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Genomic Structure and Functional Expression of a Human α_2/δ Calcium Channel Subunit Gene (*CACNA2*)

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***CACNA2* encodes the α_2/δ subunit of the human voltage-gated calcium channels and is located in the candidate region of malignant hyperthermia susceptibility type 3 (MHS3). We determined the structural organization of *CACNA2* by isolation of overlapping genomic DNA clones from a human phage library. The gene consists of at least 40 exons, 2 of which are alternatively spliced, spanning more than 150 kb of genomic DNA. Exons range from 21 to 159 bp, and introns range from 98 bp to at least more than 20 kb. We constructed a full-length cDNA and cloned it into a mammalian expression vector. Cotransfection of the *CACNA2* cDNA with α_{1A} and β_4 cDNA into HEK293 cells led to the expression of Q-type calcium currents. The α_2/δ subunit enhanced the current density 18-fold compared to cells transfected with only α_{1A} and β_4 cDNA. The sequence analysis provides the basis for comprehensive mutation screening of *CACNA2* for putative MHS3 individuals and patients with other channelopathies.** © 1999 Academic Press

INTRODUCTION

Voltage-gated calcium channels regulate the entry of extracellular Ca^{2+} ions into the cytoplasm where they participate in a variety of calcium-dependent processes including transmitter release, muscle contraction, and secretory processes (Berridge, 1997). Multiple gene families encoding the various subunits have been identified (Hofmann *et al.*, 1994). They consist of five distinct polypeptides, α_1, α_2/δ, (or briefly α_2 as the two subunits are encoded by the same gene), β, and γ (Nastainczyk *et al.*, 1990). For each polypeptide, several isoforms have been identified. All α_1 isoforms (e.g., α_{1A}, brain; α_{1C}, heart; α_{1S}, skeletal muscle) are channels as they possess an ion-conducting pore, whereas all others are auxiliary subunits (α_2, β_1–β_4, and γ). For α_2, two splice variants have been reported, α_{2A} (skeletal muscle, aorta smooth muscle) and α_{2B} (brain) (Greenberg, 1997).

The best characterized calcium channels are the dihydropyridine sensitive L-type calcium channels of heart (α_{1C}, α_{2A}, β_2) and skeletal muscle consisting of α_{1S}, α_{2A}, β_3, and γ and the P/Q-type calcium channel of brain that is composed of α_{1A}, α_{2B}, β_4, and possibly stargazin as γ_2 (Ellis *et al.*, 1988; Letts *et al.*, 1998). Each subunit is encoded by a separate gene, with the exception of the α_2/δ primary transcript, with the δ subunit arising as a result of proteolytic cleavage of the C-terminal end of the α_2/δ polypeptide (DeJongh *et al.*, 1990). The extracellularly located α_2 protein is anchored by disulfide bonds to the δ subunit that contains a single transmembrane domain (Jay *et al.*, 1991; Felix *et al.*, 1997). Although only a single gene for the $\alpha_2(\delta)$ subunit has been identified, cDNA cloning and PCR analysis have revealed different splice variants expressed in various tissues. In humans the brain expresses α_{2B}, which differs only by an insertion of 7 amino acids and a deletion of a 19-amino-acid segment between the first and the second hydrophobic domains (Kim *et al.*, 1992) [*CACNA2;* for nomenclature see Lory *et al.* (1997)]. The gene has been mapped to chromosome 7q11.23–q21.1 between the polymorphic markers D7S675 and D7S524. Previously, susceptibility to malignant hyperthermia (MH), an anesthesia-related crisis, has been linked in a large German pedigree to D7S849 also flanked by D7S675 and D7S524. Physical mapping places D7S849 within 110–380 kb of *CACNA2* (Iles *et al.*, 1994). This type of MH susceptibility is named MHS-3 (McKusick, 1997).

Malignant hyperthermia is an autosomal dominant, pharmacogenetic disorder of skeletal muscle that manifests itself as a potentially lethal crisis triggered by volatile anesthetics and depolarizing muscle relaxants. A pathologically high increase of the myoplasmic calcium concentration during exposure to the triggering agents (Iaizzo *et al.*, 1988) underlies the MH susceptibility (MHS), resulting in muscle contracture and hy-

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permetabolism with subsequent acidosis, hypoxia, hypercapnia, and hyperkalemia. In skeletal muscle, calcium release is initiated by a depolarization of the transverse tubular membrane and by a fast conformational change of intracellular loops of the α_1 subunit of the voltage-gated L-type calcium channel that opens the ryanodine receptor (RYR1), which is situated in the membrane of the sarcoplasmic reticulum (SR). This signal transmission between tubular and SR membrane is referred to as excitation–contraction coupling (Catterall, 1991). MHS-causing mutations have been identified in RYR1, the calcium release channel of the SR [MHS-1 (Manning *et al.*, 1998)], as well as in the α_{1S} subunit of the L-type calcium channel [MHS-5 (Monnier *et al.*, 1997)]. The α_2 subunit of this L-type calcium channel seems to be a reasonable candidate as a carrier of MHS-causing mutations.

