short-NT isoform is called "neuronal" in rat and "smooth muscle" in rabbit, whereas the long-NT isoform is considered "cardiac" in rabbit. However, in rat, α_{1C} protein containing the long-NT is found in both heart and brain (27). The known human α_{1C} is highly homologous to short-NT isoforms of rat and rabbit; the long-NT isoforms of rat and rabbit are also highly homologous to each other. Because the human L-type channel is up-regulated by PKC, and because the initial 20 aa of the long-NT isoform are crucial for this regulation in rabbit α_{1C} (27, 28), we reasoned that a long-NT isoform of human α_{1C} should also exist.

Here we demonstrate the presence of an exon encoding the initial 46-aa long-NT segment in the human genomic DNA. The existence of α_{1C} RNA containing this segment was confirmed by RT-PCR gel analysis and DNA sequencing of the RT-PCR product. Using the RT-PCR products, we have demonstrated that cDNA coding for a full-length long-NT α_{1C} isoform expressed in *Xenopus* oocytes produces a Ca^{2+} channel that is enhanced by a PKC activator. The identification of the long-NT isoform of human α_{1C} will enable the study of the molecular mechanisms of PKC modulation, which has previously been hampered by the inability to reconstitute this modulation in expression systems.

EXPERIMENTAL PROCEDURES

Tissues and Oocyte Culture—All experiments with human and animal tissues were approved by the Tel Aviv University Helsinki Committee and the Sackler School of Medicine Animal Use Committee, respectively. Rat atria and ventricles were obtained from 19-day-old Wistar rats after decapitation performed under ether anesthesia. *Xenopus* frogs were maintained and operated on, and oocytes were prepared as described (29). Each oocyte was injected with 2.5 ng of RNAs of α_{1C}, α_2/δ, and β2A subunits and incubated for 3–4 days at

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[1] The abbreviations used are: PKC, protein kinase C; aa, amino acid; NT, N-terminal; nt, nucleotide; UTR, untranslated region; RT, reverse transcriptase; PMA, 4β-phorbol 12-myristate 13-acetate; BIS, bisindolylmaleimide; contig, group of overlapping clones.

20–22 °C in NDE96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2.5 mM sodium pyruvate, 50 μg/ml gentamycin, 5 mM HEPES, pH 7.5).

RT-PCR Analysis—5 μg of total human cardiac RNA purchased from Ambion, Inc. (catalog no. 7966, lot 110P43B) was reverse-transcribed with SuperScript II reverse transcriptase (Invitrogen) with primer 3 (see text below and Fig. 2*A*). Each PCR reaction (50 μl) contained 2.4 μl of the product of RT reaction, 1 μl of 10 mM dNTPs, 20–50 pmol of primers, 5 μl of 10× PCR buffer, 2 μl of Mg^{2+} (2 mM), and 0.7 μl of *Taq* DNA polymerase (Promega). PCR was performed under the following conditions: 95 °C for 1 min, 49 °C for 1 min, and 72 °C for 2 min, repeated 35 times. The final elongation was performed at 72 °C for 5 min. The PCR products were analyzed on a 1% agarose gel.

The primers used for RT and PCR were: No. 1, CTTCGAGCCTTTGTTCAG (nt 4–21 from A in ATG of exon 1a); No. 2, TCAATGAGAATACGAGGA (nt 5–22 in exon 1b; numbering from ATG of the short-NT isoform $\alpha_{1C,77}$ (17)); No. 3, ATTGGTGGCGTTGGAATC (nt 468–451; numbering from ATG of exon 1b); No. 4, TAAAGTGAAATAAAGAGT (within the proposed intron between exon 1a and exon 1b; nt 95529–95546 in contig 3810573[2]; +149 nt from A in ATG of exon 1a); No. 5, ATACCACTACTGAATATA (within the same intron, nt 95896–95913 in contig 3810573; +516 nt from A in ATG in exon 1a); No. 6, AACTGAGAAAGTGGCTTT (2369 nt upstream from A in ATG of exon 1a; nt 93010–93027 in contig 3810573); No. 7, GCAGGTTAGTGTAGGAAT (1339 nt upstream from A in ATG of Exon 1a; nt 94040–94057 in contig 3810573); No. 9, CCATTCGACAATGCTGAT (nt 369–352 in exon 2; numbering from ATG of exon 1b of $\alpha_{1C,77}$); No. 10, GATACGATACGGCCATGT (within the proposed 5′-UTR of exon 1a, 147 nt upstream from A in ATG of exon 1a; nt 95232–95239 in contig 3810573).

