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Cloning, expression, and distribution of a Ca^{2+}-activated K^+ channel β-subunit from human brain

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ABSTRACT **We have cloned and expressed a Ca^{2+}-activated K^+ channel β-subunit from human brain. The open reading frame encodes a 191-amino acid protein possessing significant homology to a previously described subunit cloned from bovine muscle. The gene for this subunit is located on chromosome 5 at band q34 (*hslo-beta*). There is no evidence for alternative RNA splicing of this gene product. *hslo-beta* mRNA is abundantly expressed in smooth muscle, but expression levels are low in most other tissues, including brain. Brain subregions in which β-subunit mRNA expression is relatively high are the hippocampus and corpus callosum. The coexpression of *hslo-beta* mRNA together with *hslo-alpha* subunits in either *Xenopus* oocytes or stably transfected HEK 293 cells gives rise to Ca^{2+}-activated potassium currents with a much increased calcium and/or voltage sensitivity. These data indicate that the β-subunit shows a tissue distribution different to that of the α-subunit, and in many tissues there may be no association of α-subunits with β-subunits. These β-subunits can play a functional role in the regulation of neuronal excitability by tuning the Ca^{2+} and/or the voltage dependence of α-subunits.**

Ca^{2+}-activated K^+ channels (K_{Ca}) are present in virtually all cells and often play an important role in coupling chemical signaling to electrical signaling (1, 2). All K_{Ca} channels are activated by increases in the intracellular Ca^{2+} concentration, and many of them can also be modulated by voltage and by messenger molecules such as protein kinases, phosphatases, and G-proteins (3–6). These channels, in turn, can also function as feed-back regulators of the intracellular Ca^{2+} concentration, by hyperpolarizing the membrane voltage in close proximity to voltage-activated Ca^{2+} channels (7). In some neurons this may allow K_{Ca} channels to regulate the amount of neurotransmitter released from presynaptic terminals by modulating the duration of the presynaptic action potential (7, 8).

There are many functional subtypes of K_{Ca} channels differing in their Ca^{2+} sensitivity, toxin sensitivity, gating, and in their response to channel modulators such as protein kinases and protein phosphatases (9–11). The molecular basis underlying this functional diversity includes alternative RNA splicing of a single transcript (12–15), the combination of heterooligomers (16–18), and the association of accessory proteins with ion channels (19–22). Other voltage-gated K^+, Ca^{2+}, and Na^+ channels all have been shown to be associated with one or more types of smaller auxiliary proteins (23, 24). These subunits can play a number of functions including modulating gating kinetics and regulating channel assembly and targeting (24–27).

To determine the presence, distribution, and functional role of *hslo* β-subunits we have cloned a human K_{Ca} channel β-subunit, examined its tissue distribution, mapped its chromosomal localization, and examined the function of this protein by coexpressing it with previously cloned *hslo* alpha-subunits in *Xenopus* oocytes and in the mammalian HEK 293 cell line.

EXPERIMENTAL PROCEDURES

cDNA and Cloning. We amplified human K_{Ca} channel β-subunit cDNA fragments using a degenerate reverse transcriptase–PCR (RT–PCR) procedure as described (14). Products from the reverse transcription reaction [20 μl final volume from 200 ng of poly(A)$^+$ RNAs from human brain, hippocampus, aorta, and trachea] were used to PCR amplify *hslo-beta* sequences. Nested PCR primer pairs were designed by using bovine K_{Ca} channel β-subunit protein sequences (20, 21). The first PCR primer pair used the sequence near the start and the stop sites (5′-ACCATGGGIAARAARYTIGTIATG-3′ and 5′-GTGGATCTAICKYTGIGCIGCIARDAT-3′, respectively). The DNA products of this reaction were of the expected size (0.6 kb). Strong PCR signals were observed for RNA from aorta and trachea, and weaker signals were obtained from brain and hippocampus RNA. These RT–PCR products were used as template DNA for a second round of PCR amplification by using primers corresponding to the two putative transmembrane domains (TM1 and TM2) of the bovine K_{Ca} channel β-subunit (5′-ATGGTIGTIGGRGCIGTIATHACIIAYTA-3′ and 5′-RAAIGTIGGCCARAAIARISWRAA-3′). Strong PCR signals of the expected size (450 bp) were generated for all four tissues. The DNA generated by these four reactions were cloned into the plasmid vector pCRII (Invitrogen). One of these 450-bp brain PCR clones was used as probe to screen a human brain λgt10 cDNA library (14), resulting in the isolation of a cDNA clone containing the complete coding sequence of the β-subunit, which we refer to as *hslo-beta*. This full-length cDNA was subcloned into the pBluescript-KSII+ vector (Stratagene) for expression in *Xenopus* oocytes and subcloned into the pcDNA3 vector (Invitrogen) for expression in HEK 293 cells.