To provide the basis for a mutation screening, we determined the complete structural organization of the human *CACNA2* gene. We further constructed a full-length cDNA for functional coexpression of the human (skeletal muscle) α_2 cDNA and show that this construct enhances functional expression of calcium channels. Functional expression of a human (skeletal muscle) α_2 cDNA has not been reported yet. However, expression of a rabbit skeletal muscle α_2 cDNA (and an accessory β cDNA) resulted in an increase of the α_{1C} calcium current. The rabbit α_2 subunit also accelerated inactivation kinetics and shifted the steady-state inactivation and activation curves in the hyperpolarizing direction (Singer *et al.*, 1991; De Waard and Campbell, 1995). Since α_{1S}, the skeletal muscle isoform, and its accessory subunits β_{1A}, γ, and α_2 cannot be properly expressed in nonmuscular cell systems, we have chosen a brain-specific form of calcium channels consisting of α_{1A} and β_4 subunits with the here described first coexpression of the full-length transcript of *CACNA2* that encodes the human α_2/δ subunit, α_{2A}.

MATERIALS AND METHODS

Genomic library screening. To prepare a genomic library, DNA from human whole blood cells partially digested with *Sau*3AI was cloned into the *Bam*HI site of Lambda GET (Nehls *et al.*, 1994). Vector arms were purified by sucrose gradient centrifugation after digestion with *Xho*I and *Bam*HI to eliminate stuffer contamination of the library (Sambrook *et al.*, 1989). Genomic DNA fragments were also size-selected prior to cloning. Fragments were ligated to vector arms and packaged *in vitro* using Gigapack Gold extracts (Stratagene) and plated on *Escherichia coli* C600 bacteria.

Plaques were transferred to nylon filters and screened with a full-length DNA probe generated from *CACNA2* cDNA. Screening was performed by a standard hybridization technique using cDNA probes radiolabeled by [α-^{32}P]dCTP with a random-primed DNA labeling kit (Pharmacia) according to the manufacturer's instructions.

Plasmid subcloning. After three rounds of plaque purification, phage DNA of positive clones was converted to plasmid by infecting *E. coli* strain BNN 132 (Elledge *et al.*, 1991), which expresses cre-recombinase. The plasmid was purified using the Qiagen plasmid kit (Qiagen Inc.). To determine the insert size, the plasmid versions of Lambda GET recombinants were cut with *Kpn*I, which flanks the cloning sites and releases the 4.5-kb plasmid backbone.

Exon mapping by PCR. To determine the distance between exons, genomic DNA and positive clones containing part of the *CACNA2* locus were used as templates for long PCR amplification. To obtain the long PCR fragments required, *Taq* extender (Stratagene) and the Expand Long Template PCR system (Boehringer Mannheim) were used. The standard PCR mix in a final volume of 50 μl consisted of 50 ng DNA, 30 pmol of each primer, based on known cDNA sequences, 500 μM dNTPs, 1× *Taq* buffer, and 2 to 4 units of *Taq* polymerase. The amplification protocol consisted of 30 cycles at 94°C for 45 s, 58°C for 45 s, and 72°C for 10–15 min.

DNA sequencing. Double-stranded sequencing of plasmid DNA was carried out using the dideoxy nucleotide method and performed according to the instructions specified in the ALFexpress Auto read sequencing kit (Pharmacia) on an ALFexpress sequencer.

Construction and transfection of the expression plasmids. Using two primers, a 1-kb fragment of *CACNA2* was amplified from a human skeletal muscle tissue cDNA. This fragment was used as a probe for the screening of a human skeletal muscle cDNA library (Clontech). Screening was performed as described above. The inserts of positive phages were cut out with *Eco*RI and subcloned into pT7T3 19U (Pharmacia). Identification of inserts was checked by sequencing with vector primers flanking the cloning site. A *Bam*HI/*Alw*NI fragment of one large clone that contained the full-length human α_2 cDNA was subcloned into the *Bam*HI/*Eco*RV site of the pcDNA3 vector (Invitrogen) after blunt ending of the *Alw*NI site. HEK293 cells were transfected with α_2, α_{1A}, and β_4 cDNA using the standard calcium phosphate precipitation technique. The cells were grown in MEM, supplemented with 10% fetal calf serum (Gibco BRL).

Electrophysiological recordings and data analysis. Ca^{2+} and Ba^{2+} currents were measured using the whole-cell mode of the patch clamp method at room temperature. For data acquisition, an Axoclamp 2000 amplifier (Axon Instruments, USA) connected to a PC equipped with the Pclamp6 acquisition package (Axon Instruments) was used. The extracellular solution contained (in mM): $CaCl_2$ or $BaCl_2$, 30; *N*-methylglucamine chloride, 104; $MgCl_2$, 1; glucose, 5; Hepes, 10. The pH was adjusted to 7.4 using HCl. The patch pipettes were filled with an internal solution containing (in mM): CsCl, 112; $MgCl_2$, 1; Hepes, 5; MgATP, 1; EGTA, 10 (pH 7.4, adjusted with CsOH). Recordings were filtered at 3 kHz using a 4-pole Bessel filter and digitized at 40 kHz. Holding potentials of −100 mV were used to record ionic currents throughout the study. Steady-state inactivation of ionic currents was measured by 3-s prepulses and applying the depolarizing protocol from the same membrane potential (−100 mV) each time. Recordings were capacity-corrected. From the holding potential, four scaled hyperpolarizing prepulses were given, and the sum of the prepulses was subtracted from each recording.

Statistical analysis and curve fitting were carried out using the Pclamp6 software package, Origin (Microcal, Northampton, MA), or SigmaPlot (Jandel). All grouped data are reported as mean ± standard error of the mean (SEM).