DNA Constructs—cDNAs of α_2/δ and β2A were as described (30). cDNA of human short-NT isoform $\alpha_{1C,77}$ (Ref. 31; GenBank™ accession No. Z34815) was obtained from Dr. R. Zuhlke and subcloned into pGEM-HJ vector (which contains 5′- and 3′-UTRs of *Xenopus* α-globin flanking the polylinker). In the resulting construct, termed $\alpha_{1C,77S}$, the 5′-UTR of α-globin is followed by a *Bam*HI restriction site and then immediately by the initial ATG. The coding sequence of $\alpha_{1C,77}$ is followed by the 3′-UTR of α-globin and then by a *Sal*I site. The DNA of the long-NT, termed $\alpha_{1C,77L}$, was constructed as follows. The human heart cDNA obtained in the RT reaction described above was subjected to PCR with the reverse primer No. 3 (see above) and a forward primer ATTCGCGGATCCATGCTTCGAGCCTTTGTT that overlaps the first 18 nt of the coding part of exon 1a (starting with ATG) and also creates a *Bam*HI restriction site preceding ATG. The PCR product was digested with *Bam*HI and *Mfe*I (a unique *Mfe*I site is present in exon 3 upstream of primer No. 3) and inserted between these restriction sites into *Bam*HI/*Mfe*I-digested $\alpha_{1C,77S}$ in place of the original DNA segment flanked by these sites. The 5′ portion of the resulting DNA was sequenced. The sequence of the *Bam*HI-*Mfe*I segment obtained by this RT-PCR subcloning procedure was 100% identical to that predicted for the long-NT splice variant (based on the DNA sequence of chromosome 12), in which exon 1a is followed by exons 2 and 3 (see Fig. 1*C*). RNA was synthesized as described (29).

Electrophysiology—Whole cell currents were recorded using the Gene Clamp 500 amplifier (Axon Instruments, Foster City, CA) using the two-electrode voltage clamp technique, as described (30), in a solution containing 40 mM $Ba(OH)_2$, 50 mM NaOH, 2 mM KOH, and 5 mM HEPES, titrated to pH 7.5 with methanesulfonic acid. PMA and bisindolylmaleimide (BIS) were purchased from Sigma. Oocytes were treated with BIS essentially as described (32). In brief, the oocyte was injected with 30 nl of a water solution of BIS at 150 μM and, in addition, incubated in 5 μM BIS 2–4 h before recording. Stimulation, data acquisition, and analysis were performed using pCLAMP software (Axon Instruments).

RESULTS AND DISCUSSION

The initial exon of the previously described (17) human α_{1C} clone, which we now designate exon 1b, contains a 5′-UTR and encodes the first 16 aa of the short-NT α_{1C} protein. To find out whether DNA sequences that encode an alternative, long-NT isoform of human α_{1C} exist, we performed a standard BLAST search of human genomic DNA,[2] using as our query the cDNA sequence encoding the first 46 aa of the rabbit cardiac long-NT clone (24). The initial search showed the presence of a highly homologous sequence in the contig 3810573[3] from human chromosome 12, locus p13.3 (Fig. 1*A*; the region of the initial search is highlighted by *bold letters*). This is within the region where the gene of α_{1C}, *CACNA1C*, is located. Significantly, the sequence upstream from this segment showed significant homology to the 5′-UTR of the rabbit α_{1C} (Fig. 1*A*), supporting the possibility that it may be part of an initial exon of a human L-type channel. We have designated this tentative exon as 1a. Within the presumably protein-coding part of exon 1a, a 45-base-long DNA segment, starting at base 16 (from the initial ATG) also shares 40% homology with the protein-coding part of exon 1b (Fig. 1*A*).

The availability of the draft sequence of the human chromosome 12 on the NCBI Human Genome site allowed us to map the location of the putative exon 1a relative to the known exons of the human α_{1C} gene (Fig. 1*B*). Exon 1a precedes exon 1b and is separated from the latter by ~80 kb. We assume that this is a large intron. Two other large introns are found, according to the draft map of the chromosome, between exon 1b and exon 2 (~60 kb) and between exons 3 and 4 (~330 kb). These large introns have not been fully sequenced previously; introns of >5 and 2.4 kb have been reported (17). The location of exon 1a supports the possibility that it constitutes the first alternative exon of the *CACNA1C* gene. The screening procedure also confirmed the presence and the correct spatial location on chromosome 12 of all the of constant and alternative exons described by Soldatov in 1994 (17) (Fig. 1*B* show 10 of the 50 exons, excluding exon 1a).