Genomic Cloning. The preparation and cloning of human genomic DNA in the P1 system was performed as described by Pierce *et al.* (28). Before PCR screening this library we tested a panel of PCR primer pairs for amplification of *hslo* channel β-subunit genomic fragments from human placenta genomic DNA. AmpliTaq DNA polymerase (Perkin–Elmer) was used for PCR amplification (94°C for 45 s, 55°C for 1 min, 72°C for 1 min, 35 cycles). A primer pair, 5′-GGAACGAAAC-

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Abbreviations: K_{Ca}, Ca^{2+}-activated K^+ channel; RT–PCR, reverse transcriptase–PCR; XL–PCR, extra long PCR; FISH, fluorescense *in situ* hybridization.

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U42600–U42603).

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CAGCGTCCTATTC-3′ and 5′-AACAGAAGACAGCGTG-GATTGG-3′ that covered a 292-bp region from TM2 to 3′ untranslated region, was selected for library screening. Two P1 genomic clones were isolated. The identity of these two genomic clones were checked by Southern blots and direct DNA sequencing of extra-long PCR (XL–PCR) fragments amplified from the 2 P1 clones (XL–PCR kit from PE Express). Sequences were analyzed using SEQUENCHER (GeneCodes, Ann Arbor, MI) and Genetics Computer Group software. The procedures for fluorescense *in situ* hybridization (FISH), Southern blot analysis, and Northern blot analysis were as described (15).

cRNA Synthesis. Plasmid DNA for the splice variant hbr1 of *hslo* (15) in pBluescript-II (KS^+), and of *hslo-beta* was purified by using Qiagen (Chatsworth, CA) mini-prep columns. Linearized plasmid DNA was transcribed in the presence of the cap analog $m^7G(5')ppp(5')G$ (MEGAscript kit; Ambion, Austin, TX) as described (28). RNA samples were examined on ethidium bromide-stained denaturing agarose mini-gels to assure the presence of a single undegraded band of the expected size.

Expression of Cloned K_{Ca} Channels in *Xenopus* Oocytes. Female *Xenopus laevis* were obtained from Nasco (Fort Atkinson, WI). Stage V–VI oocytes were removed from anesthetized frogs and injected with 40 nl of *in vitro* transcribed/capped cRNA as described (29). For coexpression experiments a 4:1 molar ratio of *hslo-beta* versus *hslo-alpha* was used. Immediately before patch clamp experiments the vitelline membrane was removed with fine forceps (without using hypertonic "shrink" solution). Oocytes were maintained at 17°C in sterile Frog-92 medium (92 mM NaCl/1.5 mM KCl/1.2 mM $CaCl_2$/2 mM $MgCl_2$/10 mM Hepes/50 μg of gentamycin per ml, pH 7.6), which was changed daily.

Expression of Cloned K_{Ca} Channels in HEK 293 Cells. The *hslo* splice variant hbr1 (15) was cloned into the expression vector pcDNA3 (Invitrogen), and *hslo-beta* was cloned into the expression vector, pZeoSV (Invitrogen). The HEK 293 cell line was transfected with 10 μg of expression plasmid using lipofectamine from GIBCO/BRL according to manufactures protocol. Cells transfected with *hslo-alpha* or *hslo-beta* were selected in medium supplemented with 0.5 mg of geneticin per ml (G 418; Sigma) or in medium supplemented with 0.25 mg of Zeocin per ml (Invitrogen), respectively. RT–PCR was used to confirm the presence of *hslo-beta* in clones already expressing hbr1 channels. Naive HEK 293 cells (ATCC CRL-1573) as well as transfected HEK 293 cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM Glutamax (GIBCO/BRL) at 37°C in a humidified atmosphere of 5% CO_2/95% air. One to four days before electrophysiological experiments cells were seeded in 35-mm culture dishes containing 8–12 glass coverslips.

