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A family of potassium channel genes related to *eag* in *Drosophila* and mammals

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ABSTRACT **We have identified a conserved family of genes related to *Drosophila eag*, which encodes a distinct type of voltage-activated K^+ channel. Three related genes were recovered in screens of cDNA libraries from *Drosophila*, mouse, and human tissues. One gene is the mouse counterpart of *eag*; the other two represent additional subfamilies. The human gene maps to chromosome 7. Family members share at least 47% amino acid identity in their hydrophobic cores and all contain a segment homologous to a cyclic nucleotide-binding domain. Sequence comparisons indicate that members of this family are most closely related to vertebrate cyclic nucleotide-gated cation channels and plant inward-rectifying K^+ channels. The existence of another family of K^+ channel structural genes further extends the known diversity of K^+ channels and has important implications for the structure, function, and evolution of the superfamily of voltage-sensitive ion channels.**

Voltage-activated ion channels are members of evolutionarily conserved multigene families (1, 2). For example, the Shaker (*Sh*) family of K^+ channels comprises four related genes in *Drosophila*, each of which has one or more mammalian homologs (3). Together these genes define at least four subfamilies of voltage-activated K^+ channels within the *Sh* family. Most of our present understanding of the structure and function of K^+ channels is based on studies of the polypeptides encoded by these genes (4).

Analysis of other K^+ channels that are not members of the *Sh* family will expand our understanding of these channels. Genes encoding additional types of K^+ channels can be identified in *Drosophila* via molecular analysis of mutations affecting membrane excitability (5). Mutations of *eag*, identified by their leg-shaking phenotype, cause repetitive firing and enhanced transmitter release in motor neurons, suggesting a possible defect in K^+ channels (6, 7). Molecular studies revealed that *eag* encodes a polypeptide structurally related both to K^+ channels in the *Sh* family and to cyclic nucleotide-gated cation channels (8–10). Expression in *Xenopus* oocytes confirms that the eag polypeptide assembles into channels conducting a voltage-activated K^+-selective outward current (11, 12).

These results raise the question of whether *eag* is the prototype of a distinct K^+ channel gene family analogous to the *Sh* family. Here we use low-stringency screens and degenerate PCRs to isolate relatives of *eag* from *Drosophila*, mouse, and human tissues. Sequence alignments indicate the existence of a conserved multigene family of *eag*-related loci in *Drosophila* and mammals. The *eag* family has important implications for understanding the structure, function, and evolution of voltage-activated K^+ channels and cyclic nucleotide-gated cation channels.†

MATERIALS AND METHODS

Isolation of cDNA Clones Encoding a *Drosophila eag* Homolog. A 1682-bp *Acc* I/*Cla* I fragment of the *eag* cDNA CH20 (encoding the hydrophobic core) was used in a low-stringency screen [30% (vol/vol) formamide/5× SSCP (1× SSCP = 120 mM NaCl/15 mM sodium citrate/20mM sodium phosphate, pH 6.8)/0.1% SDS/10× Denhardt's solution/salmon sperm DNA (250 μg/ml) at 42°C; wash, 0.2× SSC/0.1% SDS at 42°C] of a *Drosophila* head cDNA library (13) (provided by B. Hamilton and E. Meyerowitz, California Institute of Technology, Pasedena, CA). Of three positive clones, one (*elk*) that hybridized to two nonoverlapping fragments from the *eag* hydrophobic core was isolated. This cDNA was used to screen a second head library (provided by T. Schwarz, Stanford University, Stanford, CA), at high stringency [2× SSCP/0.1% SDS/10× Denhardt's solution/salmon sperm DNA (250 μg/ml) at 65°C; wash, 0.2× SSC/0.1% SDS at 65°C] to obtain additional *elk* cDNAs.

