Specific Properties of T-type Calcium Channels Generated by the Human α1I Subunit*

Received for publication, February 10, 2000, and in revised form, March 30, 2000
Published, JBC Papers in Press, April 3, 2000, DOI 10.1074/jbc.C000090200

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We have cloned and expressed a human α1I subunit that encodes a subtype of T-type calcium channels. The predicted protein is 95% homologous to its rat counterpart but has a distinct COOH-terminal region. Its mRNA is detected almost exclusively in the human brain, as well as in adrenal and thyroid glands. Calcium currents generated by the functional expression of human α1I and α1G subunits in HEK-293 cells were compared. The α1I current activated and inactivated ~10 mV more positively. Activation and inactivation kinetics were up to six times slower, while deactivation kinetics was faster. The α1I subunit encodes T-type Ca2+ channels functionally distinct from those generated by the human α1G or α1M subunits and point out that human and rat α1I subunits have species-specific properties not only in their primary sequence, but also in their expression profile and electrophysiological behavior.

Voltage-dependent calcium channels control the rapid entry of Ca2+ ions into a wide variety of cell types and are therefore involved in both electrical and cellular signaling. Electrophysiological studies have identified two major Ca2+ channel types as high voltage-activated and low voltage-activated channels (1, 2) with this latter class being also identified as T-type Ca2+ channels (3). T-type Ca2+ channels were originally defined by their activation at low membrane potential, their fast time course, and their small single channel conductance (4, 5). These channels have been identified on a large variety of neurons, and it has become obvious that significant functional diversity exists in the gating behavior of T-type channels, particularly in inactivation kinetics, voltage dependence of steady-state inactivation, and pharmacology (6). The recent identification of several novel genes encoding a subset of homologous Ca2+ channel α1 subunits, e.g. the α1G subunit (7–9), the α1H subunit (10, 11), and the rat α1I subunit (12), has revealed that diversity of T-type voltage-dependent calcium channels is primarily related to the expression of distinct α1 subunits. Indeed, the expression of the various α1G and α1I subunits (7–12) produces Ca2+ currents with the typical signature of T-type channels but with specific features, such as the block by Ni2+, which discriminates between α1G and α1I currents (13). By contrast, the biophysical properties of T-type channels generated by the α1I subunit markedly differ from those made of α1G and α1H subunits (12, 14, 15), and it was postulated that the α1I subunit is responsible for native “slow” T-type currents observed in rat thalamic neurons (16). To date the α1I subunit has only been cloned from rat (12) and whether the α1I subunit encodes an atypical T-type Ca2+ channel certainly needs further investigation. We have addressed this issue by characterizing a novel α1I subunit. We have cloned and functionally expressed a full-length cDNA encoding the human α1I subunit. The properties of the Ca2+ currents generated by the human α1I subunit were compared with a neuronal isoform of the human α1G subunit cloned recently, α1G-A4 (9). The data also described several species-specific properties of the human α1I channels.