Patch Clamp Electrophysiology. K_{Ca} currents were recorded in inside–out macropatches using the patch-clamp method (30). Patch pipettes were fabricated from Corning 7052 borosilicate glass (Warner, Hamden, CT) and fire polished to resistance of 1.5–5 MΩ. Macropatch currents were amplified using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA), or an EPC-9 amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany). Excised patches were moved to an adjacent recording chamber to avoid interactions with cell-secreted molecules that can alter the open probability of expressed ion channels (data not shown). For oocyte experiments the recording solutions contained gluconate as a nonpermeant anion to prevent the activation of calcium-activated chloride channels endogenous to these cells (31). The intracellular (bath) solution was 110 mM K^+-gluconate, 5 mM Na^+-gluconate, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.35 mM EGTA, 0.5 mM Ca^{2+}-gluconate, and 10 mM Hepes (adjusted to pH 7.4 with KOH and sterile filtered into acetic acid-rinsed bottles). Free calcium concentrations were determined using Biosoft (Cambridge, U.K.) EQCAL software and stability constants published previously (30) and confirmed by using a Ca^{2+} electrode. For HEK 293 cell experiments patches were exposed to a symmetric solution containing 146 mM K^+, 144 mM Cl^-, 1 mM Ca^{2+}, 1.3 mM EGTA, 1 mM Mg^{2+}, and 10 mM Hepes. This solution results in a final Ca^{2+} concentration of 0.5 μM. Bath solutions containing 1.8 μM Ca^{2+} were generated by reducing EGTA to 1.07 mM. To generate solutions containing 30 μM Ca^{2+}, 1 mM Ca^{2+} and 1.3 mM EGTA were replaced with 30 μM Ca^{2+}. Pipette resistances were 1–3 MΩ, and R_s compensation was 80%, resulting in maximal voltage errors of 4–6 mV for 10 nA currents. All solutions were equilibrated to room temperature (22–25°C) before use. At the end of each experiment, membrane patches were reexposed to the original Ca^{2+} concentration ($[Ca^{2+}]$) and membrane voltage conditions to verify that the number of channels and/or the open probability did not change during the experiment. Records showing a more than 10% change in membrane current due to channel rundown were eliminated from data analysis.

Data Analysis. The acquisition and analysis of macropatch data was aided by PCLAMP 6.0 (Axon Instruments), ORIGIN 3.0 (MicroCal, Northampton, MA), IGOR (Wavemetrics, Lake Oswega, OR), and various custom VISUAL BASIC analysis programs developed in this laboratory. Data are expressed as means ± SEM.

RESULTS

***hslo-beta* cDNA and Genomic Cloning.** We amplified human K_{Ca} channel β-subunit cDNA fragments from brain, aorta, and trachea by using a degenerate RT–PCR procedure as described (15). Using a 450-bp PCR fragment amplified from human brain cDNA as probe (same probe used for subsequent Southern and Northern blot analyses unless otherwise stated), we screened a human brain λgt10 cDNA library, resulting in the isolation of three clones of approximately 1.1, 1, and 0.2 kb. The two largest clones were found to contain identical open reading frame sequences and are referred to as *hslo-beta* (organization of the *hslo-beta* gene is shown in Fig. 1*A*). The cloned gene contains a 576-bp open reading frame within the 1041-bp insert. Two hydrophobic regions proposed to be transmembrane domains based on hydropathy analysis are located near the N and C termini (TM1 and TM2; Fig. 1*A*). The sequence between these domains contains two N-linked glycosylation sites (20, 21). Extra-long PCR amplification of human genomic DNA shows the presence of two introns, ≈1.5 kb and 5 kb long (Fig. 1*B*). The open reading frame codes for a 191-amino acid protein that is 85% identical to the bovine β-subunit (20, 21). This interspecies homology is similar to the 92% identity shared by the human and mouse K_{Ca} channel α-subunits (15).

A Single Gene on Chromosome 5q34 Encodes Human K_{Ca} Channel β-Subunits. To determine the chromosomal localization of the *hslo-beta* genes we performed two types of experiments. The first of these was to probe a panel of somatic cell hybrids, containing individual human chromosomes. Southern blots of genomic DNA extracted from these cell lines and separately digested with four restriction enzymes demonstrated that *hslo-beta* cDNA only hybridizes to cell lines containing human chromosome 5 (discordance = 0%). All other chromosomes had discordance values of 40% or higher (data not shown).

Second, we isolated human genomic clones containing the *hslo-beta* gene by PCR screening a genomic P1 library containing human DNA fragments. Two genomic clones were isolated, and PCR analysis indicated that both contain the entire *hslo-beta* coding sequence. One of the *hslo-beta* P1 genomic clones was labeled with biotin-11-dUTP for FISH to metaphase chromosome spread from human lymphocytes (Fig. 2). This resulted in the specific labeling at a site 89%