PCR Amplification of a Mouse *eag* Homolog. Degenerate primers were *eag* oligonucleotide 1 [eag peptide sequence ACIWY—5′-CA(TC)TGGCTAGC(GATC)TG(TC)AT(ATC)-TGGTA-3′], oligonucleotide 2 [TYCDL—5′-GATGAATTC-(GATC)A(GA)(GA)TC(GA)CA(GA)TA(GATC)GT-3′], and oligonucleotide 3 [ILGKGD—5′-GCCGAATTC(GA)TC-(GATC)CC(TC)TT(GATC)CC(GATC)A(GA)(GAT)AT-3′]. PCR (35 cycles) conditions were 1 min at 95°C, 1 min at 42°C, and 1 min at 72°C with oligonucleotides 1 and 2 and random-primed mouse skeletal muscle cDNA (provided by P. Powers, University of Wisconsin, Madison) as template. An aliquot of the reaction mixture was separated on a 2% agarose gel and transferred to Zeta-Probe (Bio-Rad) membranes, and products corresponding to putative *eag* homologs were identified by cross hybridization to an *elk* probe at low stringency (low-stringency conditions as above except for 25% formamide). To enrich for a cross-hybridizing product of ≈850 bp, the remaining reaction mixture was separated on a 2% agarose gel and fragments of 750–950 bp were recovered. This product was reamplified by PCR under the conditions above but with oligonucleotides 1 and 3. The reamplified products were subcloned into pBluescriptII(KS+) (Stratagene) using the *Nhe* I and *Eco*RI sites in the primers. Subclones that hybridized at low stringency with an *elk* cDNA were partially sequenced (Sequenase version 2 kit, United States Biochemical).

Isolation and Sequence Analysis of Mammalian *eag*-Like cDNA Clones. Mouse *eag* (m-*eag*) cDNA clones were isolated from a mouse brain cDNA library (λZAPMB, provided by J. Boulter and S. Heineman, Salk Institute, San Diego). Human *erg* (h-*erg*) cDNA clones were isolated from a human hippocampus cDNA library (catalogue no. 936205; Stratagene). Both libraries were screened at high stringency (see above).

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Abbreviations: cNBD, cyclic nucleotide binding domain; ORF, open reading frame; m-eag, mouse eag; h-erg, human erg.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. *elk*, U04246; m-*eag*, U04294; h-*erg*, U04270).

After subcloning into pBluescript vectors (Stratagene), selected cDNAs were sequenced on both strands as above.

Chromosome Mapping. The chromosomal location of h-*erg* was determined by PCR using h-*erg*-specific primers and a series of hamster–human hybrid cell lines. The primers (GACGTCGTCGTGGCCATCCT, derived from an h-*erg* exon, and CAACTATGGTCGAAAGAGCT, derived from a downstream intron present in an incompletely spliced cDNA) give a 600-bp product from human but not hamster genomic DNA templates. PCR was performed on DNA from 25 hamster–human hybrid cell lines of known karyotype (Bios, New Haven, CT); lines giving the 600-bp fragment were identified by gel electrophoresis of the amplification products.

RESULTS

An *eag* Homolog in *Drosophila*. An initial low-stringency screen of a *Drosophila* head cDNA library with an *eag* cDNA probe identified a cDNA with an incomplete open reading frame (ORF) that was clearly related to the eag protein. This cDNA, designated *elk* (*eag*-like K^+ channel; Table 1), was then used to screen a second head cDNA library at high stringency. Several overlapping cDNAs were obtained yielding a composite *elk* sequence that contained a long ORF encoding a polypeptide of 1284 amino acids with a predicted molecular mass of 141 kDa (Fig. 1). Although the proposed initiator Met is not a good match to the *Drosophila* translation start site consensus [TCCCATG compared to CAA(A/C)ATG, where the Met codon is underlined; ref. 21], the strong similarity of the elk and eag polypeptides in this N-terminal region suggests that this is the true translation start site (Fig. 1).

The *elk* locus was mapped by *in situ* hybridization to polytene chromosome region 54F3–55A2 on the right arm of the second chromosome (data not shown). Flies heterozygous for deletions of this region do not exhibit any overt locomotor defects.