MATERIALS AND METHODS

Cloning of a Complete cDNA for Human α1I Subunit—An initial search of genetic data bases for cDNAs weakly homologous to α1I subunit cDNAs encoding high voltage-activated channels (9) identified several sequences homologous to the Caenorhabditis elegans gene c54d2.5 (GenBank™ accession number U37548), including three expressed sequence-tagged clones, H55225, H55223, and H55617 from human chromosome 22 (17). In the mean time, a genomic sequence covering human chromosome 22q12.3-q13.2 (GenBank™ accession number AL008716) was sequenced and released by the Sanger Center (Cambridge, United Kingdom), and more recently, a cDNA encoding the rat α1I subunit was described by the group of Perez-Reyes (Ref. 12, GenBank™ accession number AF088627). The partial intron/exon structure of the CACNA1I gene encoding the human α1I subunit was determined using the GRAIL software (18). Alignment of the rat cDNA with GenBank™ accession number AF088627 was then performed to identify more precisely the putative human α1I cDNA coding region and to design PCR primers for cDNA cloning. Pairs of primers were designed to amplify the entire coding region of the α1I subunit cDNA in five partial fragments designed PCR-I1 to PCR-I5 (PCR-I1: sense, 5′-TCTATGTTGAGACATGGCTGAG-3′; antisense, 5′-CCATATTACCATTCATGCCC-3′; PCR-I2: sense, 5′-ATGGGAGTGTCATCACATCATC-3′; antisense, 5′-AGTACCTGTGCTGCCAGGTCAC-3′; PCR-I3: sense, 5′-CCTCCCTGGAATAATGATCAG-3′; antisense, 5′-CCTCCCTGGAATAATGATCAG-3′; PCR-I4: sense, 5′-CCATATTACCATTCATGCCC-3′; antisense, 5′-CCTCCCTGGAATAATGATCAG-3′; PCR-I5: sense, 5′-CCTCCCTGGAATAATGATCAG-3′; antisense, 5′-TTGAGCTCCTGCCTTGGGCCTCC-3′; see Fig. 1A). Reverse transcription (RT)-PCR protocols were performed using mRNA from human total brain

* This work was supported in part by the Program Génôme du CNRS, Association pour la Recherche contre le Cancer (number ARC0911), Association Française contre les Myopathies (AFM). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ Supported by Prodict Roche (France) and the Groupe de Réflexion sur la Recherche Cardiovasculaire (GRRC).

§ Supported by the Programme Génome du CNRS, Association pour la Recherche contre le Cancer (number ARC 0911), Association Française contre les Myopathies (AFM). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF211189.

1 The abbreviations used are: PCR, polymerase chain reaction; RT, reverse transcription; aa, amino acid(s); bp, base pair(s); kb, kilobase(s); nt, nucleotide(s); TEA, tetraethylammonium; S, siemens.
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(CLONTECH). Nucleotide sequences were determined using automatic sequencing (Applied Biosystems) using dye terminator. A full-length cDNA encoding the human α1I subunit was constructed using the restriction sites mentioned in Fig. 1A and subsequently cloned into the mammalian expression vector pBCK-CMV (Stratagene). Sequence comparison of this α1I subunit with α1 subunits was performed with BESTFIT (Genetics Computer Group) multialignment software.

**Northern and Dot Blot Analyses—**Commercial human Northern and dot blot membranes (CLONTECH) were hybridized using a 321-bp fragment (nt 256 to nt 577) generated by RT-PCR amplification (sense, 5'-ATGCTGCTCTGCTGCTGCAAG-3'; antisense, 5'-GAATGCAGTTGATGGCCTTGGAG-3') from human brain mRNA (CLONTECH) and random-primed with [32P]dCTP. The membranes were treated according to the manufacturer's protocol, as reported previously (9). The exposure time for Northern and dot blot membranes was 6 days at −80 °C. Densitometric analysis of the autoradiograms was performed using an imaging analyzer system (Alpha Innotech Corp.) to provide semiquantitation of α1I mRNA in each condition and comparison with the α1D mRNA signal (9). No signal was observed for internal DNA controls.