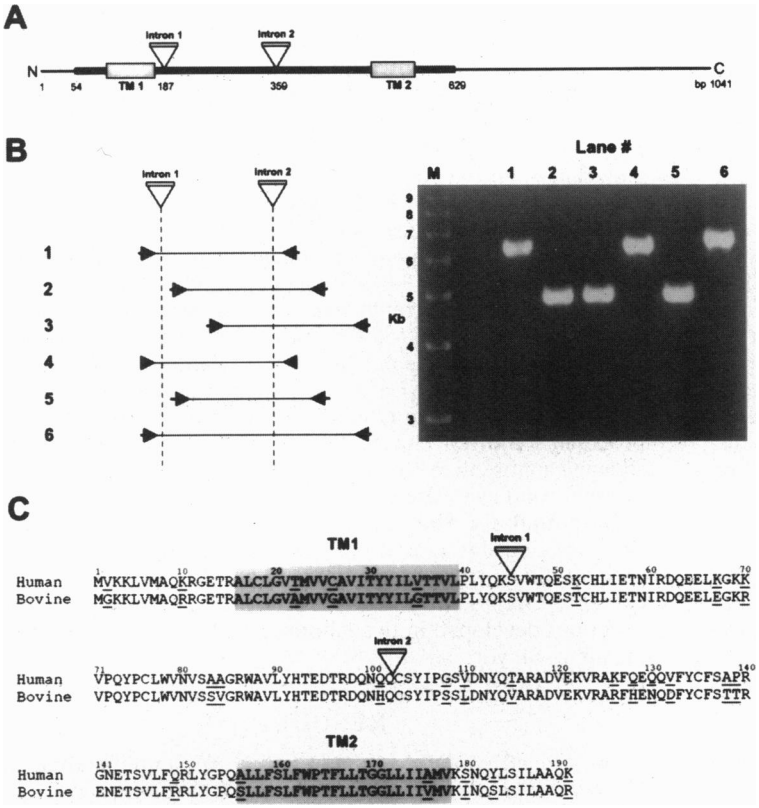

FIG. 1. Cloning of the *hslo-beta* gene from human brain, aorta, and trachea. (*A*) The *hslo-beta* cDNA clone showing the arrangement of introns and putative transmembrane domains within the coding region. (*B*) Six XL–PCR reactions were carried out by using four pairs of primers to amplify genomic fragments from two *hslo-beta* genomic P1 clones (lanes 1–3 show products from clone 1/primer pairs 1–3; lanes 4–6 show products from clone 2/primer pairs 4–6). The location of the primers (arrows) are shown aligned with the cDNA structure in *A*. The size of the resulting PCR products from these six reactions are shown on the image of the ethidium bromide-stained agarose gel. (*C*) Deduced amino acid sequences of the human and bovine K_{Ca} channel β-subunit protein sequences. Differences between the two sequences are shown underlined. The putative transmembrane domains are shaded. DNA sequences of the full-length cDNA clone and the XL–PCR-amplified genomic fragments can be accessed through the GenBank data base under the following accession numbers: U42600 for cDNA, U42601 for genomic exon 1/intron 1, U42602 for genomic exon 2/intron 2, and U42603 for genomic intron 2/exon 3.

distant from the centromere to the telomere of the long arm of chromosome 5, a location corresponding to 5q34 (n = 10).

We used Southern blot hybridization to examine whether or not a single copy gene encodes *hslo-beta*. Human genomic DNA was digested individually by four restriction enzymes (*Eco*RI, *Hin*dIII, *Pst*I, and *Taq*I). A 450-bp probe targeting the central coding region of the gene was found to hybridize to two genomic restriction fragments for each of the four enzymes used (data not shown). None of these enzymes have restriction sites within the cDNA sequence. This indicates either the presence of two homologous *hslo-beta* genes, a single gene containing one or more large introns, or both. When probing genomic DNA with either a 5′ probe or a 3′ probe, only a single restriction fragment was being hybridized for all four enzymes, indicating *hslo-beta* is encoded by a single copy gene separated by introns. The presence of two introns was confirmed by XL–PCR analysis by using a battery of primer pairs (Fig. 1*B*). The intron–exon junctions were then determined by aligning the sequences obtained from genomic DNA to cDNA sequences.

To examine the possibility of *hslo-beta* splice variants a total of 30 independent RT–PCR products from human brain RNA (7 clones), aorta (7 clones), and trachea (16 clones) were sequenced. There was no evidence for alternative splicing, as all 30 sequences were identical.

***hslo-beta* mRNA Is Differentially Distributed in the Central Nervous System and in Peripheral Tissue.** Northern blot analysis was used to show that a 1.4-kb transcript is widely but differentially expressed throughout human central and peripheral tissues (Fig. 3*A*). High expression levels were found in aorta, while expression in brain, heart, skeletal muscle, kidney, spleen, and lung was low but detectable. Within different regions of brain relatively high levels of *hslo-beta* RNA are found in hippocampus and corpus callosum and much lower levels in all other brain regions examined (Fig. 3*B*).

To examine inter-species variation in the distribution of the K_{Ca} channel β-subunit we prepared poly(A)$^+$ RNA from 32 different canine tissues and probed this by hybridization to the human K_{Ca} channel α- and β-subunit sequentially. Consistent with expression levels in human tissues (15), canine α-subunits are highly expressed in brain subregions, adrenal gland, aorta, intestine, stomach, and bladder. Lower expression levels are observed in skeletal muscle, and there is little or no expression in heart and liver (data not shown). However, contrary to human tissues, canine K_{Ca} channel α-subunit transcript was undetectable in kidney and spleen. For the K_{Ca} channel β-subunit, expression was detected only in canine smooth muscles including aorta, bladder, stomach, and intestine. We found no correlation between the distribution of β-subunit transcripts and the distribution of α-subunit splice variants in central (15) and peripheral tissues (J.T.-C., unpublished results).