Conservation of the *eag* Sequence in Mouse. The existence of an *eag* gene family in *Drosophila* raised the question of whether counterpart genes were present in mammalian genomes (see ref. 3). Because low-stringency screens of a mouse brain cDNA library with the same *eag* probe used to isolate *elk* were unsuccessful, we used a PCR screen as an alternative strategy. Three highly conserved regions between the eag and elk polypeptides were used to design degenerate oligonucleotide primers for a series of nested PCRs with mouse skeletal muscle cDNA. Among the products from the reaction, an ≈850-bp fragment hybridized to an *elk* probe at low stringency. Sequence analysis revealed that the subcloned fragment encodes a peptide segment with significant similarity to the corresponding region in *eag* and *elk* (≈56% and ≈38% identity, respectively, over a stretch of 157 amino acids).

The cloned PCR fragment was used to screen a mouse brain library at high stringency to obtain more complete cDNAs, two of which overlapped to yield the composite sequence of the complete ORF (Fig. 1). Two closely spaced Met codons are present at the N terminus. Comparison of the sequences flanking the first Met (GTCGGGAGGATGG) and second Met (AGGATGACCATGA) codons (underlined) with the vertebrate translation start site consensus sequence [GCCGCC(A/G)CCATGG, refs. 21 and 22] indicates that translation of the mouse cDNA could begin at either one to specify a polypeptide of either 989 or 987 amino acids with a predicted molecular mass of ≈111 kDa (Fig. 1). However, because the second Met codon aligns with the initiating Met codon of *eag* and *elk*, we believe it is more likely that translation starts there. The very strong similarity of the polypeptide encoded by this cDNA to that specified by *eag* (Table 1) suggests that the mouse gene is the counterpart of *eag*, and therefore, we name it mouse-*eag* (m-*eag*).

Isolation and Mapping of a Human *eag* Homolog. Because ion-channel structural genes are potential targets for neurological disorders that have a hereditary component (see ref. 23), it was of interest to identify human homologs of *eag*. A human hippocampus cDNA library was screened at high stringency with the m-*eag* PCR fragment. The single cDNA recovered was used to isolate several overlapping cDNAs in a second screen of the library. Although sequence analysis showed none of the cDNAs was complete, each shared substantial similarity to members of the *eag* family. The composite ORF assembled from these cDNAs was clearly incomplete because it began in-frame within a conserved domain at the N terminus without a Met codon. Missing sequence was obtained by screening a genomic cosmid library with a human cDNA fragment. A positively hybridizing cosmid clone was digested with *Sau*3A and probed with a fragment from the 5′ end of the cDNA. An exon was identified that overlapped the composite cDNA by 47 bp and

Table 1. Amino acid identity between ion channel families

	eag	m-eag	h-erg	elk	KAT1	AKT1	cAMP	cGMP	slo	Shab	ShB	Shal	Shaw
eag	**100**	**65**	**49**	**49**	25	26	24	20	15	17	12	13	10
m-eag		**100**	**49**	**47**	26	27	27	25	13	16	13	12	12
h-erg			**100**	**49**	24	27	24	23	16	15	15	17	13
elk				**100**	24	26	25	20	17	14	11	13	12
KAT1					**100**	**62**	23	24	19	11	11	12	13
AKT1						**100**	25	24	18	11	14	14	14
cAMP							**100**	**68**	15	11	13	14	11
cGMP								**100**	14	11	14	13	11
slo									**100**	19	21	19	18
Shab										**100**	**46**	**39**	**42**
ShB											**100**	**43**	**47**
Shal												**100**	**41**
Shaw													**100**

