Raven Press, Ltd., New York

Molecular Cloning, Expression, and Pharmacological Characterization of humEAA1, a Human Kainate Receptor Subunit

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Abstract: Kainate is a potent neuroexcitatory agent; its neurotoxicity is thought to be mediated by an ionotropic receptor with a nanomolar affinity for kainate. In this report, we describe the cloning of a cDNA encoding a human glutamate ionotropic receptor subunit protein from a human hippocampal library. This cDNA, termed humEAA1, is most closely related to rat and human cDNAs for kainate receptor proteins and, when expressed in COS or Chinese hamster ovary cells, is associated with high-affinity kainate receptor binding. We have successfully established cell lines stably expressing humEAA1. This is the first report of establishment of stable cell lines expressing a glutamate receptor subunit. The relative potency of compounds for displacing [^{3}H]kainate binding of humEAA1 receptors expressed in these stable cell lines was kainate > quisqualate > domoate > L-glutamate ≫ (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid > dihydrokainate > 6,7-dinitroquinoxaline-2,3-dione > 6-cyano-7-nitroquinoxaline-2,3-dione. Homooligomeric expression of humEAA1 does not appear to elicit ligand-gated ion channel activity. Nevertheless, the molecular structure and pharmacological characterization of high-affinity kainate binding of the humEAA1 expressed in the stable cell line (ppEAA1-16) suggest that the humEAA1 is a subunit protein of a human kainate receptor complex. **Key Words:** Excitatory amino acid receptor—High-affinity kainate receptor—Human glutamate receptor subunit—cDNA cloning—Mammalian expression.
J. Neurochem. **62,** 1–9 (1994).

Excitatory amino acid (EAA) receptors are found throughout the mammalian brain and represent the predominant transmitter receptors mediating synaptic excitation in the CNS. The majority of excitatory synapses in the CNS use glutamate as their neurotransmitter. Glutamate receptors play an important role in CNS physiology and pathology (Monaghan et al., 1989). Based on electrophysiological and pharmacological studies, these receptors have been classified into four main subclasses: *N*-methyl-D-aspartate (NMDA), (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and metabotropic types. Molecular studies have now demonstrated that these receptors are highly heterogeneous. At least six different G protein-coupled (metabotropic) glutamate receptors are known to exist, each as a single protein with seven putative membrane-spanning domains (Masu et al., 1991; Tanabe et al., 1992). Even more complex are the ionotropic glutamate receptors, which are heteromeric protein complexes with multiple subunits, each possessing four transmembrane regions, and arranged to form a ligand-gated ion channel (Barnard and Henley, 1990). Based on molecular structure and selective agonist activation, subunit proteins for ionotropic glutamate receptors can be divided into NMDA, AMPA, and kainate types. The recent molecular cloning studies of EAA receptor subunits from each of the major subclasses have confirmed this classification. NMDA receptor protein subunits that exhibit homomeric or heteromeric channel activity when activated have recently been cloned from the rat (Moriyoshi et al., 1991; Monyer et al., 1992; Sugihara et al., 1992) and mouse (Kutsuwada et al., 1992; Meguro et al., 1992; Yamazaki et al., 1992). AMPA receptor proteins that have been cloned include rat GluR1, GluR2, GluR3, and GluR4. When expressed as homomeric or heteromeric complexes, they form ligand-gated cation-permeable ion channels that are activated by the agonists AMPA, kainate, or quisqualate (Hollman et al., 1989;

Received January 27, 1993; revised manuscript received April 27, 1993; accepted May 20, 1993.

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Abbreviations used: 1*S*,3*R*-ACPD, 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CHO, Chinese hamster ovary; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EAA, excitatory amino acid; NMDA, *N*-methyl-D-aspartate; PCR, polymerase chain reaction.

Boulter et al., 1990; Keinanen et al., 1990). The human homologues of rat GluR1, designated GluH1 (Puckett et al., 1991) and HBGR1 (Sun et al., 1992), have also been cloned.