Coexpression of *hslo-beta* with *hslo-alpha* in *Xenopus* Oocytes and in HEK 293 Cells. cRNA coding for one of the *hslo-alpha* subunit splice variants (hbr1; ref. 15) was injected either alone or in combination with *hslo-beta* cRNA into *Xenopus* oocytes. To test the voltage dependence of the *hslo* channels, currents were measured by using inside–out macropatches containing 20–600 functional channel molecules. Fig. 4*A* shows currents produced by expression of hbr1 alone (*Upper*; n = 6), or in combination with *hslo-beta* (*Lower*; n = 5). From the raw current records shown in Fig. 4*A* it can be seen that the presence of *hslo-beta* alters the voltage dependence of these channels, as evidenced by the channel openings at hyperpolarized voltages in these experiments performed under symmetrical K^+ concentrations. Since, in the presence of *hslo-beta*, K_{Ca} channels are open at 0 mV, hyperpolarizing voltage jumps lead to an initial deactivating current component reflecting the kinetics of channel closure. For the sake of clarity some of these deactivating currents have been truncated in Fig. 4*A*. Mock-injected oocytes gave rise to negligible currents under these recording conditions (data not shown). Fig. 4*B* shows normalized G/V relationships for *hslo-alpha* alone or when expressed together with *hslo-beta*. It can be seen that the presence of the β-subunit results in a left-shift of the voltage dependence (46 ± 14 mV; n = 4), without any significant change in the steepness of the voltage dependence (14.8 ± 1.6

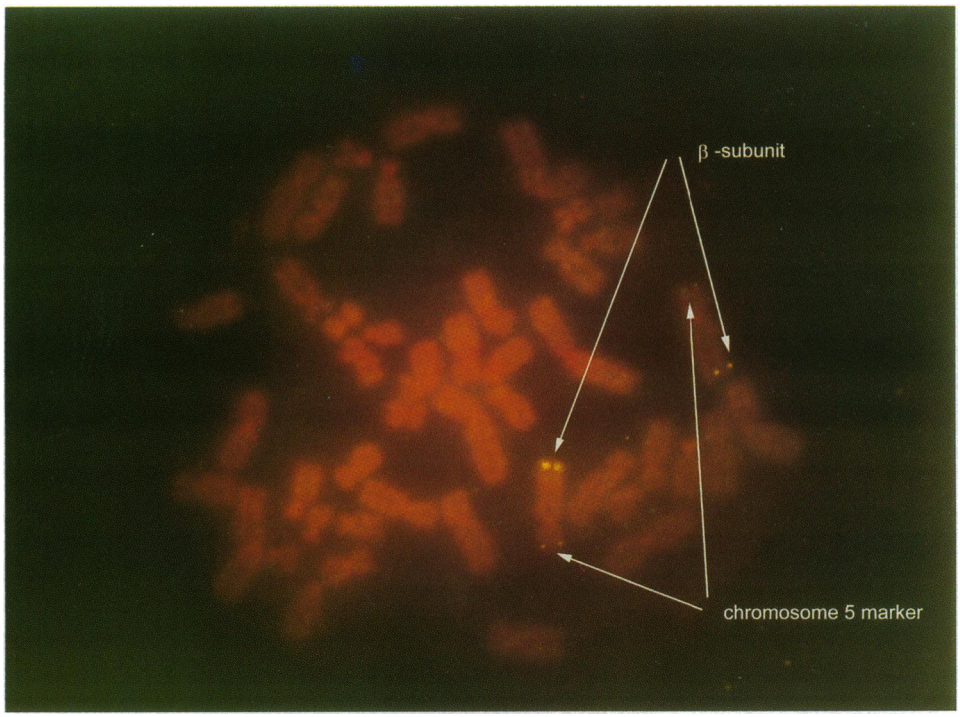

FIG. 2. Localization of the *hslo-beta* gene by FISH. Two probes were used for cohybridization to metaphase chromosomes of phorbal 12-myristate 13-acetate-stimulated peripheral blood lymphocytes. One probe was a human genomic P1 clone containing the *hslo-beta* gene. The other was a control probe of a human P1 clone containing the *cri du chat* locus located at 5p15, the distal end of the chromosome 5 short arm. The *hslo-beta* gene is localized on human chromosome 5, subregion 5q34.

mV per e-fold, 15.6 ± 1.8 mV per e-fold change in p_o in the presence of *hslo-beta*).