Multiple sequence alignment of the hydrophobic cores (S1–S6) of the following ion channel polypeptides was generated using the PILEUP multiple sequence analysis program from the GCG sequence analysis software package version 7.0 (14): eag family members eag, m-eag, h-erg, and elk; the bovine retinal cGMP-gated cation channel (15); the rat olfactory cAMP-gated cation channel (16); the inward-rectifying potassium channels AKT1 and KAT1 from plants (17–19); the *Drosophila* slo calcium-activated potassium channel (20); and the Sh voltage-activated potassium channel family members Kv4, Shaw, RK5, Shal, Rck1, ShB, Drk1, Shab, Ik8, and K13 (2). This table was derived from the multiple sequence alignment using the DISTANCES multiple sequence analysis program from the GCG package (14). The percentage of amino acid identity between each pairwise combination was determined by dividing the number of identical amino acid residues by the length of the shorter sequence without gaps. The percent identity for each ion channel family is shown in boldface type.

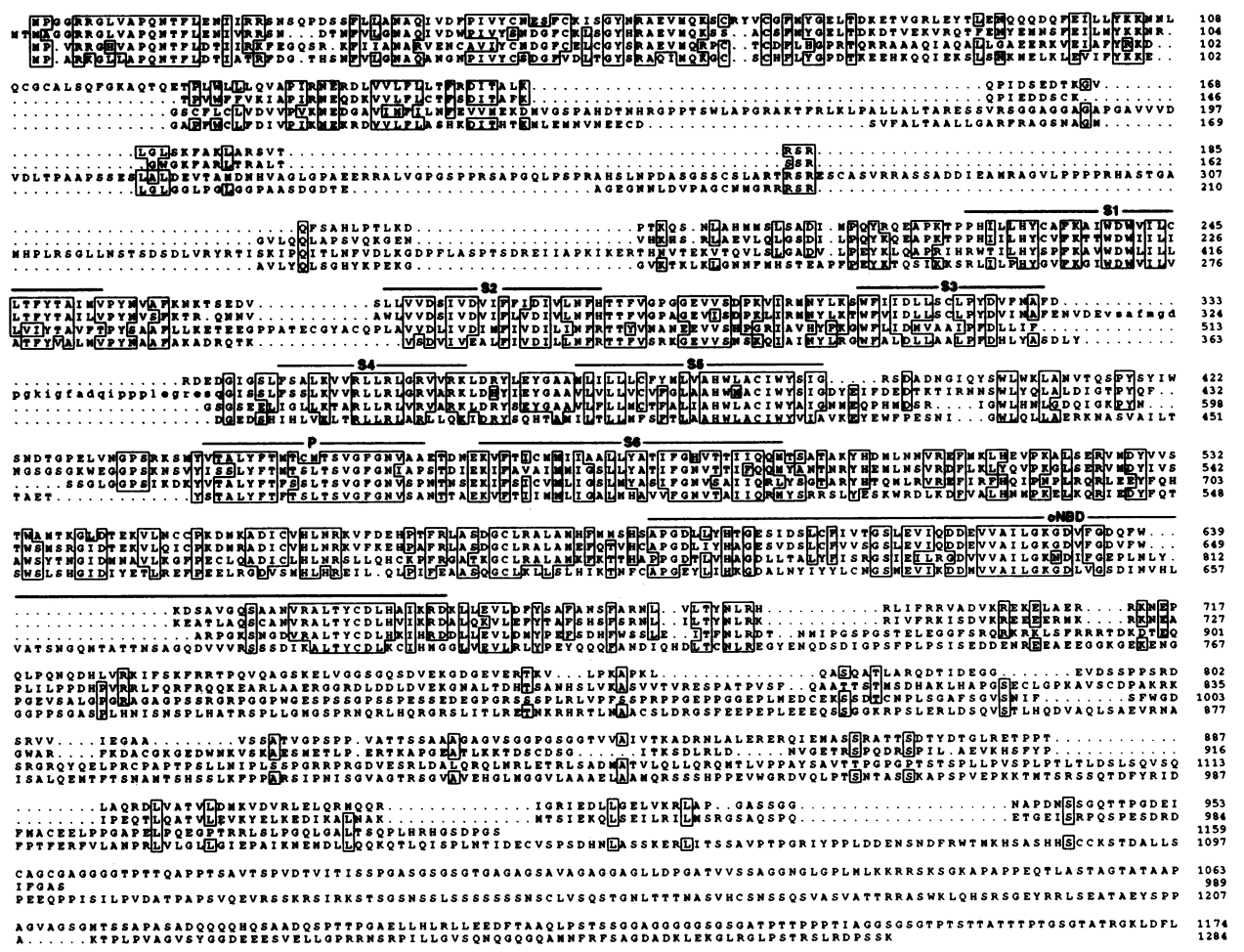

FIG. 1. Amino acid sequence alignment of the *eag* family of K^+ channel polypeptides. The alignment was generated using the PILEUP multiple sequence analysis program from the GCG sequence analysis software package version 7.0 (14). Positions with identical amino acids in at least three of the four sequences are boxed. Gaps in the alignment are indicated by periods. Amino acid residues are numbered on the right. Residues 1–13 of h-erg were determined from genomic DNA; the remaining sequence was determined from overlapping cDNA clones (see text). The seven hydrophobic domains and the putative cNBD are overlined. Amino acid residues in lowercase type in the m-eag sequence denote an alternatively spliced exon identified by comparison of different cDNAs. All four polypeptides share at least two potential postranslational modifications: a consensus site for N-glycosylation (Asn-424 in eag, Asn-433 in m-eag, Asn-444 in elk, and Asn-598 in h-erg) and a consensus site for potential protein kinase C phosphorylation (Thr-499 in eag, Thr-509 in m-eag, Ser-513 in elk, and Thr-670 in h-erg).