**Transient Expression Experiments and Electrophysiology—** Transfection experiments were performed with the pBCK-CMV construct that encodes for the α1I subunit, cotransfected with a plasmid encoding the reporter gene CD8 or GFP, in a 10:1 ratio. Human embryonic kidney cells (HEK 293) were grown in Dulbecco's modified Eagle's medium (Eurobio) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (v/v). Cells were transfected using a calcium phosphate precipitation procedure when needed. Extracellular solutions were 120 mM NaCl, 2.7 mM CaCl2, 12 mM KCl, 1 mM MgCl2, 4.7 mM glucose, and 0.8 mM Na2HPO4, pH 7.2 with TEAOH. Membrane potential was reduced toward 0 mV by bathing the cells in a high potassium solution containing (in mM): 140 potassium gluconate, 10 EGTA, 10 glucose, 1 MgCl2, 10 HEPES (pH to 7.2 with KOH). The sampling frequency for acquisition was 10 kHz, and data were filtered at 1 kHz. Data were analyzed using pCLAMP6, Excel (Microsoft), and GraphPad Prism (GraphPad Inc.) software. Activation and inactivation curves were fitted with a Boltzmann equation. Apparent dissociation constants (Kd) and for the transmembrane domains (white box) and complete proteins (black box) were indicated by the position of the three expressed sequence tag clones that were sequenced (GenBankTM accession number AF086827 (12)), as well as to the human α1G (GenBankTM accession number AF126965 (9)) and α1H (GenBankTM accession number AF051946 (10); GenBankTM accession number AF073931 (11)) subunits. The position of the five partial cDNA fragments isolated by RT-PCR and named PCRI1-I5 (see “Materials and Methods”). The percentage of homology calculated for the complete sequence. This percentage number for the transmembrane domains (white box) and complete proteins (black box) were indicated by the position of the three expressed sequence tag clones that were sequenced (GenBankTM accession number AF086827 (12)), as well as to the human α1G (GenBankTM accession number AF126965 (9)) and α1H (GenBankTM accession number AF051946 (10); GenBankTM accession number AF073931 (11)) subunits. The position of the five partial cDNA fragments isolated by RT-PCR and named PCRI1-I5 (see “Materials and Methods”). The percentage of homology calculated for the complete sequence.

**RESULTS AND DISCUSSION**

A putative cDNA encoding the human α1I subunit was predicted from the genomic sequence of chromosome 22q12.3-q13.2 (GenBankTM accession number AL008716), and the partial intron/exon structure of the human gene encoding the α1I subunit, CACNAI1, was identified (19, 20). Five pairs of primers were designed to clone overlapping cDNA fragments that covered its complete coding sequence. The five partial cDNA clones were obtained by using RT-PCR with human total brain mRNA and subsequently sequenced and assembled (Fig. 1A). Finally, the full-length cDNA encoding the α1I subunit was sequenced (GenBankTM accession number AF211189) and was cloned into the pBCK-CMV expression vector. The human α1I subunit has an open reading frame of 5943 bp and encodes a protein of 1981 aa (calculated molecular mass of 220,747 Da). It is highly homologous to the rat α1I subunit (GenBankTM accession number AF073931 (11)), as well as to the human α1G (GenBankTM accession number AF126965 (9)) and α1H (GenBankTM accession number AF051946 (10); GenBankTM accession number AF073931 (11)) subunits.

**TABLE I.**

| Subunit | Hum. α1I | Hum. α1G | Hum. α1H | Rat α1I | Cytoplasmic Loop |
|---------|----------|----------|----------|---------|-----------------|
| α1I     | 63.7     | 64.7     | 64.9     | 63.9    | 75.8            |
| α1G     | 59.2     | 64.0     | 63.9     | 64.9    | 75.8            |
| α1H     | 63.7     | 64.7     | 64.9     | 63.9    | 75.8            |

**FIG. 1.** Cloning and molecular properties of the human α1I subunit. A, the cloning strategy is illustrated (see “Materials and Methods”). The percentage of homology calculated for the complete sequence. This percentage number for the transmembrane domains (white box) and complete proteins (black box) were indicated by the position of the three expressed sequence tag clones that were sequenced (GenBankTM accession number AF086827 (12)), as well as to the human α1G (GenBankTM accession number AF126965 (9)) and α1H (GenBankTM accession number AF051946 (10); GenBankTM accession number AF073931 (11)) subunits. The position of the five partial cDNA fragments isolated by RT-PCR and named PCRI1-I5 (see “Materials and Methods”). The percentage of homology calculated for the complete sequence.
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1720–1736) was not retrieved in the human subunit, both in our cDNA cloning experiments and from the analysis of the human genomic sequence. Second, the rat $\alpha_{II}$ subunit was described with a rather short COOH-terminal region, as compared with other T-type $\alpha$ subunits (12). Here we describe that COOH-terminal region of the human $\alpha_{II}$ subunit is 158 aa longer. The stop codon identified by Lee et al. (12) is likely to shorten prematurely the rat $\alpha_{II}$ protein, since pairwise alignment of the human and rat cDNAs can predict a novel 3′-end for the rat $\alpha_{II}$ cDNA sequence, which is highly homologous to the human cDNA. The corrected rat sequence was obtained by cDNA cloning and/or sequencing of the 3′ end of this corrected rat sequence most likely indicates the position of each mRNA and/or sequencing of the 3′-end sequence of the rat $\alpha_{II}$ cDNA should be performed again before further investigations.