Rat GluR5 homomeric receptor channels show a very small response to glutamate only when its cDNA is expressed in *Xenopus* oocytes (Bettler et al., 1990). Using a mammalian expression system, Sommer et al. (1992) showed that rat GluR5 is capable of forming homomeric channels that can be gated by domoate, kainate, and glutamate. In addition, a rat kainate receptor protein (GluR6) has been identified, which forms homomeric receptor-operated ion channels that are activated by kainate, quisqualate, and glutamate but not AMPA (Egebjerg et al., 1991). Recently cloned rat GluR7 (Bettler et al., 1992) exhibits high sequence identity with glutamate receptor subunits GluR5 and GluR6. The structurally related proteins rat KA1 (Werner et al., 1991), rat KA2 (Herb et al., 1992), mouse γ2 (Sakimura et al., 1992), and human EAA2 (Kamboj et al., 1992) have also been cloned. Rat GluR7, rat KA1, rat KA2, mouse γ2, and human EAA2 exhibit [^{3}H]kainate binding but when expressed in a homomeric manner do not exhibit any receptor-operated ion channel properties. Nevertheless, the molecular structure and binding pharmacology of these glutamate receptor subunits suggest that these subunits are a component of the heteromeric receptor complex. Rat KA2 (Herb et al., 1992) and mouse γ2 (Sakimura et al., 1992) subunits have been shown to interact with the GluR6 subunit when coexpressed, resulting in new properties, such as AMPA-sensitive channels, including the formation of agonist-sensitive channels.

The molecular cloning of these cDNAs has led to significant advances in our understanding of various subtypes of glutamate receptors. Human glutamate receptors are of great medical importance because of their postulated role in the mediation of learning and memory acquisition. Also, EAAs can be highly toxic to neurons, and dysfunction of this neurotransmitter system has been implicated in several neurological disorders, such as Alzheimer's disease, Huntington's chorea, epilepsy, Parkinson's disease, amyotrophic lateral sclerosis, AIDS encephalopathy, and dementia complex (Gasic and Hollman, 1992). The central involvement of EAA receptors in the human brain has prompted our efforts toward the isolation, cloning, and functional characterization of these receptors from human brain. Availability of these human brain cloned receptors would allow in vitro drug screening and drug design to discover and develop new receptor-specific drugs. Nonhuman mammalian models are not suitable for this purpose despite high homology of receptor sequences, as minute differences can cause dramatic pharmacological variation between species homologues of the same receptor (Oksenberg et al., 1992). Therefore, drug–receptor interactions should not be extrapolated from animal to human species without verification.

Here we report the cloning, molecular structure, expression in mammalian stable cell lines, and pharmacological characterization of a human kainate receptor protein, termed humEAA1. This humEAA1 appears to be a human homologue of rat KA1 (Werner et al., 1991) and exhibits [^{3}H]kainate binding characteristics suggesting that it is a subunit protein of a human kainate receptor complex.

MATERIALS AND METHODS

Isolation of cDNA for humEAA1

To isolate the cDNA for humEAA1 receptor subunit, the nucleotide sequences of the rat GluR1 receptor subunit (Hollmann et al., 1989), chicken kainate binding protein (Gregor et al., 1989), and frog kainate binding protein (Wada et al., 1989) were compared, to identify regions of homology capable of serving as primer annealing sites for polymerase chain reaction (PCR)-based amplification as described by Kamboj et al. (1992). The following oligonucleotide primers having nonhybridizing flanks bearing *Hin*dIII restriction sties were then synthesized:

5′ GGGGTTTAAGCTTGAGCGT-

CGTCCTCTTCCTGGT 3′

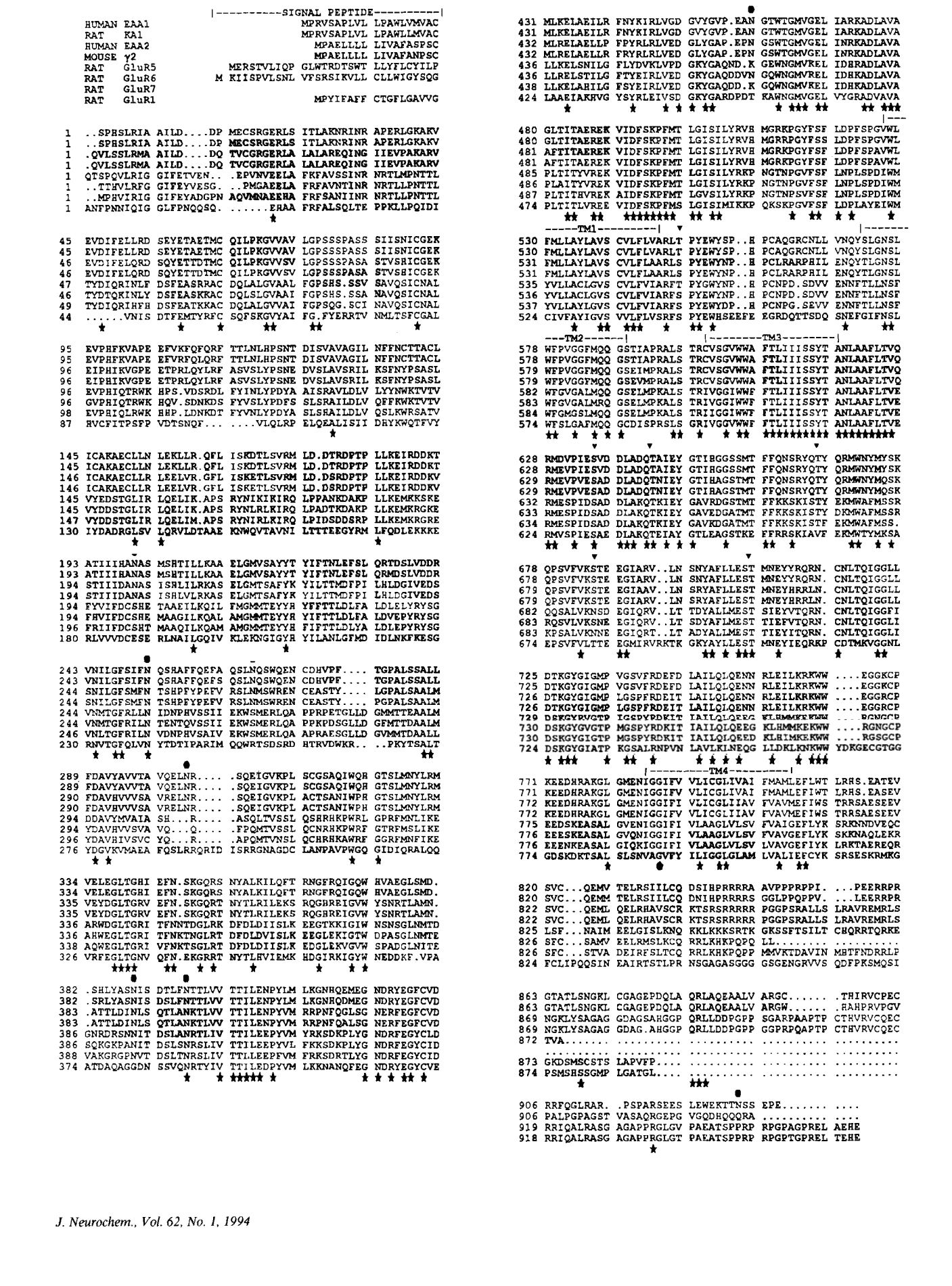
5′ GGGGTTTAAGCTTGTGAAG-

AACCACCAGACGCCG 3′

Using human hippocampal cDNA as template and the oligonucleotide primers and reaction conditions for PCR as described by Kamboj et al. (1992), the resulting PCR product having an expected nucleotide length (239 bp) was then purified following agarose gel electrophoresis and subcloned into the phagemid vector pTZ19 (Pharmacia). A comparison of the nucleotide sequence (amplified fragment) with the rat GluR1 revealed only ~60% identity, indicating that a fragment from a novel human gene had been isolated.

To isolate the cDNA encoding the entire human receptor subunit, a bacteriophage λgt10-based library of human hippocampus cDNA (Clontech) was screened using a PCR-generated, radiolabeled ([α-^{32}P]dCTP) version of the 239-bp amplification product. Of 1×10^6 clones screened, probing identified 60 putative clones under the high-stringency hybridization conditions as described by Kamboj et al. (1992). DNA inserts were subcloned into the pTZ18 vector for DNA sequence analysis. Sequencing revealed one partial clone harboring, internally, a region with a nucleotide sequence identical to the sequence of the 239-bp probe. Because the cDNA library did not appear to contain a full-length clone, an alternative human hippocampus cDNA library constructed in bacteriophage λZAPII (Stratagene) was screened by using a PCR-generated radiolabeled version of the subclone. Screening of 1×10^6 clones of this library by hybridization under the stringency conditions described by Kamboj et al. (1992) led to the selection of 50 positive clones. For sequencing, pBluescript-SK phagemids carrying the inserts were excised. DNA sequence analysis identified two clones sharing a sequence overlap. One clone carrying a 2.2-kb insert and representing a 5′ region of the open reading was designated pBS/RKLS181. The overlapping clone,