that extended the ORF up to and including a putative initiating Met codon. We believe this is the initiator Met because the additional 13 amino acids, including the position of the initiating Met, align well with the other three *eag* family members and also because the sequence preceding this codon (GGGCTCAGGATGC) is a good match to the vertebrate translation start site consensus sequence. Starting from this Met codon, the human gene encodes a polypeptide of 1159 amino acids with a predicted molecular mass of 127 kDa (Fig. 1). Because the human sequence is equidistant from the other three *eag* family members (47–49% identity within the hydrophobic core; see Table 1), it is probably not the human counterpart of either *eag* or *elk* but represents a third branch of the *eag* family. Therefore, we designate it *h*uman-*eag*-*r*elated gene (h-*erg*).

A set of oligonucleotide primers that specifically amplified an ≈600-bp genomic fragment of the human gene was used to determine its presence or absence in each of 25 hamster–human hybrid cell lines carrying a known subset of human chromosomes. Only human chromosome 7 showed perfect concordance for the h-*erg* PCR product, indicating that h-*erg* is located on this chromosome (data not shown).

Structural Features of the *eag* Family of K^+ Channel Subunits. The additional *eag* family members isolated here are shown in a protein sequence alignment with *Drosophila eag* in Fig. 1. Table 1 summarizes the degree of amino acid identity both within this family and with a large group of other ion channel proteins. It is clear from Fig. 1 that all *eag* family members share a similar overall structure. This includes a central hydrophobic core that contains seven hydrophobic domains corresponding to the six putative transmembrane α-helical domains (S1–S6) and the pore region (P) analogous to those found in the *Sh* family of voltage-activated K^+ channels (4). The hydrophobic core is flanked by long presumptive cytoplasmic domains at the N and C termini. Although the overall length of the four polypeptides is comparable, ranging between 989 and 1284 amino acids, the length of the flanking cytoplasmic domains is variable. h-*erg* has the longest N-terminal domain but the shortest C-terminal region. The C terminus of the m-eag polypeptide, the smallest family member, is also much shorter than that of either eag or elk. All of the hydrophobic segments are well conserved among the family members, although some segments show an especially high degree of similarity. The positively charged amino acids in the S4 segment, the putative voltage sensor, are identical among the four polypeptides with only two exceptions: an Arg → Gln substitution at position 386 in the elk polypeptide and an Arg → His substitution at position 370 in the m-eag polypeptide. The pore region is also highly conserved, including a stretch of 8