Northern blot and dot blot analyses of a large variety of human tissues showed that $\alpha_{II}$ mRNA is almost exclusively present in the brain (Fig. 2). The $\alpha_{II}$ mRNA was found as a single band of approximately 10.5 kb (Fig. 2, A and B). By contrast, the rat $\alpha_{II}$ mRNA was detected as at least two bands (10.5 and 8 kb) in several tissues, including brain, kidney, and liver (12). A complementary analysis of the $\alpha_{II}$ mRNA expression profile was performed using a dot blot membrane, which confirmed the lack of $\alpha_{II}$ expression in human peripheral tissues with the exception of adrenal and thyroid glands (Fig. 2C). In addition, it is worth noting that in brain the intensity for $\alpha_{II}$ signal in fetal tissue is ~50% higher than for adult brain (compare A1 and G1; Fig. 2, C and D). Such a difference was not retrieved for the human $\alpha_{II}$ mRNA signal in identical experimental conditions (9).

Expression of human $\alpha_{II}$ channels was performed in HEK-293 cells, and the properties of the resulting current ($\alpha_{II}$ current) were compared with those of the current generated by the human $\alpha_{II}$ subunit, in the presence of 2 mM $Ca^{2+}$ (Fig. 3). Isoform “a” is the major isoform of the $\alpha_{II}$ subunit expressed in the human brain and differs from the $\alpha_{IIb}$ isoform described in Monteil et al. (9) in its cytoplasmic III-IV loop. Superimposition of $\alpha_{II}$ current traces obtained for depolarizing pulses from −90 to +50 mV (holding potential −110 mV) revealed an activation at low voltages (−60 mV) and a crossing over of the current traces (Fig. 3A) that are typical features of T-type currents (21).

Peak of the normalized current-voltage curve for $\alpha_{II}$ current (n = 12) was obtained near −24 mV, while for $\alpha_{II}$ current, the peak of the normalized current-voltage relationships was 12 mV more negative (Fig. 3B; Table I). The conductance-voltage curves (Fig. 3C) were then deduced from each current-voltage relationships to estimate the activation parameters. For $\alpha_{II}$ and $\alpha_{II}$ currents, the potentials for half-maximal activation ($V_{0.5}$) were −40 mV and −51 mV, respectively, (Table I). Steady-state inactivation properties for $\alpha_{II}$ and $\alpha_{II}$ currents (Fig. 3C) were determined by potentials for half-inactivation ($V_{0.5}$) of −69 mV for $\alpha_{II}$ currents (n = 8) and −75 mV for $\alpha_{II}$ currents (n = 5) that were significantly different (Table I). For $\alpha_{II}$ current, similar steady-state inactivation properties were obtained ($V_{0.5}$ = −70 mV; n = 5) when using a 15-s prepulse duration. These data demonstrate that the human $\alpha_{II}$ subunit generate channels with significant differences in steady-state activation and inactivation properties as compared with the channels made of $\alpha_{II}$ subunit, with activation and inactivation $V_{0.5}$ values more positive by at least 11 and 5 mV, respectively. As a consequence of its steady-state activation and inactivation properties, the $\alpha_{II}$ subunit can generate a window current at membrane potentials in the range of −60 / −50 mV, i.e. −10 mV more positive than the one related to the $\alpha_{II}$ subunit (−70 / −55 mV). Considering the expression profile of the $\alpha_{II}$ subunit, this channel could therefore regulate intracellular $Ca^{2+}$ concentrations at resting membrane potential in endocrine tissues where it could play a role in hormone secretion (22), as well as in neuronal tissues for the induction of burst firing (5).