Our working hypothesis was that exons 1a and 1b are alternative initial exons of the human *CACNA1C* gene (Fig. 1*C*). Exons 2 and 3 are probably constant (17), as supported by the conservation of the corresponding protein sequences in known long- and short-NT α_{1C} isoforms in human, rat, rabbit, and mouse (Fig. 1*D* and Refs. 23, 27, and 28). The protein sequences of N termini of the proposed human α_{1C} short- and long-NT isoforms are shown in Fig. 1*D* and are compared with the rabbit long-NT α_{1C}. The segment corresponding to exon 2 starts after aa 46 in the long-NT isoform and after aa 16 in the short-NT isoform. The protein segment encoded by exon 3 starts at aa 155, exactly at the proposed junction between the cytosolic NT and the first transmembrane segment of the channel, IS1 (24).

To further substantiate our working hypothesis, we performed RT-PCR on total human cardiac RNA. cDNA was obtained by reverse transcription using primer No. 3 (Fig. 2*A*), which corresponds to the 3′ end of exon 3. The analytical PCR was done with primers corresponding to segments of chromosome 12 DNA, as published at the NCBI Human Genome site, within the putative exons and introns of the 5′ region of *CACNA1C*. The location of primers is shown in Fig. 2*A*. Clear bands were detected for all DNAs that contained exon 1a (Fig. 2*B*): protein-coding sequences of [exon 1a + exon 2 + exon 3] (*lane 1*) and [exon 1a + exon 2] (*lane 3*); [exon 1a including 5′UTR + exon 2] (*lane 5*). The sizes of the DNAs corresponded to those expected for an RNA transcript that contains exons 1a, 2, and 3 *without* 1b. Control reactions, with primers within the presumed intron regions (*lanes 6* and *7*) and ~1.4–2.4 kb upstream from the beginning of exon 1a (*lane 8*) did not yield signals. Another cDNA segment that included protein-coding sequences of exon 1a, 2, and (part of) 3 was obtained for subcloning purposes (see "Experimental Procedures") and sequenced. The size and the DNA sequence of this RT-PCR product were exactly as expected for [exon 1a + exon 2 + exon 3 up to *Mfe*I site] *without* exon 1b and encoded the first 46 aa of the

[2] Found on the Web at ncbi.nlm.nih.gov/BLAST.

[3] NCBI accession number AC005342.