The effects of the β-subunit on channel properties were also studied in stable HEK 293 cell lines expressing either *hslo-alpha* subunit alone (HEK-hbr1) or *hslo-alpha* in combination with *hslo-beta* (HEK-hbr1/β). Stable clones were generated by transfection, and expression of both α- and β-subunits was confirmed by RT–PCR using specific α- and β-subunit primer pairs. Two double-stable HEK 293 clones expressing both α- and β-subunit were selected for electrophysiological characterization. In addition to these two single clones, we analyzed a pool of double-resistant clones. Inside–out patch clamp analysis indicated that the expression level of functional K_{Ca} channel tetramers is similar in both of these cell lines. The average current amplitude in patches obtained from HEK-hbr1 was 4.2 ± 3.1 nA (n = 32; V_m = 100 mV), a value consistent for 25 passages. The average current amplitude in HEK-hbr1/β cells was similar (5.7 ± 3.8 nA; n = 10; V_m = 100 mV). For both cell lines one can calculate that individual membrane patches contain 40–600 ion channels.

The steady-state activation of these channels by voltage was measured at potentials ranging from −150 mV to +150 mV, and the tail currents were determined by a following step to −150 mV (Fig. 5). The midpoint of voltage ($V_{1/2}$) for activation in HEK-hbr1 cells was found to be 90 ± 5.5 mV, 22.6 ± 11.4 mV, and −36.4 ± 7.1 mV in 0.5 μM, 1.8 μM, and 30 μM Ca^{2+}, respectively (n = 6–11). K_{Ca} currents in HEK-hbr1/β cells were shown to be activated at much more hyperpolarized voltages than K_{Ca} currents in HEK-hbr1 cells. In these cells the $V_{1/2}$ for activation was found to be 69.2 ± 1.7 mV, −41.3 ± 5.7 mV, and −90.2 ± 8.7 mV in 0.5 μM, 1.8 μM, and 30 μM Ca^{2+}, respectively (n = 5–9). Thus, the β-subunit shifted the activation curves leftward by an average of 20.8 mV, 63.9 mV, and 53.8 mV in 0.5 μM, 1.8 μM, and 30 μM Ca^{2+}, respectively. Further, the β-subunit conveyed a pronounced slowing in the relaxation of the tail currents (Fig. 5). In the pooled clones the

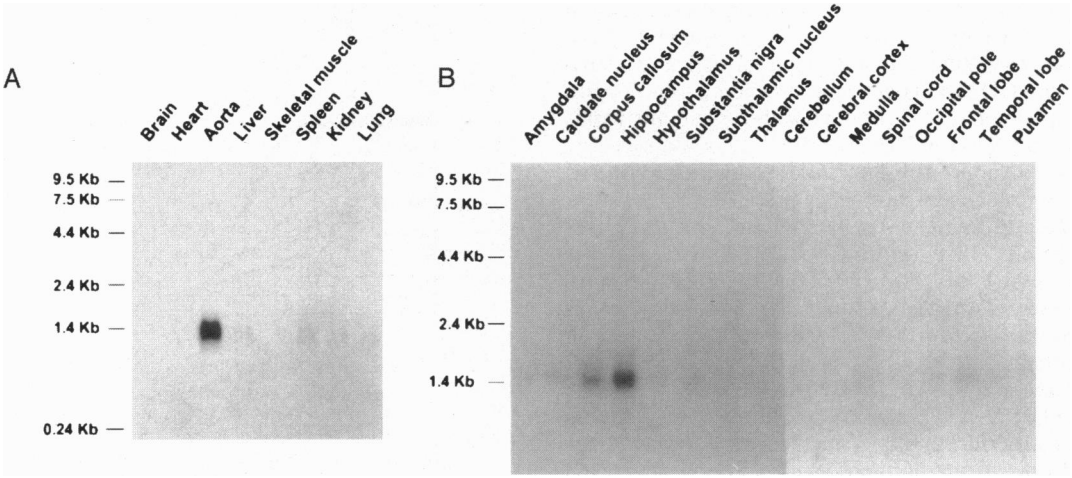

FIG. 3. Northern blot analysis of *hslo* mRNA expression in neural and nonneural adult human tissue. (*A*) Each lane contains 5 μg of poly(A)$^+$ RNA from the indicated tissue. The blots were hybridized with a 450-bp cDNA probe spanning the TM1 domain to the TM2 domain. (*B*) Each lane contains 2 μg of poly(A)$^+$ RNA from the indicated neural region. The blots were hybridized with the same probe used in *A*. Consistent loading of poly(A)$^+$ RNA for both blots was checked by hybridization to a human β-actin probe.