RESULTS

Exon/Intron Structure

To define the genomic structure of *CACNA2*, a genomic library of human blood cell DNA constructed in Lambda GET was screened with different fragments from the *CACNA2* cDNA. About 2×10^6 clones were screened, and approximately 200 independent clones were analyzed. Positive clones containing part of *CACNA2* were converted to plasmids by infecting *E. coli* strain BNN132, which expresses cre-recombinase (Elledge *et al.*, 1991). Exon/intron boundaries were determined by sequencing plasmids using oligonucleo-

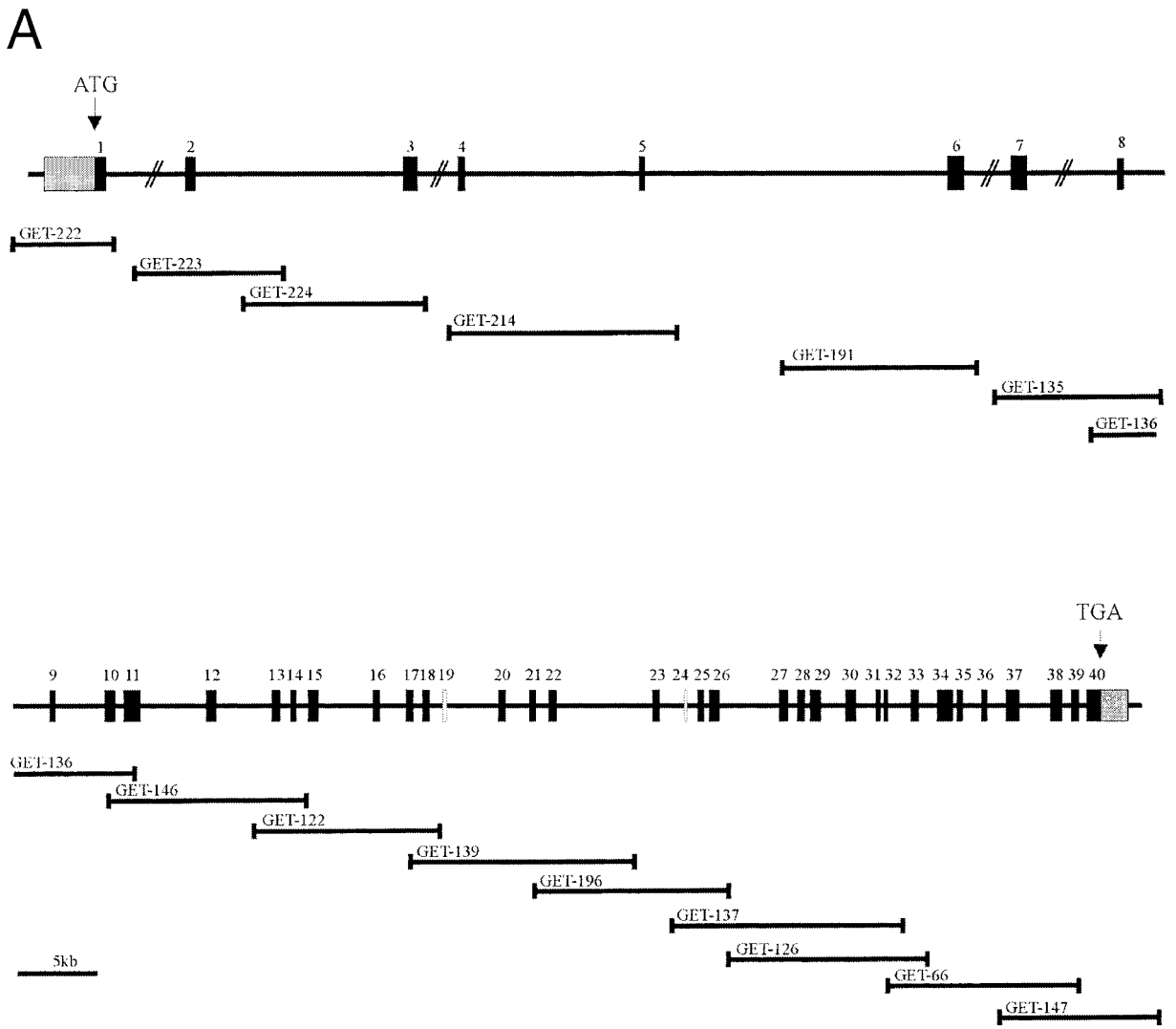
TABLE 1

Exon No.	3′ Splice acceptor	5′ End of exon	Exon size (bp)	3′ End of exon	5′ Splice donor	Intron size (kb)
1			95	CGGCCGTCAC	**gt**aagtcggc	nd
2	atttctgc**ag**	TATCAAATCA	82	GCTTGTTGAT	**gt**gagtaaaa	14[a]
3	tttcacac**ag**	ATTTATGAGA	117	ATCTAAAGCC	**gt**gagtttaa	nd
4	tgtttttc**ag**	GCCTGGCATT	59	AGATTTTGCA	**gt**aagaaccc	10[a]
5	ttttttgt**ag**	AGCAATGAAG	42	TGATCTCGAT	**gt**gagtatta	18[a]
6	ttctatat**ag**	CCTGAGAAAA	130	TATGAGGGCT	**gt**aagtaaaa	nd
7	tttttgtt**ag**	CAACAATTGT	132	TATTATCCAG	**gt**aggtgctt	nd
8	tttttttt**ag**	CTTCACCATG	70	GAAGACCATG	**gt**aagaattt	2.3[a]
9	tttgtttc**ag**	GTACATCCAA	51	TGGTGGATGT	**gt**gagttgtt	4.0[a]
10	ctatttct**ag**	GAGTGGAAGT	100	TGTAGCTTCA	**gt**aagtataa	0.7[a]
11	tcttttgc**ag**	TTTAACAGCA	159	GCTGCTTAAT	**gt**aagtatca	5.7[a]
12	ttttaac**ag**	TATAATGTTT	105	AGATAAAAAA	**gt**gagtgtaa	4.2[a]
13	attcatcc**ag**	GTACGTGTAT	79	GAAAACAAAG	**gt**aacaaatc	1.1[a]
14	tatatttc**ag**	GTTATTATTA	50	CAATACTCAG	**gt**atgattat	1.5[a]
15	ccataaac**ag**	GAATATTTGG	90	GGATGCATTG	**gt**aagaggtt	4.6[a]
16	ctatttttt**ag**	GAACTGGGAC	78	AAACTTAAAG	**gt**taaaagtt	2.1[a]
17	cttattat**ag**	AACCAGCTGA	75	ACGTTTTACA	**gt**aagatgaa	0.3
18	tcttttcc**ag**	CTGTGCCCCA	75	TCAGCCAAAG	**gt**cagtatat	0.9[a]
19	gttgcttc**ag**	CCTATTGGTG	57	CAATATCCAG	**gt**aactgtga	4.6[a]
20	ttctgtgt**ag**	AACCCCAAAT	72	TAAAGTGGAG	**gt**gagtggaa	2.4[a]