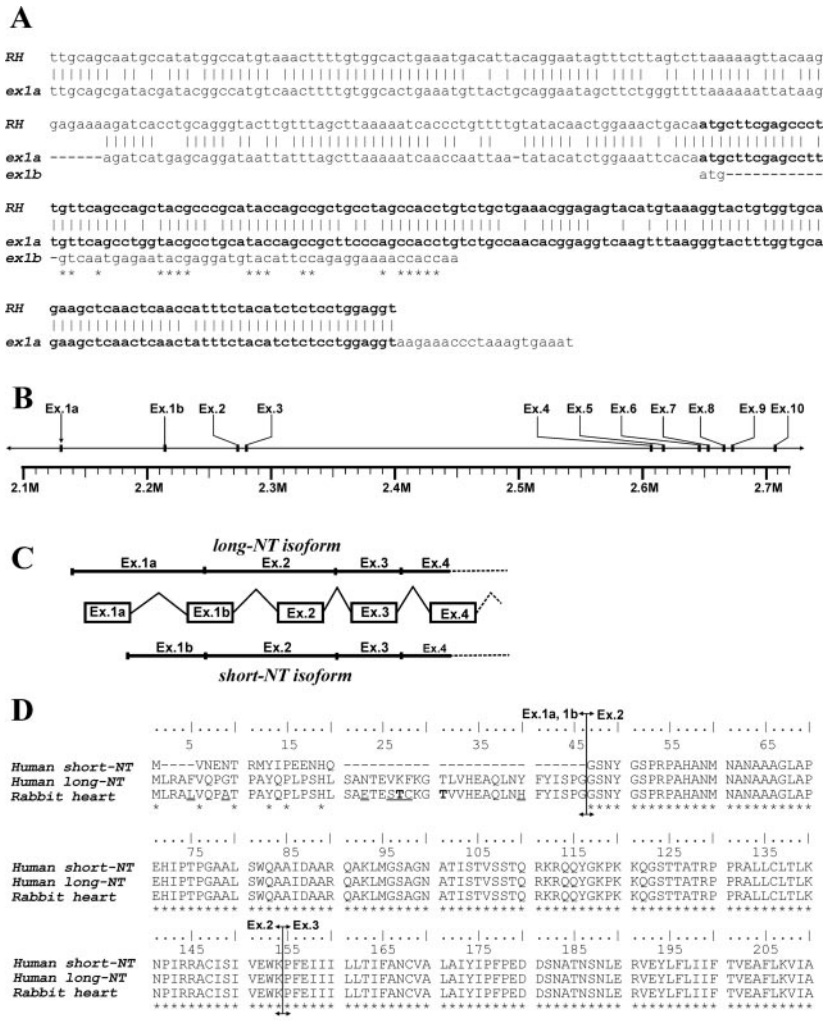

FIG. 1. **The newly identified exon 1a and the proposed DNA and protein structure of two NT isoforms of human α_{1C}.** *A*, nucleotide homology between rabbit heart (*RH*) long-NT cDNA, the homologous part of human chromosome 12 corresponding to the proposed exon 1a (*ex1a*), and the coding sequence of the known exon 1b (*ex1b*). Full homology is indicated by *asterisks*. *Bold letters* show the protein-coding sequence of long-NT isoforms. *B,* the location of exons and introns according to the draft map of chromosome 12 available at the NCBI site. *C*, the DNA sequences corresponding to proposed alternative splice variants encoding long- and short-NT isoforms of α_{1C}. *The middle section* shows the 5′ part of the *CACNA1C* gene, with *boxes* representing exons and *angled lines* representing introns. *D*, comparison of the protein sequences of rabbit cardiac α_{1C} and the two proposed isoforms of the human α_{1C}. *Asterisks* show fully conserved aa; *bold letters* highlight the PKC phosphorylation sites responsible for the inhibitory effect of PMA in rabbit α_{1C} (35); the *underlined* residues are those that differ in rabbit and human long-NT α_{1C}.

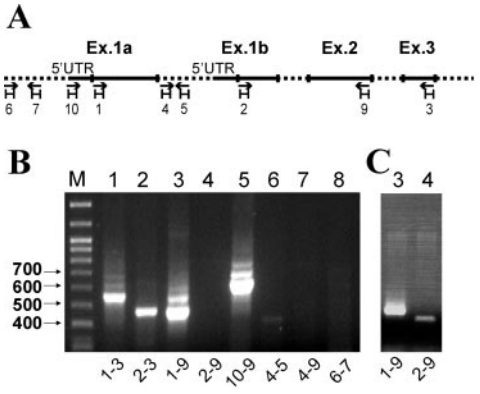

FIG. 2. **RT-PCR supports the existence of human long-NT isoform.** *A*, the location of primers used for RT-PCR. *B*, RT-PCR products obtained with the primer pairs shown *below* the lanes. Primers were added to the PCR reactions at 50 pM. The sizes of DNAs expected from the hypothesis of Fig. 1*A* were, by *lanes* (in nt): *1*, 547; *2*, 463; *3*, 449; *4*, 365; *5*, 591; *6–8*, no PCR products were expected. *C*, RT-PCR with 20 pM primers, showing *lanes 3* and *4* as in *panel B*.

proposed long-NT isoform of α_{1C} followed by protein sequences highly conserved in all known isoforms of α_{1C}. These results strongly support the presence in human cardiac tissue of an RNA species encoding the proposed long-NT isoform shown in Fig. 1, *C* and *D*.

A band corresponding to the DNA of exons [1b + 2 + 3] was also obtained; the size was exactly as expected for a short-NT isoform, *without* 1a (Fig. 2*B*, *lane 2*). Under standard conditions the band corresponding to [exon 1b +exon 2] was not detected (Fig. 2*B*, *lane 4*), but varying the conditions of PCR revealed this band, although it still remained relatively weak (Fig. 2*C*). Thus, RNA of the previously described short-NT isoform is also present in human cardiac tissue.

Previously, the existence of a long-NT α_{1C} protein in rat has been demonstrated by Western blot with a polyclonal antibody directed against the unique initial 46 aa of the rabbit long-NT isoform (27). The same antibody detected a >220 kDa protein (presumably the L-type Ca^{2+} channel) in a human colon cancer cell line (33). These results corroborate the presence of the long-NT α_{1C} protein in human tissues. Taken together, our data and those of Ref. 33 support the notion that human cardiac (and probably other) tissues contain two N-terminal isoforms of α_{1C} protein, a long-NT and a short-NT one, which are products of alternatively spliced RNA transcripts of the 5′-terminal region of the *CACNA1C* gene (as shown in Fig. 1, *C* and *D*).