FIG. 1. Nucleotide sequence of the cDNA encoding the humEAA1 subunit and its deduced amino acid sequence. Nucleotides are numbered in the 5′- to 3′-terminal direction, starting with the first nucleotide of the codon for the putative amino-terminal residue of the mature subunit. Nucleotides −1 to −60 encode a putative signal peptide. Numbers of the nucleotide and amino acid residues are given to the right of each line.



carrying a 3.1-kb insert and representing the remaining 3′ region of the open reading frame, was designated pBS/RKLS161. These overlapping clones were used to construct a full-length cDNA containing the entire open reading frame.

Expression of humEAA1 in mammalian cells

For transient expression in mammalian cells, cDNA coding for the human EAA1 receptor was incorporated into the mammalian expression vector pcDNA1 (Invitrogen). COS-1 cells were transfected with 8 μg of DNA (as pcDNA1-humEAA1) per 10^8 COS cells, by diethylaminoethyl-mediated DNA transfection, and were treated with chloroquine essentially as described by Kamboj et al. (1992).

For stable expression in mammalian cells, cDNA coding for the human EAA1 receptor was incorporated into the mammalian expression vector pRC/CMV (Invitrogen). Chinese hamster ovary (CHO) cells were transfected with 3 μg of DNA (as pRC/CMV-humEAA1) per 5×10^5 CHO cells, by the calcium phosphate-DNA coprecipitation procedure, as described by Sambrook et al. (1989). Cells resistant to neomycin were selected in 10% fetal bovine serum-supplemented α-minimal essential medium containing G418. Individual colonies of G-418-resistant cells were isolated ~2–3 weeks later and then propagated for northern blot analysis and for subsequent use in ligand binding assays. A cell line stably expressing humEAA1, designated as ppEAA1-16, was established and propagated for further ligand binding studies.

[^{3}H]Kainate binding

Binding studies were performed essentially as described by Kamboj et al. (1992). Frozen CHO cells from the stable cell line expressing the human EAA1 subunit were lysed, by suspension in ice-cold purified water, and then centrifuged for 20 min at 50,000 *g*. The resulting membrane pellets were frozen at −80°C for at least 24 h. For binding assays, to remove any endogenous glutamate, the membrane preparations were washed by resuspending in >100 volumes of 50 m*M* Tris-HCl buffer (pH 7.5 at 5°C) and centrifuged for 10 min at 50,000 *g*.

[^{3}H]Kainate binding experiments (including displacement studies using nonradioactive competitive ligands) were performed by incubating washed membranes (100–150 μg of protein per sample) with [^{3}H]kainate (5 n*M*) in the same buffer as used for washing, in a total volume of 1 ml. L-Glutamate (1 m*M*) was used to define nonspecific binding. The binding reaction was performed in an ice bath for 60 min after addition of the membrane suspension. Bound ligand was separated from free ligand by rapid filtration through Whatman GF/B filters. The saturation and Scatchard data were analyzed with the aid of the computer software program INPLOT (Graph Pad, San Diego, CA, U.S.A.).

Materials

AMPA, kainic acid, NMDA, domoic acid, quisqualic acid, dihydrokainate, 6,7-dinitroquinoxaline-2,3-dione (DNQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) were purchased from Tocris Neuramin (Essex, U.K.). L-Glutamate (disodium salt) was from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

RESULTS

The PCR-mediated DNA amplification, using human hippocampal cDNA as template, the oligonucleotide primers, and reaction conditions for PCR as described by Kamboj et al. (1992), resulted in generation of an amplified product having an expected nucleotide length (239 bp). A comparison of the nucleotide sequence (amplified fragment) with the rat GluR1 (Hollmann et al., 1989) revealed only ~60% identity, indicating that a fragment from a novel human gene had been isolated. A human brain cDNA library derived from hippocampus was screened using this PCR-generated probe.

Two overlapping cDNA clones encoding a human glutamate receptor subunit, designated as humEAA1, were isolated and subjected to DNA sequence analysis. The nucleotide sequence analysis of the cloned cDNA revealed an open reading frame encoding 956 amino acid residues (Fig. 1). An analysis of the deduced amino acid sequence of humEAA1 shows that the amino-terminus has a stretch of hydrophobic amino acids, serving as a putative leader sequence. The first 20 amino acids are likely to be cleaved off to form the mature protein, which is predicted to start with a serine residue at the amino-terminus (von Heijne, 1986). The proposed mature protein consists of 936 amino acids, with a calculated molecular weight of 105,080.

