New Ether-à-go-go K⁺ Channel Family Members Localized in Human Telencephalon*

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A cDNA encoding a novel voltage-gated K⁺ channel protein was isolated from human brain. This protein, termed BEC1, is 46% identical to rat elk in the ether-à-go-go K⁺ channel family. The BEC1 gene maps to the 12q13 region of the human genome. Northern blot analysis indicates that BEC1 is exclusively expressed in human brain, where the expression is concentrated in the telencephalic areas such as the cerebral cortex, amygdala, hippocampus, and striatum. By in situ hybridization, BEC1 is detected in the CA1–CA3 pyramidal cell layers and the dentate gyrus granule cell layers of the hippocampus. Specific signals are also found in neocortical neurons. Transfection of mammalian L929 and Chinese hamster ovary cells with BEC1 cDNA induces a voltage-gated outward current with a fast inactivation component. This current is insensitive to tetraethylammonium and quinidine. Additionally, a second related gene BEC2 was isolated from human brain. BEC2 is also brain-specific, located in the neocortex and the striatum, and functional as a channel gene. Phylogenetic analysis indicates that BEC1 and BEC2 constitute a subfamily, together with elk, in the ether-à-go-go family. The two genes may be involved in cellular excitability of restricted neurons in the human central nervous system.

Voltage-gated K⁺ channels play an essential role in controlling cellular excitability in the nervous system, and regulate a variety of neuronal properties such as interspike membrane potential, action potential waveform, and firing frequency (1). These results indicate the important functions of voltage-gated K⁺ channels in neuronal signal transduction and processing. Their contribution to behavioral phenotypes such as learning and memory have also been studied (2, 3).

To date, many voltage-gated K⁺ channel genes have been identified from different tissues such as heart and brain, and constitute an evolutionarily related multigene superfamily (4–6). This superfamily is classified into two groups, the Shaker and the ether-à-go-go (eag) families. The Shaker family is quite heterogeneous and mainly consists of four subfamilies, Kv1–Kv4. Their protein structures are characterized by six transmembrane regions (S1–S6) with a voltage-sensing S4 region and an ion-conducting pore region located between S5 and S6. K⁺ channels in the eag family also have similar structural features. However, overall sequence similarity between eag and Shaker-type K⁺ channels is very low. Members of the eag family are related to cyclic nucleotide-gated cation channels, hyperpolarization-activated cation channels and plant hyperpolarization-activated K⁺ channels, rather than Shaker-type channels (6). Indeed, a common feature of the C terminus of eag-type channels is a putative cyclic nucleotide-binding (CNB) domain, a characteristic of such ion channels.

The eag family consists of eag, eag-related gene (erg), and elk (6–8). In Drosophila, genetic mutations of eag or erg induces a hyperekctile phenotype (9–11). Human erg maps to LQT2, the locus of inherited long-QT syndrome, an abnormality of cardiac rhythm involving the repolarization of the action potential (12). This gene is expressed not only in the heart but also in the brain and parasympathetic ganglia (8). In a dorsal root ganglionic cell line, pharmacological blocking of erg currents causes the disappearance of spike-frequency adaptation of firing (13). This finding suggests that mammalian erg also contributes to the regulation of neuronal excitability. Consequently, we attempted to identify other novel erg-related genes expressed in mammalian brain. As a result, a new gene encoding a voltage-gated K⁺ channel was isolated, which is more closely related to elk than erg and is exclusively expressed in human telencephalon. This study reports the molecular cloning, distribution, and channel activity of this gene, BEC1. In addition, the identification of another novel gene closely related to BEC1 is described, indicating the existence of a new subfamily in the eag K⁺ channel family.

EXPERIMENTAL PROCEDURES

Molecular Cloning of Human BEC1—A BLAST search of the expressed sequence tag (EST) data base of GenBank, using human erg as a query amino acid sequence, retrieved two sequences with the accession number R35526 (387 bases) and M79045 (321 bases). To identify the 5’ and 3’ ends of the cDNA corresponding to each EST, 5’ and 3’ rapid amplification of cDNA ends (RACE) were performed using the Human Brain Marathon-Ready cDNA (CLONTECH) and primers derived from each EST sequence. Amplified fragments were directly cloned into the plasmid pCR2.1 (Invitrogen). Sequencing the RACE products revealed that the two EST were part of a cDNA. Determined sequences were assembled into a large contig (3610 bases) with an open-reading frame encoding 1083 amino acids, which we called human BEC1.