Eag	Y	V	T	A	L	Y	F	T	M	T	C	M	T	S	V	G	F	G	N	V	A	A	E	T	D	N	E
M-Eag	Y	I	S	S	L	Y	F	T	M	Y	S	L	T	S	V	G	F	G	N	I	A	P	S	T	D	I	E
H-Erg	Y	V	T	A	L	Y	F	T	F	S	S	L	Y	S	V	G	F	G	N	V	S	P	N	T	N	S	E
Elk	Y	S	T	A	L	Y	F	T	F	T	S	L	T	S	V	G	F	G	N	V	S	A	N	T	T	A	E
KAT1	Y	V	T	A	L	Y	W	S	I	T	T	L	T	T	T	G	Y	G	D	F	H	A	E	N	P	R	E
AKT1	Y	V	T	S	M	Y	W	S	I	T	T	L	T	T	V	G	Y	G	D	L	H	P	V	N	T	K	E
cAMP	Y	I	Y	C	L	Y	W	S	T	L	T	L	T	T	I	G	-	E	T	P	P	P	V	K	D	E	E
cGMP	Y	V	Y	S	L	Y	W	S	T	L	T	L	T	T	I	G	-	E	T	P	P	P	V	R	D	S	E
Slo	Y	W	T	C	V	Y	F	L	I	V	T	M	S	T	V	G	Y	G	D	M	Y	C	E	T	V	L	G
Shab	I	P	E	A	F	W	W	A	G	I	T	M	T	T	V	G	Y	G	D	I	C	P	T	T	A	L	G
ShB	I	P	D	A	F	W	W	A	V	V	T	M	T	T	V	G	Y	G	D	M	T	P	V	G	V	W	G
Shal	I	P	A	A	F	W	Y	T	I	V	T	M	T	T	V	G	Y	G	D	M	V	P	E	T	I	A	G
Shaw	I	P	L	G	L	W	W	A	L	V	T	M	T	T	V	G	Y	G	D	M	A	P	K	T	Y	I	G

FIG. 2. Amino acid sequence of the pore forming region of various members of the superfamily of voltage-sensitive K^+ channels. Amino acid residues identical to the eag sequence are boxed (see Table 1 for description of channel genes included here).

amino acids (residues 452–459 of eag) in which there is only a single conservative substitution in m-eag (Figs. 1 and 2). Several of the amino acids within this region, such as Ser-453, Phe-456, and Asn-458, uniquely distinguish the pore of the *eag* family from all known members of the *Sh* family. Another very highly conserved hydrophobic segment in the *eag* family occurs in S5 (residues 410–421 of eag). In this region of 12 amino acids, all family members have at least 10 amino acid identities and no more than one nonconservative substitution. Again, this region is distinctive for the *eag* family because it has no similarity to the corresponding region of the *Sh* family. The presumed extracellular segments linking S1 to S2 and S3 to S4 vary in length and sequence within the family. In contrast, the linkers between S2 and S3 and between S4 and S5, which are presumed to be cytoplasmic, have considerable sequence similarity and are identical in size.

The similarity among members of the *eag* family also extends beyond the hydrophobic core of the polypeptides. Although the length of the N-terminal sequence is variable, the first ≈150 amino acids have been very well conserved. Also, in the cytoplasmic region downstream from S6, all four polypeptides contain a segment that shares substantial similarity to the cyclic nucleotide-binding domain (cNBD) present in cyclic nucleotide-gated cation channels from vertebrate photoreceptors and olfactory epithelia (24, 25). This segment represents one of the most conserved regions within the *eag* family. The putative cNBD of the elk, m-eag, and h-erg polypeptides share 45%, 84%, and 51% identity to that of eag, respectively. In the C-terminal region downstream from the putative cNBD, there is little sequence similarity among family members.