Activation and inactivation kinetics were also markedly different for the $\alpha_{II}$ and $\alpha_{II}$ currents (Fig. 3D). For $\alpha_{II}$ current, activation kinetics was voltage-dependent with a 10–90% rise time ranging from 65 to 8 ms for potentials between −50 and +30 mV (Fig. 3E), i.e. ~8-fold slower than $\alpha_{II}$ current (7.6 to 1 ms). Inactivation kinetics for $\alpha_{II}$ current was also strongly

Fig. 2. Northern and dot blot analyses. A 321-bp fragment covering the domain I of the human $\alpha_{II}$ cDNA was used to probe from a variety of tissues on commercial membranes (see “Materials and Methods”). A and B, Northern blot membranes that contain approximately 2 μg of poly(A)−RNA per lane are used here as described under “Materials and Methods.” Internal controls were performed using two probes, ubiquitin and β-actin (9). Considering the panel of tissues, expression was detected only in brain (A). For neuronal tissues (B), expression was observed in most of the structures. The stronger signals were obtained for occipital and frontal lobes, cerebellum, caudate nucleus, and amygdala. The fainter signals were observed for spinal cord, corpus callosum, thalamus, substantia nigra, lateral geniculate body, hypothalamus, and medulla oblongata. C and D, the position of each mRNA sample on the membrane is provided by the manufacturer. The dot blot membrane, for which the normalized amount of poly(A) RNA per dot ranges from 100 to 500 μg, shows specific signals for several neuronal tissues, including whole brain (A1; amygdala, A2; caudate nucleus, A3; cerebellum, A4; cerebral cortex, A5; frontal lobe, A6; hippocampus, A7; occipital lobe, B1; putamen, B2; substantia nigra, B3, temporal lobe, B4; nucleus accumbens, B6; fetal brain, G1. The only peripheral tissues positive for $\alpha_{II}$ mRNA were the adrenal gland, D5, and the thyroid gland, D6. Note that the thalamic structures showed faint signals for $\alpha_{II}$ both in Northern blot (B, right panel) and in dot blot (thalamus, B5), contrasting with the strong signals identified in these lines and dots for the $\alpha_{II}$ mRNA (9). No signal was obtained for the cardiac, smooth, and skeletal muscles (C1–C8), as well as other peripheral tissues, including testis, ovary, and pancreas (D1–D9), pituitary gland (D4), salivary gland (D7), mammary gland (D8), kidney (E1), liver (E2), small intestine (E3), spleen (E4), thymus (E5), peripheral leukocytes (E6), lymph node (E7), bone marrow (E8). The RNA/DNA controls in row H: yeast RNA (H1–H2), Escherichia coli rRNA (H3), E. coli DNA (H4), and human genomic DNA (H7 and H8) were also negative.
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Molecular identification of the human α1I and α1G subunits as expressed in HEK-293 cells provide compelling evidence that the diversity of T-type channel activity in native neurons (6) is primarily related to the expression of distinct subunits. Although these two channel proteins are highly homologous and do not display striking differences in their primary sequence, we have demonstrated here that they do not display striking differences in their kinetic properties. For α1I and α1G subunits that generate currents with significant difference in their activation and inactivation kinetics and steady-state properties, as well as in their deactivation kinetics and recovery from inactivation, should offer the opportunity to identify the molecular determinant(s) responsible for the hallmark properties of T-type Ca2+ channels.