Finally, to verify that human BEC1 cDNA including the entire open-reading frame could be cloned from an independent source, human poly(A)⁺ RNA (CLONTECH) was used for reverse transcriptase-polymerase chain reaction (RT-PCR). Reverse transcriptase reaction was primed with the random hexamer. Primers for PCR were designed from kb, kilobases; CHO, Chinese hamster ovary; contig, group of overlapping clones.
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the 3610-bp contig as follows: 5′-GGATCCCTAAGATCGCCCAAGCTG-3′ and 5′-GCTCTAGAGCTCATGGTGGGCAGCA-3′, which contains artificial sequences with EcoRI and XbaI sites, respectively. (italic) Cycles were as follows: initial denaturation at 96 °C for 1 min; and 35 cycles of denaturation at 98 °C for 10 s, annealing at 63 °C for 30 s, and extension at 72 °C for 30 s. To amplify products were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. The plasmid (0.1 ng) carrying BEC1 or BEC2 cDNA was used as a control template. The efficiency of cDNA synthesis was estimated by RT-PCR (23 cycles) of the glyceraldehyde-3-phosphate dehydrogenase cDNA (18).

Molecular Cloning of Rat BEC1 and BEC2—To isolate rat homolog cDNA, RT-PCR was performed using primers designed from two nucleotide sequences of BEC1 conserved with elk, 5′-GACCTTCTCGACACCATTGCACATC-3′ and 5′-CCAACCACCACCGGTATCGTAT-3′, in which lowercase letters are indicated mismatches with elk. These sequences correspond to amino acid residues 13–19 (TFLDTHIA) and 492–498 (MHAVVFGG) of human BEC1, which are shared only with elk among the eag family (Fig. 1). Rat brain poly(A)+ RNA was prepared by guanidinium thiocyanate extraction followed by oligo(dT)-cellulose chromatography (15), and converted into random-primed cDNA. The RT-PCR amplified two fragments of 1.5 and 1.4 kb, which encode polyepitides with 97% and 59% identities to human BEC1, respectively. Because the N and C termini were missing, 5′- and 3′-RACE were performed on the basis of each determined sequence using the Rat Brain Manual (Clontech). Sequences were assembled into a contig (3715 bases) with an open reading frame of 1087 amino acids, which we named pME-E1. The amplified fragment was digested with EcoRI and XbaI, and cloned into pME18S, a plasmid for mammalian expression (14). The resulting BEC1 expression vector was termed pME-E1.

Northern Blot and RT-PCR Analysis of mRNA—Northern blot analysis with an amino acid sequence of human erg (6). R35526 (231 bases) is a conserved region of rat BEC1 cDNA with its 5′- and 3′-cDNA ends were determined by RACE. The results revealed a contig (3920 bases) of human BEC2 cDNA and the presence of only a silent mismatch (T17C) in the reverse primer. To construct an expression vector for human BEC2, the open reading frame in pCRII.1 and sequenced. The sequence has an open reading frame encoding 1087 amino acids with 96% identity to rat BEC2, suggesting that this cDNA encodes human BEC2. The internal pipette solution contained 125 mM KCl, 2 mM CaCl2,2m M glucose, 10 mM HEPES-Na (pH 7.4). All recordings were done at room temperature (25 °C). Tetraethylammonium (TEA) chloride and quinidine gluconate were used. The amplified fragment amplifies a 655-bp fragment corresponding to amino acids 598–815 of BEC2. Random-primed cDNAs were synthesized from each EST sequences causing to identification a cDNA (3610 bases) with an open-reading frame encoding 1083 amino acids (Fig. 1), showing 28–46% identity to K+ channels of the eag family. The greatest similarity (46% identity) is to rat

RESULTS

Identification of BEC1 and BEC2 Genes—Two erg-related sequences, the accession numbers R35526 and MT9045, were identified in the EST data base of GenBank using a BLAST search with an amino acid sequence of human erg (6). R35526 (3587 bases) has two regions encoding amino acid sequences with 45% and 52% identities to 22- and 23-amino acid portions around the S1 and S2 regions of human erg, respectively (smallest sum probability p < 0.0032). As described under “Experimental Procedures,” a series of RACE studies using primers derived from each EST sequences caused to identification a cDNA (3610 bases) with an open-reading frame encoding 1083 amino acids (Fig. 1), showing 28–46% identity to K+ channels of the eag family. The greatest similarity (46% identity) is to rat
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elk, which has been identified only recently (23). Rat eag and human erg share 30% and 33% sequence similarity with BEC1, respectively. We named this new protein the brain-specific eag-like channel 1, BEC1. Additionally, rat counterpart of the BEC1 gene was identified in rat brain mRNA, which encodes 1087 amino acids with 95% identity to human BEC1.