All *eag* family members have numerous potential sites for posttranslational modification. At least two of these potential posttranslational modifications are shared among all four polypeptides (Fig. 1): a consensus site for N-glycosylation located 12–17 amino acids upstream of the pore region and a consensus site for protein kinase C phosphorylation located 2–4 amino acids downstream of S6.

A Multigene Family of K^+ Channel Subunits. A dendrogram displaying the similarities among various ion channel polypeptides is shown in Fig. 3. Genes encoding voltage-activated K^+ channels (the *Sh* and *eag* families), a Ca^{2+}-activated K^+ channel (slo), inward-rectifying K^+ channels (KAT1 and AKT1), and cyclic nucleotide-gated cation channels are thought to share a common ancestral origin (2). The dendrogram shows that this large group of channel proteins can be seen as two distinct groupings: the first composed of the *Sh* family of voltage-activated K^+ channels plus the slo Ca^{2+}-activated K^+ channel and the second consisting of ion channel polypeptides with a functional or putative cNBD. However, it is important to note that these groupings do not depend on the presence of the cNBD itself, because the dendrogram was constructed solely on the basis of the hydrophobic cores.

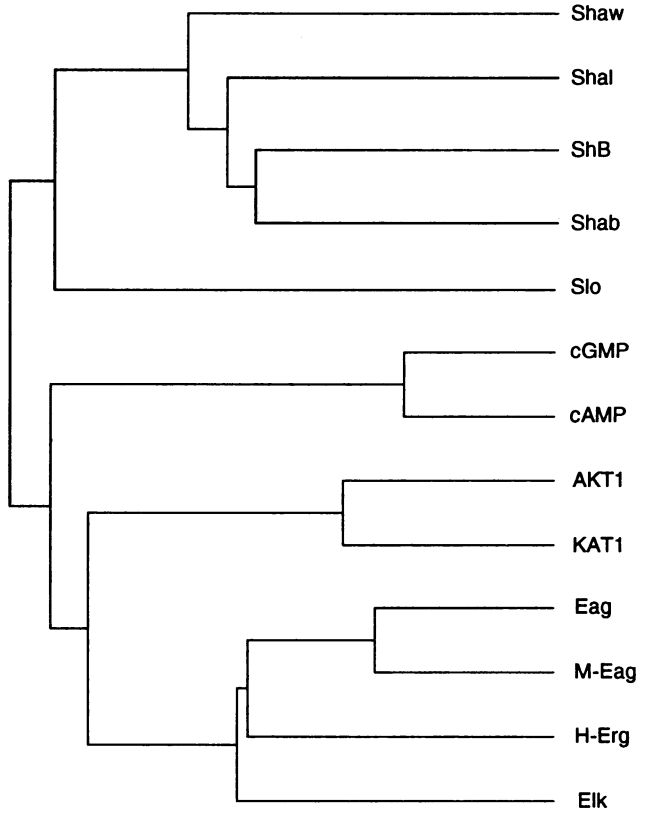

FIG. 3. Dendrogram of representative members of the superfamily of voltage-sensitive ion channels. The horizontal branch lengths are inversely proportional to the similarity between the sequences. This dendrogram is derived from an unweighted pair-group method using arithmetic averages comparison of the S1–S6 domains of the channels listed in Table 1 using the PILEUP multiple sequence analysis program from the GCG sequence analysis software package version 7.0 (14).

Sequence similarities have previously been noted between the *Sh* family and the plant inward-rectifying channels, AKT1 and KAT1 (17–19), as well as the vertebrate cyclic nucleotide-gated cation channels (26). However, as indicated in Fig. 3 and summarized in Table 1, sequence comparisons based only on the hydrophobic core demonstrate that these other channel types are more closely related to the *eag* family than to the *Sh* family. For example, in the hydrophobic core there are 36 amino acid identities between AKT1 and ShB, 58 between AKT1 and a cGMP-gated cation channel, and 68 between AKT1 and eag (data not shown). Although the similarities between *eag* and the plant channels are generally distributed throughout the entire hydrophobic core, the proximal portion of the pore region (residues 438–445 of eag) is especially well conserved. In this region, KAT1 and eag share 6 amino acid identities and two conservative substitutions (Fig. 2), whereas Sh has only limited similarity to either KAT1 (two identities) or eag (one identity).