Our study also suggests that there are functional differences between the currents generated by the human and rat α1I subunits. Voltage-dependent and was described by single exponential function with time constants ranging from 273 to 95 ms for potentials between −50 and +30 mV (Fig. 3F). Inactivation kinetics of α1I current was 6-fold slower than for α1G current at membrane potentials up to −30 mV. The difference was even more pronounced at lower voltages (inactivation kinetics of α1I current −13-fold slower at −50 mV). The recovery from fast inactivation was determined using a double pulse protocol that comprises an inactivating pulse followed by a test pulse at variable interpulse durations. Plot of the relative peak current amplitude as a function of the interpulse duration (Fig. 3G) was described by a single exponential for α1I current (τ = 297 ± 43 ms, n = 11) but not for α1G current, which was best fitted by a two exponential function (τ1 = 56 ± 5 ms (64%) and τ2 = 238 ± 32 ms (36%)). A distinctive feature between T-type channels is their slow deactivation kinetics (21). For α1I current, however, deactivation was fast (~3-fold) as compared with α1G current and showed little voltage dependence (Fig. 3H). In addition, we have also found that the sensitivity to Ni2+ and mibebradil was significantly different between human α1I and α1G channels (Table 1). Dose-response curves for mibebradil indicated IC50 values of 2.3 and 1 μM for α1I and α1G currents, respectively. Similarly, α1I current was blocked by higher concentration of Ni2+, as compared with α1G current (IC50 of 184 and 133 μM, respectively; Fig. 3J). Finally, single channel currents measurements performed as indicated under "Material and Methods" (Fig. 3F) revealed a slope conductance of 11.2 pS (n = 3).
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Table I: Summary of the electrophysiological and pharmacological data obtained for human α1I and α1Gα currents and expressed as mean values ± S.E.
The number of cells is presented as (n).

| Property                           | α1I current          | α1G current          |
|-----------------------------------|----------------------|----------------------|
| Peak current (I-V curve)          | -23.7 ± 0.9 mV (12)* | -36.0 ± 1.0 mV (10)* |
| V<sub>0.5</sub> activation        | -40.6 ± 0.3 mV (11)* | -51.2 ± 0.1 mV (10)* |
| Slope activation factor (k)       | -5.6 ± 0.1 mV (11)*  | -4.2 ± 0.1 mV (10)*  |
| V inactivation<sub>50</sub>       | -68.9 ± 0.9 mV (8)*  | -74.7 ± 0.4 mV (5)*  |
| Slope inactivation factor (k)     | 5.6 ± 0.1 mV (8)     | 5.3 ± 0.3 mV (5)     |
| Current kinetics at -50 mV:       |                      |                      |
| Activation rate (rise time 10-90) | 65.8 ± 3.9 ms (24)*  | 7.6 ± 0.5 ms (10)*   |
| Inactivation rate (t<sub>1</sub>) | 272.9 ± 23.5 ms (7)* | 19.9 ± 1.3 ms (7)*   |
| Current kinetics at -30 mV:       |                      |                      |
| Activation rate (rise time 10-90) | 19.9 ± 1.4 ms (26)*  | 2.5 ± 0.1 ms (10)*   |
| Inactivation rate (t<sub>1</sub>) | 95.8 ± 3.9 ms (21)*  | 15.2 ± 0.9 ms (8)*   |
| Current kinetics at -10 mV:       |                      |                      |
| Activation rate (rise time 10-90) | 9.7 ± 0.8 ms (19)*   | 1.4 ± 0.1 ms (7)*    |
| Inactivation rate (t<sub>1</sub>) | 89.1 ± 2.8 ms (21)*  | 14.1 ± 0.9 ms (7)*   |
| Recovery from inactivation        | τ = 297 ms (11)      | -                       |
|                                  | ± 44 ms (11)         | -64% amplitude       |
|                                  | z<sub>2</sub> = 235  | ± 32 ms (5)          |
|                                  |                      | 36% amplitude        |

The statistical significance of the differences between two sets of averaged values was estimated using a Student’s unpaired t test, where p < 0.05 was considered significant.