Another related gene, termed BEC2, was identified in rat or human brain mRNA (see “Experimental Procedures”). Both human and rat BEC2 consist of 1017 amino acids with 48% identity to BEC1. Amino acid sequences of BEC2 proteins are highly conserved between human and rat (89% identity), although the C-terminal part of BEC2 with about 400 amino acids is rather divergent, compared with BEC1.

**Primary Structure of BEC1 and BEC2—**The sequence alignment of BEC1 and BEC2 is shown in Fig. 1. BEC1 and BEC2 contain a hydrophobic core corresponding to the six transmembrane regions, S1–S6, and the pore region of voltage-gated K\(^+\) channels. The hydrophobic core is highly conserved between BEC1 (residues 227–508) and BEC2 (residues 229–482) with 70% identities. This sequence similarity is comparable to that among members in a given subfamily of all the known K\(^+\) channel superfamily genes. The putative voltage-sensing S4 and ion-conducting pore regions are also shared by BEC1 and BEC2. In the S4 region, only three amino acids are different between BEC1 and BEC2. The S4 regions of both BEC1 and BEC2 contain five positively charged residues found at every third position. Additionally, other two positively and one negatively charged residues are found at common positions. The pore regions of BEC1 and BEC2 have a GFG triplet, a common motif of eag-type K\(^+\) channels (6), and contain four distinct amino acids from each other. Potential N-glycosylation sites were found in the hydrophilic segments between the S5 and pore region; BEC1 contains three sites and BEC2 one site. An additional site was identified between the S3 and S4 region of BEC2. In the C-terminal region, there is a sequence with significant similarity to the CNB domain of cyclic nucleotide-binding proteins such as cyclic nucleotide-gated cation channels and hyperpolarization-activated cation channels. Although the CNB domains of BEC1 and BEC2 are homologous to eag-type K\(^+\) channels, they are markedly similar to each other (57% identity) among the cyclic nucleotide-binding proteins. These results indicate that BEC1 and BEC2 may be members of a new subfamily of eag-type K\(^+\) channels. Also, phylogenetic analysis suggests that these novel genes have a common ancestor with eag-type channels and represent an additional branch in the eag family (Fig. 2).

**Assignment of the BEC1 Gene in Human Genome—**Human EST R35526, which corresponds to BEC1, is a partial sequence of the clone 37299 in the human infant brain cDNA library 1NIB arrayed by IMAGE consortium (24). This clone has been termed DRES61 and assigned to the 12q13 region of the human genome using fluorescence in situ hybridization analysis by Banfi et al. (25). The complete sequence of DRES61 was recently submitted to GenBank (accession number U69184), and consists of 1088 bases, except a sequence of the adapter used in constructing the library. We performed sequence alignment of human BEC1 and DRES61. The alignment revealed that the 3‘-end sequence of DRES61 with 879 bases was identical to the 5‘-end sequence of human BEC1 upstream of codon 282, except a silent mismatch in codon 10 (CTT to CCG) of BEC1. An unique chromosomal region has been identified by fluorescence in situ hybridization with DRES61 cDNA, indicating that BEC1 is derived from an identical gene to DRES61. Taken together, the BEC1 locus is located in the 12q13 region.