According to our initial hypothesis, the long-NT α_{1C} should be up-regulated by the activation of PKC. To test this prediction, we constructed a cDNA encoding a long-NT α_{1C} on the basis of a human short-NT α_{1C} DNA, $\alpha_{1C,77}$ (19). Both cDNAs were subcloned into the pGEM-HJ vector and verified by DNA sequencing (see "Experimental Procedures"). The long-NT clone was designated $\alpha_{1C,77L}$ and the short-NT clone $\alpha_{1C,77S}$. The corresponding RNAs were synthesized *in vitro* and injected into *Xenopus* oocytes with RNA of the α_2/δ subunit with or without RNA of the β2A subunit (30). The voltage-dependent Ba^{2+} currents (I_{Ba}) via the expressed $\alpha_{1C,77L}$ and $\alpha_{1C,77S}$ Ca^{2+}

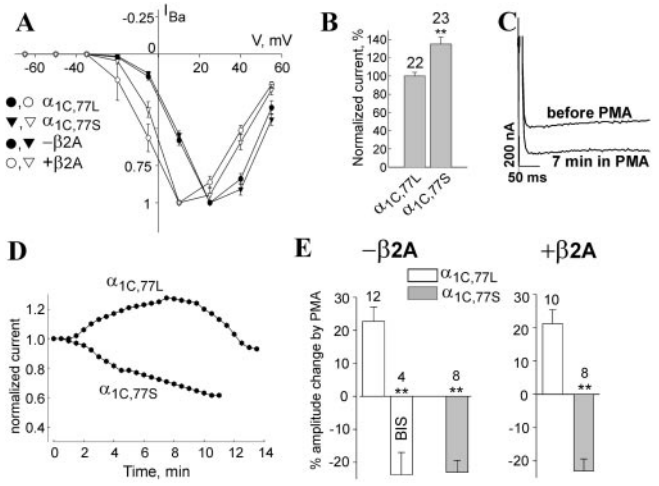

FIG. 3. **PKC up-regulates the long-NT isoform of human α_{1C}.** In all cases, data are shown as the mean ± S.E., and the numbers of cells tested are indicated *above* the *bars*. **, indicates $p < 0.01$ (by *t* test). *A*, current-voltage relations of $\alpha_{1C,77S}$ and $\alpha_{1C,77L}$ expressed with α_2/δ with or without β2A. *B*, relative amplitudes of peak I_{Ba} of $\alpha_{1C,77S}$ and $\alpha_{1C,77L}$ (with α_2/δ + β2A) measured at +20 mV in four oocyte batches. In each oocyte, I_{Ba} was normalized to the average current amplitude of the $\alpha_{1C,77L}$ group of the same batch. *C*, the effect of 10 nM PMA in a representative oocyte expressing $\alpha_{1C,77L}$ + α_2/δ + β2A. I_{Ba} was measured by 300-ms steps to 20 mV from a holding potential of −80 mV delivered every 30 s. *D*, the time course of PMA effect in two oocytes of the same donor expressing the two α_{1C} isoforms (with α_2/δ + β2A). PMA was applied at time point zero after stabilization of the leak and Ba^{2+} currents (see Ref. 13 for details of PMA use). *E*, summary of experiments done with PMA.

channels ranged from 100 to 800 nA without β2A and from 1500 to 7000 nA with β2A. The kinetics of I_{Ba} of $\alpha_{1C,77L}$ and $\alpha_{1C,77S}$ (*e.g.* Fig. 3*C*) were very similar to each other and to those directed by rabbit cardiac α_{1C}. The other parameters also resembled those previously reported for L-type Ca^{2+} channels of various species. For instance, as described previously for the rabbit α_{1C} (30, 34), coexpression of the β2A subunit increased the current amplitude 9.5 ± 0.4-fold in $\alpha_{1C,77L}$ and 9.3 ± 0.6-fold in $\alpha_{1C,77S}$, and shifted the voltage dependence of activation to more negative potentials, as shown by normalized current-voltage curves (Fig. 3*A*). Peak I_{Ba} was similar in the long-NT and short-NT isoforms; the small difference was statistically significant (Fig. 3*B*), but at present we do not know whether this reflects differences in protein expression or in the gating properties of the two isoforms.