Subunits, although the human subunit retained most of the properties described initially for the rat α1I subunit (12). It was recently reported that the rat α1I and α1G<sub>α</sub> currents have identical steady-state inactivation properties with a potential for half-maximal inactivation (V<sub>0.5</sub>) of -72 mV (14). Here we report that the human α1I and α1G<sub>α</sub> currents have significantly different V<sub>0.5</sub> values (-69 and -75 mV, respectively). It is of course important to consider that the rat and human α1G<sub>α</sub> subunits used in the present study and in Ref. 14 are highly homologous (α1G<sub>α</sub>-isoforms; 95% of homology on the complete sequence), while the α1I subunits are potentially different in their carboxyl terminus (Fig. 1C). Also, while rat α1I current was more sensitive to the block by Ni<sup>2+</sup> than rat α1G<sub>α</sub> current (IC<sub>50</sub> of 216 and 250 μM, respectively; Ref. 13), we found a reverse sequence of sensitivity for the currents generated by the human subunits (α1G<sub>α</sub> > α1I). It will be important to determine precisely whether species specific differences in the proteins can account for functional differences described here.

The specific features described here for the currents generated by the recombinant α1I and α1G<sub>α</sub> subunits should be useful for the identification and the discrimination of the corresponding currents in native cells. According to the expression profile of α1I and α1G<sub>α</sub> mRNA in rat brain (23), it was suggested that α1I and α1G<sub>α</sub> currents could coexist in thalamic structures. Indeed, it was reported that the LVA current in rat thalamic reticular and laterodorsal neurons could be separated into fast and slow components (16, 24, 25). By contrast, our Northern and dot blot data have not confirmed that human thalamic structures expressed the α1I mRNA at high level. By comparing α1I and α1G<sub>α</sub> mRNA expression profiles in human brain (this study and Ref. 9), we found that the occipital lobe, putamen, and nucleus accumbens are the regions that exhibit rather high level of both α1I and α1G<sub>α</sub> transcripts (not shown). Unfortunately, these human brain structures remains poorly explored, and no slow T-type current has been reported in any human neurons to date. Altogether, our data provide important new insights into the molecular properties and the function of T-type Ca<sup>2+</sup> channels in humans, suggesting that there are species specificity in the structure, the expression profile, and the function of the Ca<sup>2+</sup> channels generated by the α1I subunit. Functional expression of this human channel also offers a bioassay to investigate in detail the pharmacological profile of this peculiar T-type calcium channel and should help further in the identification and interpretation of the physiological role(s) of α1I T-type Ca<sup>2+</sup> channels in humans.

Interest for the characterization of human α1I subunit Ca<sup>2+</sup> channels is also raised by the identification of several neuronal disorders mapped to human chromosome 22q13. The CACNA1I gene is located in 22q12.3-q13.2, a susceptibility locus for familial schizophrenia (28) and a form of spinocerebellar ataxia, SCA10 (27). Another Ca<sup>2+</sup> channel gene, CACNG2, encoding the G2 subunit and localized on chromosome 22q13 has been hypothesized as a candidate for SCA10 (28). Similarly, it is tempting to suggest that CACNA1I is a candidate gene for these human diseases and linkage analysis of this gene with human neuronal disorders should be investigated.

Acknowledgments—We gratefully acknowledge Drs. F. A. Rassendren, S. Dubel, S. Richard, F. Chatail, F. Grigorescu, and C. Lautier for valuable technical support and helpful comments. We are most grateful to S. Spieesser for excellent technical assistance.

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J. Biol. Chem. 2000, 275:16530-16535.
doi: 10.1074/jbc.C000090200 originally published online April 3, 2000

Access the most updated version of this article at doi: 10.1074/jbc.C000090200

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