The AMPA/kainate/NMDA receptor subunits are thought to conform to a structure in which a large amino-terminal extracellular domain is followed by a region containing four transmembrane domains (TM1–TM4). The locations of these transmembrane domains in the humEAA1 protein sequence are similar to those proposed for various subunits of AMPA/kainate/NMDA receptors (Keinanen et al., 1990) (Fig. 2). Based on this assignment, humEAA1 consists of a 526-amino-acid N-terminal extracellular domain, followed by a region containing four putative transmembrane domains (TM1, spanning residues

FIG. 2. Alignment of the deduced amino acid sequence of the human EAA1 receptor subunit with seven published rat, mouse, and human glutamate receptor subunits. The sequences of rat GluR1, rat GluR5, rat GluR6, rat GluR7, rat KA1, mouse γ2, human EAA2, and the human EAA1 polypeptide were aligned with the aid of the computer program Pileup [the sequence analysis software package by Genetics Computer Group, Inc. (Devereux et al., 1984)]. Dotted lines indicate gaps introduced for better alignment. Asterisks indicate positions at which the identical amino acid is found. All polypeptide sequences are numbered from the proposed mature N-terminus. The predicted signal peptide sequences and transmembrane regions TM1–TM4 are marked. Solid circles indicate potential *N*-linked glycosylation sites in the humEAA1; solid triangles indicate calmodulin-dependent protein kinase type II consensus phosphorylation sites (30) in the predicted intracellular domains (between TM1 and TM2 and between TM3 and TM4).

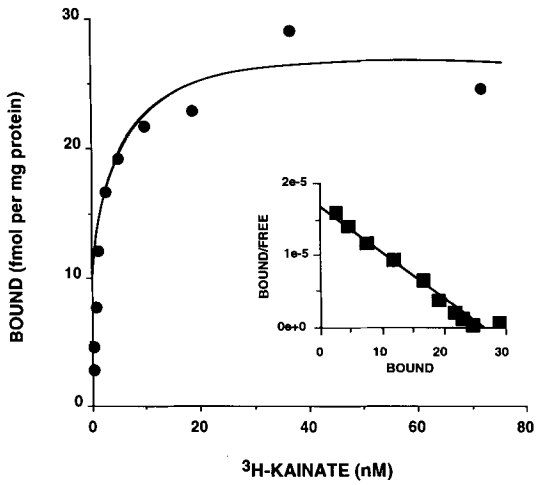


FIG. 3. Representative saturation and Scatchard (**inset**) plots of [^{3}H]kainate binding to humEAA1-expressing stable cell line CHO cell membranes. Washed membranes were incubated with [^{3}H]-kainate in the absence (total binding) or presence (nonspecific binding) of 1 m*M* L-glutamate in a 1-ml volume at 0°C for 1 h. Bound ligand was separated from free ligand by rapid filtration. Data are expressed as amount of specific [^{3}H]kainate bound (total minus nonspecific).

527–546, inclusive; TM2, spanning residues 571–589; TM3, spanning residues 600–618; and TM4, spanning residues 785–805), and, finally, an extracellular carboxy-terminal domain of 131 amino acid residues.

The predicted human EAA1 polypeptide shares significant amino acid identity with rat and human glutamate receptor subunits: GluR1, 35.3%; GluR2, 35.4%; GluR3, 35.5%; GluR4, 34.4%; GluR5, 41.8%; GluR6, 44.2%; GluR7, 41.6%; KA1, 94.2%; KA2, 69.0%; humEAA2, 69.0%; and NMDAR1, 25.2% (see Hollman et al., 1989; Keinanen et al., 1990; Bettler et al., 1990, 1992; Egebjerg et al., 1991; Moriyoshi et al., 1991; Werner et al., 1991; Herb et al., 1992; Kamboj et al., 1992). The human EAA1 has ~37% amino acid sequence identity with the cloned chicken kainate binding protein (Gregor et al., 1989) and frog kainate binding protein (Wada et al., 1989). Sequence conservation is most striking within the region encompassed by the transmembrane domains, where various cloned AMPA/kainate receptor subunits share >50% sequence identity with the human EAA1 receptor subunit. This would predict that the humEAA1 polypeptide is a glutamate-gated ion channel receptor subunit. The human EAA1 receptor subunit shares ~94% amino acid identity with rat KA1 (Werner et al., 1991) and appears to be a human homologue of rat KA1. The human EAA1 receptor subunit differs most strikingly from this rat receptor in the proposed carboxy-terminal extracellular domain (Fig. 2). A stretch of 40 amino acid residues at the extreme carboxy-terminus, starting at residue Cys^{896}, has no significant identity with the corresponding region in rat KA1. Also, the proposed mature rat KA1 protein is composed of 935 amino acid residues, whereas the predicted human EAA1 mature protein is