**Tissue Distribution of BEC1 and BEC2—**Northern blot analysis revealed the presence of a 4-kb BEC1 transcript in human brain poly(A)\(^+\) RNA (Fig. 3A). The size of the mRNA concurs with the length of our identified cDNA. No signals were detected in other human tissues including the heart, placenta, liver, lung, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes. Within human brain, 4-kb transcripts of BEC1 were detected in cortical structures such as the cerebral cortex, amygdala, and hippocampus, and the striatal regions including the putamen and caudate nucleus (Fig. 3B). In these brain regions, an additional 6-kb transcript was identified, but it possessed weak signals. Expression of only the 6-kb transcript was found in human cerebellum, but the expression level was very low. BEC1 transcripts were not detectable in the spinal cord or in the corpus callosum, which contains primarily axons and glia. Thus, the expression of the BEC1 transcripts is largely restricted to the telencephalon in human tissues. The expression pattern of BEC1 contrasts with that of human erg, which displays a more ubiquitous distribution in the brain (Fig. 3B).

**Tissue distribution of BEC2 was also elucidated by Northern blot analysis of human poly(A)\(^+\) RNA. Multiple signals were detected only in the brain, with 4.4, 7.5, and 10 kb (Fig. 3A). We have identified BEC2 cDNA with 3920 bases, as described under “Experimental Procedures.” Considering an addition of a poly(A) tail, the 4.4-kb transcript probably corresponds to the identified cDNA. Since the signals were weak, tissue distribution of BEC2 was further determined by RT-PCR analysis. A
655-bp fragment corresponding to BEC2 was amplified only from the brain mRNA (Fig. 4). This fragment was generated when the plasmid carrying BEC2 cDNA was used as a template, but not BEC1 cDNA. These results suggest that BEC2 is exclusively expressed in the brain, as well as BEC1. Northern blot analysis of the brain regions indicated the BEC2 expression restricted to the telencephalon, similar to BEC1 (Fig. 3B). All size BEC2 transcripts were predominantly detected in the striatal regions such as the putamen and caudate nucleus. In addition, hybridization signals for BEC2 were detected in the cerebral cortex and hippocampus. The expression pattern of BEC2 is nearly parallel to that of BEC1, although the expression levels appear to be different in each region.

Cellular Localization of BEC1 within the Brain—In situ hybridization was performed using rat brain sections to determine the cellular localization of BEC1 within the brain. Hybridization signals were prominently found in the hippocampus, when only the antisense probe specific to BEC1 was used (Fig. 5A and B). Specific signals were also detectable in the cerebral cortex. In the hippocampus, in situ hybridization showed that BEC1 transcripts were concentrated in the pyramidal cell body layers of the CA1 and CA3 field and in the granule cell layers of the dentate gyrus. In the cerebral cortex, BEC1 signals were widely present from layer II to layer VI. Specific signals were detected in cell bodies of neurons with typical pyramidal shapes in the cerebral cortex (Fig. 5, C and D).

Electrophysiological Characteristics of BEC1—To characterize electrophysiological properties of BEC1 using the whole-cell voltage-clamp method, L929 cells were transiently transfected with the BEC1 expression vector. The cells were clamped at a holding potential of −90 mV and were depolarized to voltages between −60 and 100 mV. Depolarizing steps induced an outward current in BEC1-transfected cells (Fig. 6A, lower traces). When the voltage was stepped to potentials above 20 mV, the
outward current was rapidly inactivated and relaxed to a sustained plateau. The peak current amplitude induced by depolarization to 100 mV was 0.14–7.3 nA ($n=41$). Such responses were not observed in control cells (Fig. 6A, upper traces). Fig. 6B shows averaged current-voltage curves for the peak current within 40 ms and the current at the end of the 200-ms voltage pulse, which correspond to transient and steady-state currents, respectively. The transient current amplitude increased from −60 to 100 mV voltage-dependently. In contrast, the steady-state current amplitude increased up to −20 mV and then decreased with further depolarization.

For tail current analysis of BEC1, transfectants were depolarized to 80 mV from a holding potential of −70 mV, and then were repolarized to voltages between −20 and −120 mV (Fig. 6C). The BEC1 current was activated and inactivated by the 200-ms-long depolarizing steps. Following repolarization allowed recovery from inactivation and induced a tail current. The tail current reversed at approximately −80 mV in the bath solution containing 5.4 mM K$^+$. Considering the Nernst potential (−87 mV, 25 °C) for K$^+$, this result supports that BEC1 is a member of the K$^+$ channel family.