21	ttactctt**ag**	ATTCGAAATA	72	TCAAGATGAG	**gt**aagaattg	1.2[a]
22	ttctttct**ag**	AGATATATTG	62	CAGATTACAG	**gt**aattaact	6.4[a]
23	tctattgc**ag**	TTTGGCCTTG	77	CAGGCCAGAT	**gt**aagtactg	1.5[a]
24	attttcac**ag**	CAAAAAAGGG	21	AAAATGAAGG	**gc**aagtattt	0.7
25	atctttgc**ag**	ATTCGGAAAC	61	TAGCACCAAG	**gt**tggttttg	0.7[a]
26	ttttttc**ag**	AGATTACTGC	98	AACCCATCAT	**gt**aagtatgc	3.0[a]
27	tctgtgtt**ag**	GTAACGCGGA	88	AGAAAAATAT	**gt**aagtattt	1.3[a]
28	tctgtttc**ag**	CAAGGGAGTG	63	ATCCCAAAGA	**gt**aagttcaa	0.9[a]
29	tcctcctt**ag**	GGCTGGAGAA	104	TACTTTAACA	**gt**acgtattg	1.2[a]
30	tttttttt**ag**	AAAGTGGACC	87	AAACCTGCAG	**gt**aaggataa	1.6[a]
31	aatttttc**ag**	TTGTTGGAAT	68	CAGAGATCCG	**gt**aagatatt	0.4
32	tgtctttc**ag**	TGTGCTGGTC	39	AAACAGTGAC	**gt**aagaaaac	1.6[a]
33	attttgat**ag**	GTAATGGATT	72	TACTAATCAG	**gt**atgtacct	1.5[a]
34	tcttcacc**ag**	ATTGGAAGAT	153	AGCATATGTG	**gt**cagtaata	0.1
35	tattttat**ag**	CCATCAGTAG	53	CTGCTGCCTG	**gt**aagtcaaa	1.7[a]
36	aaaaattc**ag**	GTCTATTCTA	56	CTTGAGGCAG	**gt**atgtaaat	0.9[a]
37	gccacccc**ag**	TTGAGATGGA	130	ACTGTTCCAG	**gt**aatttatt	2.3[a]
38	tattttgc**ag**	AATCTTTCAT	110	GAGCAGACTT	**gt**atcctttg	10.5
39	tctcttttt**ag**	CTGACGGTCC	83	CAATGTCTTG	**gt**aagatcac	
40	ttaacaac**ag**	GAGGATTATA	117			

Note. Exon sequences are indicated as uppercase letters, and intron sequences are in lowercase letters.
[a] Approximate length was determined by PCR and restriction digestion mapping.

tides derived from the gene and comparison of the genomic sequence to the *CACNA2* cDNA sequence. Splice site consensus junctions were identified for each exon/intron junction except for intron 24, which is unusual in that the normally invariant GT dinucleotide is replaced by GC. *In vitro* studies of the 5′ splice donor junctions showed that the substitution of the GT dinucleotide by GC is the only one that allows the 5′ site to be accurately cleaved, albeit more slowly than the usual GT sequence (Aebi *et al.*, 1987).