The prediction that the long-NT human α_{1C} should be enhanced by PKC has been fully confirmed. The main effect of the phorbol ester β-PMA (a PKC activator) was to increase the Ba^{2+} current of $\alpha_{1C,77L}$ coexpressed with α_2/δ with or without β2A (Fig. 3, *C–E*). As in the rabbit α_{1C} channel (13), the increase reached a maximum within 5–8 min and was followed by a decrease, sometimes below the basal current level (Fig. 3*D*). In contrast, the short-NT $\alpha_{1C,77S}$ channels were inhibited by PMA (Fig. 3*D*), in line with the previous report (15). On average, PMA increased the current via the long-NT channels by 22.7 ± 4.4% in the absence of β2A subunit and by 21.2 ± 4.2% in its presence (Fig. 3*E*). The PMA-induced increase in I_{Ba} was fully blocked by the specific PKC inhibitor, bisindolylmaleimide (Fig. 3*E; bar* marked *BIS*), supporting the notion that the enhancement of I_{Ba} by PMA was indeed mediated by PKC. Thus, in general, the modulation of the human long-NT isoform by PKC is similar to that of the rabbit cardiac channel. With rabbit cardiac L-type channel, coexpression of β2A substantially weakens the enhancement of the current by PMA (13), whereas in the human channel this action of β2A is less pronounced. It would be of interest to see whether this is due to one of the few differences in the primary amino acid composition of the 46-aa-long initial NT segment in human and rabbit (Fig. 1*D, underlined* amino acids).

In a different expression system (human embryonic kidney cell line tsA-201), only inhibition of the rabbit long-NT α_{1C} by PMA has been observed (35). The inhibition crucially depended on the presence of each of the two threonines, Thr^{27} and Thr^{31} (shown in *bold* in Fig. 1*D*), in which phosphorylation by PKC was proposed to underlie this modulation. It has been proposed that the lack of PKC-induced enhancement in tsA-201 cells may result from the absence of a specific PKC isozyme (35). At present, we do not know whether the phosphorylation of Thr^{31} (Thr^{27} is absent in the long-NT human α_{1C}; Fig. 1*D*) plays a role in any of the PKC effects observed in *Xenopus* oocytes. We suspect that the mechanism of PMA-induced inhibition of α_{1C} in oocytes is different from that observed in tsA-201 cells, because a short-NT isoform of rat α_{1C} was not sensitive to PMA in this system (35), whereas the homologous short-NT human α_{1C} is inhibited by PKC in *Xenopus* oocytes (Ref. 15 and Fig. 3). Furthermore, the decrease of I_{Ba} is still observed in the presence of BIS (Fig. 3*E*), whereas in tsA-201 cells PKC inhibitors blocked the effect of PMA (35). The PMA-induced inhibition of human L-type Ca^{2+} channels observed in *Xenopus* oocytes requires further study.

Because the most widely observed effect of PKC activators on human L-type Ca^{2+} channels is an enhancement of the current (sometimes accompanied by an inhibition (5–10)), and because this regulation is reproduced with the long-NT α_{1C} in *Xenopus* oocytes, we propose that the long-NT α_{1C} is the isoform that underlies the PKC-induced enhancement. The homologous long-NT rabbit α_{1C} isoform behaves in the same way when expressed in oocytes (13, 28), probably by means of an identical molecular mechanism as in human α_{1C}. The four aa following the initial methionine in rabbit long-NT α_{1C}, LRAL, are necessary for PKC-induced enhancement. Because this protein segment contains no putative phosphorylation sites, we have proposed that PKC phosphorylation occurs elsewhere in α_{1C} or in an auxiliary protein, whereas the first five aa play a role in PKC anchoring or in channel gating (27). The short-NT isoform lacks these four amino acids; its initial 15-aa segment (following the first methionine) carries a partial homology to amino acids 6–20 of the long-NT isoform (Fig. 1*D*). In rabbit long-NT α_{1C}, the first 20 aa play the role of the inhibitory gating element, which reduces the open probability of the L-type channel[4] (27, 28). It would be of great interest to see whether this is also the case in human long-NT α_{1C}. We hope that the discovery of the long-NT isoform of human α_{1C} and the demonstration of its modulation by PKC, as described in this report, will provide the basis and the incentive for future studies of PKC targets (α_{1C} or an auxiliary protein?) and of the interaction between the phosphorylated and gating parts of α_{1C} in PKC modulation.

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