We examined the effect of two classical K$^+$ channel blockers, TEA and quinidine, on the BEC1 channel. Kv2.1 was used as a control K$^+$ channel. TEA (10 mM) reduced current amplitude of the Kv2.1 channel but did not affect that of the BEC1 channel in response to depolarization pulses (data not shown). In general, K$^+$ channels of the eag family are less sensitive to TEA, compared with the Shaker family (26, 27). Our results indicate that BEC1 also has the common feature. Conversely, sensitivities to quinidine are heterogeneous in the eag family. Quinidine potently inhibits the erg current with an IC$_{50}$ value of 0.9 μM (28), whereas the sensitivity of erg current is low (27). BEC1 was insensitive to 10 μM quinidine, which inhibited the channel activity of Kv2.1 (data not shown).

Stable BEC1 transfectants were constructed using dihydrofolate reductase gene-deficient CHO cells and the gene amplification induced by methotrexate. The 5C1–5 clone was selected as a transfectant expressing high amount of BEC1. This cell produced an outward current in response to depolarization

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Fig. 5. Cellular localization of BEC1 in rat brain. A and B, in situ hybridization analysis of rat brain coronal sections. Cx, the cerebral cortex; DG, the granule cell layer of the dentate gyrus; CA1 and CA3, the pyramidal cell layer of the CA1 and CA3 field. C, D, hybridization signals in rat cerebral cortex. The sections were hybridized with a digoxigenin-labeled antisense (A, C) or sense (B, D) probe corresponding to amino acids 840–1013 of rat BEC1. Scale bars: A and B, 1.5 mm; C and D, 50 μm.

Fig. 6. Electrophysiological studies of BEC1. A, voltage-dependent outward currents in L929 cells untransfected (control; upper traces) and transfected with BEC1 cDNA (lower traces). Cells were held at −90 mV, depolarized to voltages between −60 and 100 mV, and returned to −120 mV. B, the current-voltage relationships of the peak current within 40 ms (circle) or the current at the end of the 200-ms voltage pulse (square). The current amplitude at each potential is normalized to amplitude of the peak current recorded at 100 mV. Each point represents mean ± S.E. of 23–25 cells. C, tail current of the BEC1 channel. Cells were depolarized to 80 mV to activate and inactivate BEC1 channels, and then were repolarized to voltages between −20 and −120 mV to give a tail current. Holding potential was −70 mV.
BEC1 current was not observed in the BEC2 current. The inactivation process such as the inactivation process such as the depolarization steps induced an outward current in L929 cells and the whole-cell voltage-clamp method were used isolated a full-length cDNA of a third human gene, BEC3. Identification of BEC1 channel in this neural circuit.

**DISCUSSION**

This study describes new members of the voltage-gated K⁺ channel superfamily, BEC1 and BEC2, which are exclusively expressed in human brain. Although a number of voltage-gated K⁺ channel genes have been isolated from the mammalian brain, almost all of those genes are also expressed in other tissues such as the heart and skeletal muscle. Among the Shaker-type channel genes identified in the brain, Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv2.2, Kv4.2, and Kv4.3 are detectable in heart or skeletal muscle (29–31). Kv1.3 and Kv3.1 are expressed in lymphocytes (32, 33). Expression of mammalian erg also is predominant in heart (Ref. 12; Fig. 3A). Exclusive expression in the brain is a remarkable feature of BEC1 and BEC2. In addition, both messages are highly concentrated in the telencephalon of the human brain, suggesting that this channel may contribute to excitability of restricted neurons in the central nervous system. BEC1 messages in the hippocampus are prominently detected in the CA1 and CA3 pyramidal neurons and the dentate gyrus granule neurons, which constitute the trisynaptic excitatory pathway, a neural circuit important to establish long term synaptic potentiation and depression (34). Given the contribution of voltage-gated K⁺ channels to learning and memory, it is of high interest to study involvement of BEC1 channel in this neural circuit.

BEC1 belongs to the eag family of voltage-gated K⁺ channel. Identification of BEC2, a gene closely related to BEC1, reveals the existence of a new subfamily of eag-type channels. Both BEC1 and BEC2 are homologous to elk, a Drosophila putative K⁺ channel gene. The recently identified rat homolog of elk, rat elk1 (23), is not identical but is the most closely related to BEC1 and BEC2. Intriguingly, we have already isolated a full-length cDNA of a third human gene, BEC3. Rat elk1 seems the rat ortholog of BEC3, since this gene is 91% identical to BEC3. Thus, this subfamily consists of at least three mammalian homologs of elk.

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