The genomic sequences corresponding to the intron/exon boundaries are presented in Table 1. *CACNA2* is distributed over at least 150 kb of genomic DNA. It is encoded by 40 exons with very large introns in the 5′ region between exons 1 and 8. Exons range from 21 to 159 bp in size. The ATG start codon and the signal peptide are included in exon 1. Exon 40 contains the termination codon followed by 494 bp of the 3′ untranslated region where no adenylation signal was found. Exons 37–40 encode the putative δ-part, which is post-transcriptionally cleaved from the α-portion of the protein. The membrane-spanning part of the δ-portion is encoded by a single exon (exon 40). The *CACNA2* gene contains two sequences that can be alternatively spliced (Kim *et al.*, 1992). In our analysis we found that both were distinct exons (see Fig. 1b). We have included them both in our gene structure map as exons 19 (57 bp) and 24 (21 bp). One of the alternatively spliced exons was not accounted for in the original human *CACNA2* cDNA (Williams *et al.*, 1992). We have added these 19 amino acids, which are encoded by exon 19 and are inserted after Lys-510 only in the skeletal muscle splice product. The sizes of intervening introns were determined either by restriction enzyme mapping or by PCR amplification of genomic DNA clones using intron spanning primers. In some cases



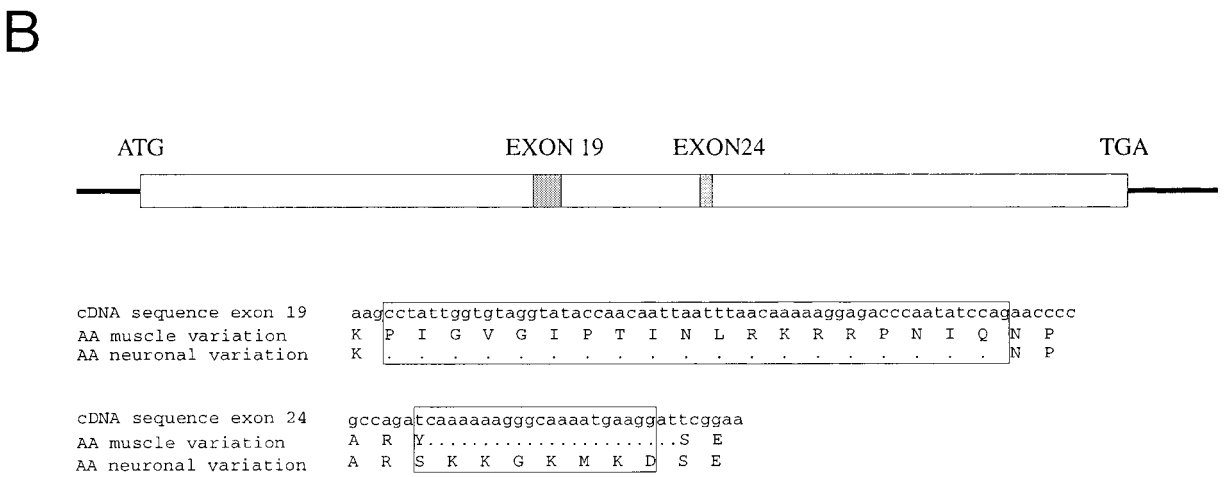


FIG. 1. (**A**) Genomic organization of the human CACN2A gene. The location of exons is shown with respect to the size of the introns. The exact sizes of the first and last exons are not known. Position of exons is shown by numbered filled boxes. Open rectangles indicate alternative spliced exons 19 and 24. Hatched boxes represent partially sequenced exons containing 5′ and 3′ untranslated sequences. Heavy lines represent the relative position of studied genomic phage clones (GET). (**B**) (**Top**) Localization of the two differentially spliced exons. (**Bottom**) cDNA sequence and deduced amino acid sequence of the two exons.

TABLE 2

Revised Nucleotide and Amino Acid Sequences

Original	Correction	Change	AA Position[a]
Ser	(AGC) to Arg (CGC)	Base substitution	99
Arg	(AGG) to Thr (AGC)	Base substitution	386
Glu	(GAG) to Asp (GAC)	Base substitution	395
Arg	(CGG) to Arg (CGC)	Base substitution	1900
Pro	(CCT) to Pro (CCC)	Polymorphism	1038

[a] Amino acid positions with respect to published sequence (Accession No. M76559).

intron length could not be detected due to extended size.

Screening for Mutations and Isolation of cDNA

Knowledge of the intron sequences flanking the exons enabled us to develop primer pairs for amplification of exons from genomic DNA from patients of the MHS family linked to the above-mentioned region on chromosome 7q (Iles *et al.*, 1994). We analyzed both the DNA of the patient who had suffered from the MH crisis and that of additional affected members of this family. However, so far no mutation has been detected in *CACNA2*. Sequence analysis revealed neither differences in the coding region of the gene nor differences in sequences flanking exon/intron boundaries. We could not detect any sequence alteration in the first 300 bases of the putative promoter region. The complete promoter region remains to be analyzed, and the possibility of an intronic mutation must be considered. Our sequence analysis identified errors in the published (Accession No. M76559) human *CACNA2* cDNA (these are corrected in Table 2), which altered amino acid residues but did not affect numbering. Furthermore, a silent mutation at nucleotide position 1038 (a T to C transition in exon 39) was found that did not segregate with the MH phenotype in the family. This nucleotide transition affects the third base of a proline codon. The change eliminates a *Bfa*I site and can be used as an intragenic polymorphism. It can easily be detected by *Bfa*I digestion of the PCR product obtained from amplification of exon 39 with flanking primers.