DISCUSSION

Our results show that *eag*, m-*eag*, *elk*, and h-*erg* define a family of K^+ channel genes in *Drosophila* and mammals. Because of their high degree of similarity, *eag* and m-*eag* probably represent equivalent genes in *Drosophila* and mouse. In contrast, h-*erg* diverges equally from *eag* and *elk* and thus represents a distinct family member in humans. A mammalian counterpart of *elk* and a *Drosophila* counterpart of h-*erg* are predicted. In fact, we have recently isolated a mammalian cDNA encoding a member of the *elk* subfamily.

Thus, the *eag* family seems to contain at least three subfamilies analogous to those of the *Sh* family. Genes defining additional *eag* subfamilies may be identified by more exhaustive screens.

The striking hyperexcitability of *eag* mutants demonstrates the importance of eag channels in maintaining normal neuronal excitability in *Drosophila* (6, 7). The strong conservation of eag polypeptide sequences from *Drosophila* to mammals suggests preservation of *in vivo* functions as well. Mapping h-*erg* more precisely on chromosome 7 is of interest to determine whether it is linked to any heritable neurological disorders, such as those associated with seizures.

The existence of an *eag* family of K^+ channel genes indicates that K^+ channel diversity *in vivo* is even greater than has previously been inferred. If coassembly of polypeptides occurs within the *eag* family or between the *eag* and *Sh* families, the potential number of distinct K^+ channels becomes still larger. Reduction in the amplitude of four distinct K^+ currents by *eag* mutations, including the A-current abolished by *Sh* mutations, may indicate that *eag* encodes a shared component of different K^+ channels (27, 28). However, further experiments are necessary to determine whether this interpretation is correct.

Inspection of the amino acid similarities and differences between the *eag* and *Sh* families provides some interesting insights into structure–function relationships. For example, in the pore region, all *eag* family proteins have Asn at the position corresponding to residue 458 of eag (Fig. 2). In contrast, all *Sh* family proteins contain Asp at this site (2). Substituting Asn for Asp at this position in the *Sh* family proteins abolishes channel activity (29). Nonetheless, the activity and K^+ selectivity of the eag channel demonstrate that Asn at this position is not necessarily incompatible with K^+ channel function (11, 12). Elucidating the basis of this difference between the pore region properties should prove informative. Conversely, the invariant amino acids between the *eag* and *Sh* family proteins could identify critical residues under severe evolutionary constraints.

A distinctive feature of the *eag* family is the homology to cNBDs of cyclic nucleotide-gated cation channels and cyclic nucleotide-activated protein kinases (24, 25). A 10–15% increase in macroscopic current in the presence of 2 μM cAMP but not cGMP has been reported for eag channels in inside-out macropatches and it is suggested that this modulation may involve direct binding to the channel (12). However, unlike the vertebrate cyclic nucleotide-gated cation channels, which are relatively voltage-insensitive, activation of eag channels shows a very steep voltage dependence (11, 12). In addition, whereas cyclic nucleotide-activated cation channels show little selectivity among monovalent and divalent cations, eag is strongly selective for K^+ over Na^+. The *eag* family may thus be an evolutionary link between voltage-activated K^+ channels and cyclic nucleotide-gated cation channels with intermediate structural and functional properties.

The phylogeny of these ion channel families as well as the inward-rectifying K^+ channels in plants is still uncertain. However, conservation of a putative cNBD and a portion of the pore among the *eag* family, the plant channels, and cyclic nucleotide-gated channels indicates that these features are ancient, predating the evolutionary separation of plants and animals. Additional studies of the *eag* family and its relationship to other channel families should, therefore, provide further insights into the structure, function, and evolution of K^+ channels and their immediate relatives.

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