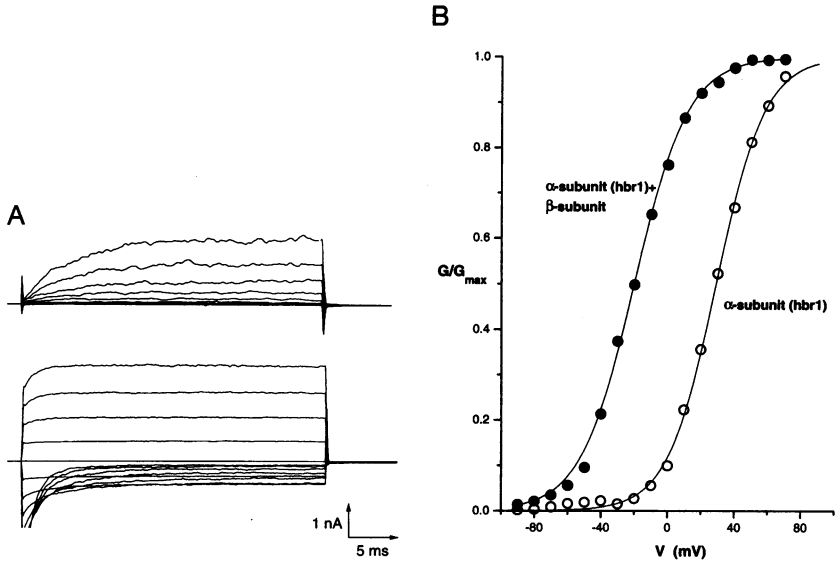

FIG. 4. Expression of *hslo-beta* in *Xenopus* oocytes. (*A*) mRNA coding for hbr1 alone (*Upper*) or in combination with *hslo-beta* (*Lower*) was injected into *Xenopus* oocytes, and current expression was assayed by inside–out patch clamp analysis. Current recordings were performed in symmetrical 110 mM K-gluconate buffers containing 1 μM free Ca^{2+}. Membrane patches were held at 0 mV and depolarized from −90 mV to +70 mV for 50 ms in 10 mV steps every 2s. Data were filtered at 2 kHz, digitized at 4 kHz, and are not leak-subtracted. (*B*) The voltage-dependence of hbr1 channels expressed alone or in combination with *hslo-beta* subunits. Conductance values (*G*) were obtained by extracting peak current values from 50-ms voltage steps as shown in *A* and experimentally determining the driving force, which was always close to 0 mV under these ionic conditions. These data were plotted and fit with a Boltzmann function to estimate G_{max}. The data points shown are the calculated G/G_{max} values, and the continuous lines are optimized fits to the Boltzmann function.

average shifts in $V_{1/2}$ were smaller, as expected since the cells expressed various amounts of the β-subunit, and the shifts amounted to −14,6 and −30.0 mV in 0.5 and 1.8 μM Ca^{2+} (n = 8), respectively.

DISCUSSION

These experiments have shown that numerous human tissues express a protein shown to be closely associated with large-conductance Ca^{2+}-activated K^+ channels (20, 21, 32). We have cloned the gene coding for this protein (*hslo-beta*), mapped it to the human genome, examined its tissue distribution, and characterized its function when coexpressed with previously cloned *hslo* Ca^{2+}-activated K^+ channel α-subunits. *hslo-beta* codes for a 191-amino acid protein containing two regions of hydrophobic amino acids presumed to be transmembrane domains. *hslo-beta* does not share significant sequence homology with α- and β-subunits of other ion channels, nor with any other protein presently in sequence data bases. This protein is believed to be closely associated with the α-subunits since the β-subunit can be coimmunoprecipitated and copurified together with the α-subunit (20, 21). The close association of α- and β-subunits is highlighted by the finding that charybdotoxin, a peptide pore blocker of the α-subunit, is incorporated into the β-subunit by bifunctional cross-linking agents (32). Further evidence that the extracellular pore region of these ion channels is in close proximity to β-subunits comes from experiments showing that the K_{Ca} channel activator dehydrosoyasaponin I (DHS-I) is active only when α- and β-subunits are coexpressed (33). Presumably the β-subunit forms part of the binding site for DHS-I.

Two independent approaches, somatic cell hybridization and FISH, were used to show that a gene on chromosome 5 encodes *hslo-beta* in humans. We cannot rule out the possibility that there are other distantly related *hslo-beta* channel genes not revealed by the stringency of our assays or that more than a single *hslo-beta* gene is located at 5q34. Furthermore, the analysis of 30 *hslo-beta* PCR clones reveals that we can detect only a single isoform of this protein. This is in contrast to *hslo-alpha* subunits for which extensive splicing of a single gene was found (15). *hslo-beta* maps close to γ-aminobutyric receptor isoforms α1, α6, β2, and γ3 and the tyrosine kinase genes FLT4 and FMS on human chromosome 5 and possibly on the syntenic region of mouse chromosome 11 (34–37).