Electrophysiological Characterization of the Gene Product

For expression studies we inserted the full-length muscle *CACNA2* cDNA into the pcDNA3 vector. The human skeletal muscle α_2 cDNA (human α_{2A}) when coexpressed in HEK293 cells with the brain-specific α_{1A} subunit did not lead to measurable currents. Yet the addition of β_4 subunits led to quite large ion currents of greater than 2 nA (Fig. 2). The mean current density was 65 pA/pF $\pm$ 18 (n = 12). For comparison, the combination of α_{1A}, β_4, and an α_2 from rabbit brain ($=\alpha_{2B}$) resulted in a significantly lower current density of 41 pA/pF $\pm$ 9 (n = 32). In contrast, after transfection of the cells with the cDNA of the α_{1A} and β subunit without an α_2 subunit the mean current density amounted to 3.4 pA/pF, and the expression efficiency was low: less than 10% of the transfected cells showed calcium currents when tested. Coexpression of α_{1A}, β_4, and α_{2A} subunits raised the expression rate to about 25%.

The α_{2A} subunits influenced the voltage dependence of activation and inactivation. They induced a shift in hyperpolarizing direction in activation (7 mV) and inactivation (4 mV) (see Table 3; Fig. 3), but the change in current inactivation was not statistically significant. We further looked at the gating-related channel characteristics. The addition of the α_{2A} subunits accelerated the inactivation kinetics. To analyze this action in detail, we estimated the time course of the voltage-dependent inactivation of the current during a 2-s activating pulse to +20 mV by fitting the decaying current with a 2-exponential equation. A significant difference was seen on the slow component of the decay. α_{2A} reduced the time constant τ_2 by 34% (Table 3).

DISCUSSION

A growing number of diseases are recognized as being caused by mutations in genes encoding voltage-gated ion channels of skeletal muscle (Hoffman *et al.*, 1995). Naturally occurring mutations in channel genes or antibodies directed against channel proteins have also been found for other excitable tissues such as the heart and the brain as well as unexcitable organs such as the kidney (Greenberg, 1997; Lehmann-Horn and Jurkat-Rott, 1999). Mutations in calcium channels or channel subunits cause malignant hyperthermia susceptibility types 1 and 5 (Monnier *et al.*, 1997; Manning *et al.*, 1998), hypokalemic periodic paralysis, and various forms of hemiplegic migraine, episodic ataxias, and epilepsies (Jurkat-Rott *et al.*, 1994; Ptacek *et al.*, 1994; Terwindt *et al.*, 1998).

In a large German pedigree, malignant hyperthermia susceptibility (MHS type 3) was found to be tightly linked to the gene on chromosome 7q encoding the $\alpha 2/\delta$ subunit of voltage-gated calcium channels (Iles *et al.*, 1994; McKusick, 1997). To perform a mutation screening with MH3 susceptible individuals, we performed a detailed analysis of the sequence and genomic structure of the CACNA2 gene. Yet, in the MH patients we tested up to now, we did not identify a mutation in the coding region of *CACNA2* or in sequences flanking the exon/intron boundaries. Still, the promoter region remains to be analyzed, and the possibility of an intronic mutation must be considered. Independent of the molecular genetic results for this family, *CACNA2* remains a prime candidate for MH for susceptible individuals of other families that show no mutation in the RYR1 or the calcium channel α_1 subunit of skeletal muscle. The genomic structure reported here allows for improved screening for mutations in those cases of

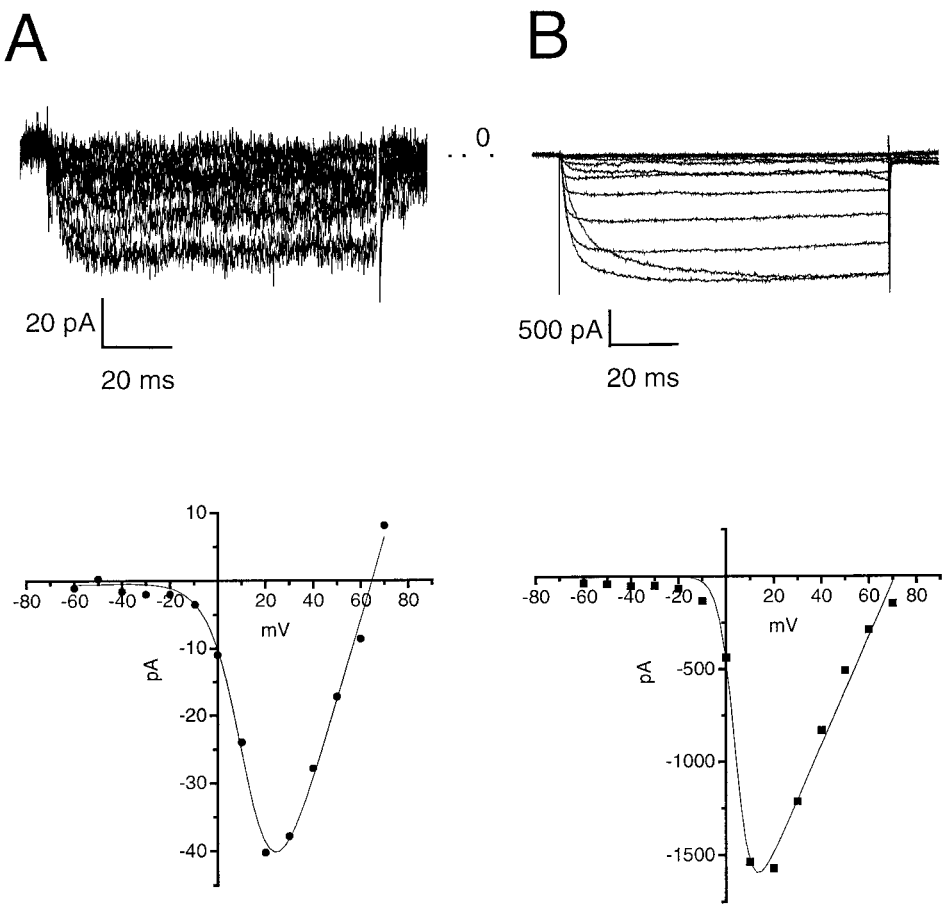


FIG. 2. Expression of ionic current in HEK 293 cells transfected with α_{1A}, β_4 and α_{1A}, β_4, α_{2A} subunits. Representative current traces in response to a series of voltage steps (−60 mV to +70 mV in 10-mV increments) and current voltage relation. (**A**) Cells transfected with α_{1A}, β_4. (**B**) Cells transfected with α_{1A}, β_4, and α_{2A}. The current voltage data were fitted by a modified Boltzmann function: current $I = G^*(V - E_0)/\{1 + \exp[(V_{0.5} - V)/k]\} + V^*G_{leak} + I_{off}$, with $V_{0.5}$ as midpoint voltage, k as slope factor, and G_{leak} and I_{off} as correction factors for leak and offset.

other diseases that are caused by an altered cell excitability, particularly where no mRNA will be available.

The expression studies were intended to prove the functional integrity of our isolated gene product and to test for the modifying capabilities of the α_{2A} subunit on calcium currents. The skeletal muscle variant of the voltage-gated calcium channel consisting of the pore-forming subunit α_{1S} cannot be properly expressed in heterologous expression systems. Therefore we used the combination of our isolated form α_{2A} with the brain-specific type α_{1A} and β_4 subunits for our expression studies, thus forming P/Q-type calcium channels. The physiological properties of the channels (P- or Q-type) hereby are determined by the type of the auxiliary subunits and the type of splice variant of the α_{1A} subunit. With our combination we obtained currents that were similar to the Q-type. The characterization of the α_2 influence on this brain-specific type of calcium channel may contribute to an understanding of how aberrant α_2 subunits may induce neurological disorders.

The most obvious manifestation of the interaction of α_2 with α_{1A} subunits was the expression of high-amplitude currents in the transfected cells. Even though the α_{1A} subunits theoretically should be able

TABLE 3

Effects of the Human α_{2A} Subunit on the Biophysical Properties of the α_{1A} Currents

Properties	α_{1A}/β_4	$\alpha_{1A}/\beta_4/\alpha_{2A}$
Peak current (pA)	65 ± 18 (16)	810 ± 78 (12)*
Potential for half-activation (mV)	19 ± 2 (18)	11 ± 0.9 (32)*
Potential for steady-state half-inactivation (mV)	−4 ± 5 (6)	−8 ± 1.2 (13)
Time constant of decay τ_1 (ms)	244 ± 38 (6)	217 ± 30 (12)
Time constant of decay τ_2 (ms)	2640 ± 190 (6)	1750 ± 157 (12)*

Note. Numbers of experiments in parentheses.
* Significant differences ($P < 0.05$).