The analysis of *hslo-beta* RNA distribution has revealed that the tissue expression levels and patterns of *hslo-beta* are different to the expression of *hslo-alpha* subunits. Whereas the expression of *hslo-alpha* is highest in the brain, the expression of *hslo-beta* is virtually undetectable in this tissue, and is highest in aorta. Furthermore, there are clear interspecies differences in the expression of *hslo-beta*, exemplified by relatively high expression level in the human hippocampus, whereas canine hippocampus shows virtually undetectable levels.

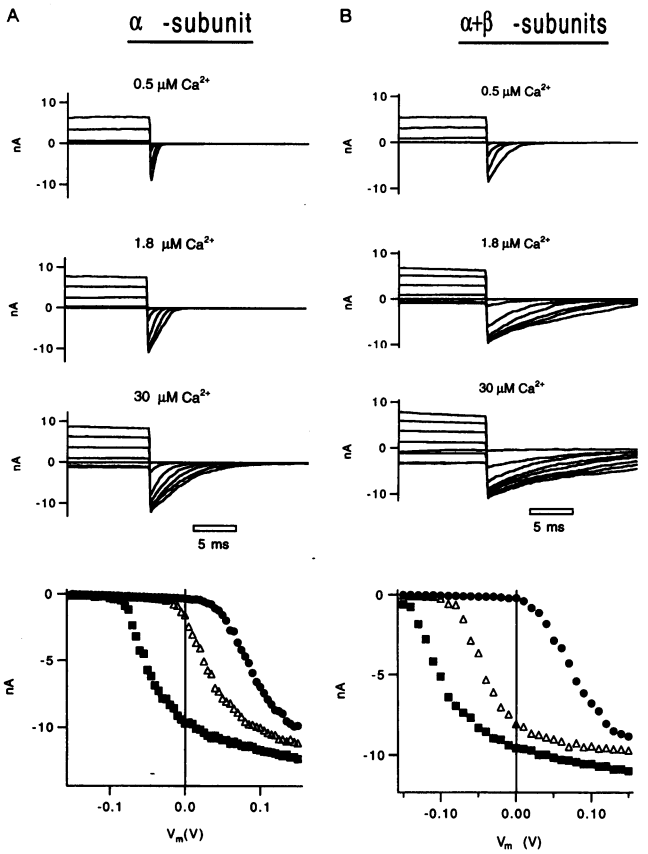

FIG. 5. Expression of *hslo-beta* in HEK 293 cells. Inside–out patches of HEK 293 cells stably transfected with either hbr1 alone (*A*) or in combination with *hslo-beta* (*B*). Patches were exposed to either 0.5 μM, 1.8 μM, or 30 μM Ca^{2+}. Currents were activated by voltage steps from −150 mV to +130 mV in 40 mV increments, always followed by a step change to −150 mV (V_H = −100 mV). The effects of Ca^{2+} were determined on peak tail currents, which were fit to the Boltzmann function to estimate $V_{1/2}$ values.

The coexpression of *hslo-alpha* subunits (hbr1) together with *hslo-beta* results in a pronounced increase in the Ca^{2+} and/or voltage sensitivity of these channels. The steepness of the voltage dependence of these channels is not significantly altered by the β-subunit (12–16 mV per e-fold change in p_o); however, the midpoint for voltage activation ($V_{1/2}$) is left-shifted. This differential sensitivity to voltage and/or Ca^{2+} may allow *hslo* $\alpha\beta$ complexes to play unique roles within different brain regions or within distinct functional domains of a single cell. Consistent with this finding is the nonuniform distribution of K_{Ca} channel splice variants within different brain regions. The mechanism by which *hslo-beta* alters the Ca^{2+} sensitivity of K_{Ca} channels is unresolved. Many other calcium-binding proteins contain a helix–turn–helix Ca^{2+}-binding motif referred to as an EF hand (38). While no EF-hand motif can be found in *hslo*, it is possible that glutamate and aspartate residues contribute to the Ca^{2+} binding domains in these proteins and that *hslo-beta* subunits can act to "fine-tune" the Ca^{2+} concentrations needed to activate the α-subunit.

Although we have shown that *hslo-beta* subunits can alter the functional properties of coexpressed α-subunits, this protein–protein interaction may have other roles as well. For instance, $\alpha\beta$ complexes may be a mechanism for targeting particular channel isoforms to specific brain regions, and may even be used to target specialized areas within a single cell type (23).

There is evidence that K_{Ca} channels may be present in close proximity to Ca^{2+} channels (8, 39–42), particularly at presynaptic terminals (7). Hence, the proximity of these channels to each other, as well as the sensitivity of the Ca^{2+} sensor on the K_{Ca} channels will determine the exact contribution made by K_{Ca} channels to the overall electrical activity of a cell.

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