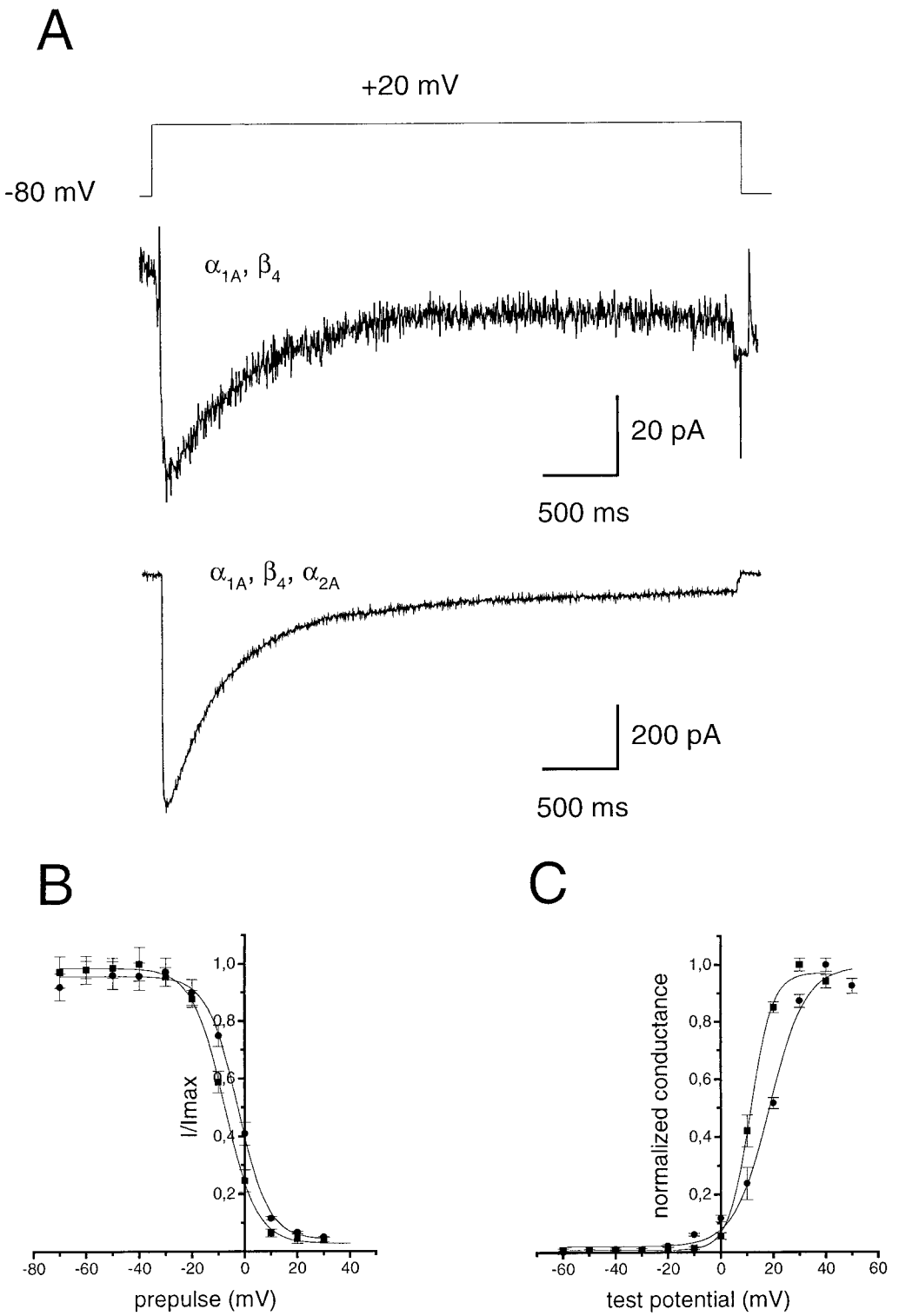


FIG. 3. Effects of α_{2A} subunits on currents through α_{1A}-type channels. (**A**) Currents evoked by a depolarizing pulse to +20 mV (upper trace) of a cell transfected with α_{1A}, β_4 (middle trace) and α_{1A}, β_4 and α_{2A} (lower trace). (**B**) Steady-state inactivation. The steady-state inactivation was determined by examining the amplitude of the current elicited by a test pulse to 20 mV after prepulses to various potentials. The data were fitted by a Boltzmann function: $I/I_{max} = 1/\{1 + \exp[(V_{hold} - V_{0.5})/k]\}$. $V_{0.5}$ was estimated as −2.4 and −5.6 mV; the slope factor k = 5.5 and 3.5, respectively. (**C**) Conductance plot. The conductance (**G**) was calculated according to $G(V) = I_v/(V - V_{rev})$, where I_v is the peak I_{Ca} as a function of the test potential, and V_{rev} is the reversal potential. Results of fitting procedures are summarized in Table 2. The symbols in A and B correspond to data for α_{1A}, β_4 (●) and α_{1A}, β_4, α_{2A} (■).

to form a functional ion pore, in expression systems the accessory subunits α_2 and β are necessary to form a population of channels in the cell membrane that develop measurable currents. There is only one report on the expression of reasonable amounts of "pure α_{1A}" current in *Xenopus* oocytes after the injection of very large amounts of cRNA (De Waard and Campbell, 1995).

In the combination α_{1A} plus β, the currents were barely measurable, and in accordance with previous reports (Mori *et al.*, 1991), we were not able to record any current on α_{1A} plus α_2 transfected cells without an additional β subunit. We suppose that the effect of current enhancement by the α_2 subunit, in our system by a factor of almost 20, is analogous to cardiac L- and neuronal E-type calcium channels where it has been shown that the auxiliary subunits facilitate membrane insertion (Gao *et al.*, 1999). In those combinations it also has been shown that the gating characteristics of the channels are modified (Bangalore *et al.*, 1996; Shistik *et al.*, 1995; Qin *et al.*, 1998). In our case, the interaction of the α_{2A} with α_{1A} subunits led to a hyperpolarizing shift in activation and steady-state inactivation as well as in an acceleration of the inactivation kinetics. In sum, the regulatory action of the α_{2A} subunit results in a gain of function of the channels, a drastic increase in current amplitudes, and an activation threshold at lower voltages.

The marked influence of the α_2 subunit on channel properties makes it plausible that even minor changes in the α_2 subunit affecting the interaction with the α_1 subunit could lead to abnormal channel function and disease. The functional interaction of the α_2 subunit with the pore forming unit is also underscored by the fact that the anticonvulsant drug gabapentin exerts its pharmacological action by binding to the α_2 subunit (Gee *et al.*, 1996).

In addition to its effects on high-voltage-activated calcium channels, it seems that the α_2 subunit has a significant influence on the low-voltage-activated T-type currents (Wyatt *et al.*, 1998). The overexpression of α_2 modified the properties of different native voltage-gated calcium channels including T-type channels, whereas the β subunits had only minor effects, supporting the hypothesis that native T-type channels are associated with α_2. T-type channels are important in heart and are also found in sensory neurons and in brain, e.g., cerebellar Purkinje cells or thalamic neurons, where they are involved in neuronal firing regulation. An involvement of T-type channels has also been proposed in mechanisms of genetic forms of epilepsy, e.g., absence epilepsy (Futatsugi and Riviello, 1998). Therefore even if no mutations during the screening of MHS patients could be found in the *CACNA2* gene, the gene still should be considered as a candidate for other muscular or neuronal inherited diseases.

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