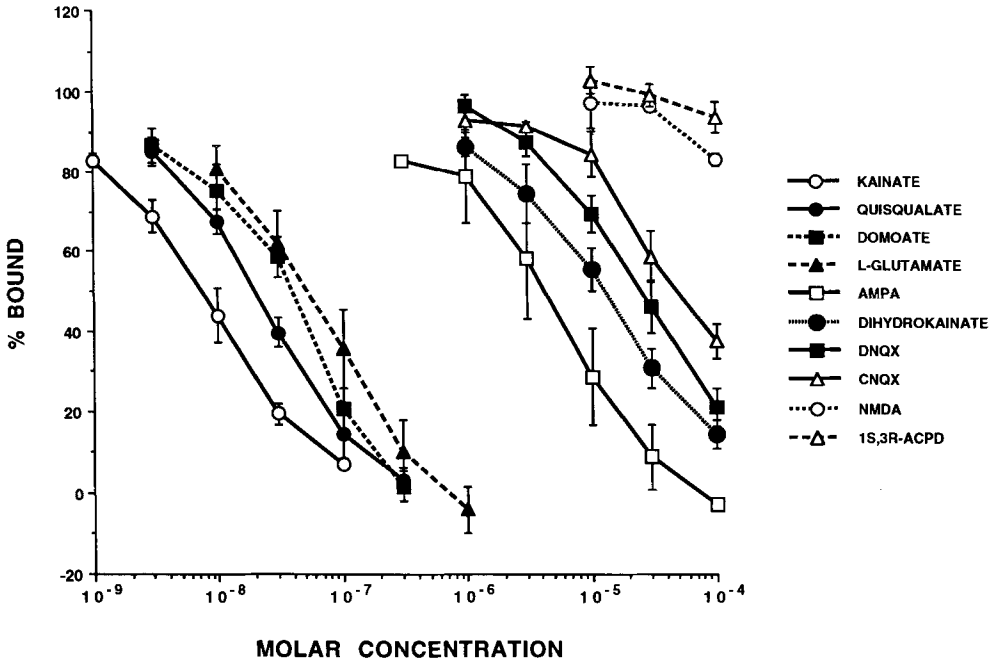


FIG. 4. Displacement curves of [^{3}H]kainate binding to humEAA1-expressing stable cell line CHO cell membranes by EAA analogues. Washed humEAA1-expressing stable cell line CHO cell membranes were incubated with [^{3}H]kainate (5 n*M*). Nonspecific binding was determined with 1 m*M* L-glutamate. Data are mean ± SE (bars) values, expressed as percentages of specific [^{3}H]kainate binding from three experiments, performed in triplicate.

TABLE 1. *Affinities of EAA analogues for [^{3}H]kainate binding to humEAA1 receptors expressed in CHO cells from a stable cell line (ppEAA1-16)*

Compound	K_i (n*M*)	Hill coefficient
Kainate	2.29 ± 0.43	0.936 ± 0.042
Quisqualate	6.43 ± 1.36	1.036 ± 0.043
Domoate	10.5 ± 2.22	1.111 ± 0.073
L-Glutamate	18.3 ± 6.59	1.147 ± 0.077
AMPA	1,970 ± 1,260	1.093 ± 0.068
Dihydrokainate	3,840 ± 983	0.859 ± 0.139
DNQX	8,490 ± 2,550	0.961 ± 0.013
CNQX	17,200 ± 4,490	0.920 ± 0.043
1*S*,3*R*-ACPD	>100,000	—
NMDA	>100,000	—

Data are mean ± SE values of three separate experiments, each performed in triplicate. [^{3}H]Kainate binding was conducted with a radioligand concentration of 5 n*M*. Reactions were initiated by addition of washed membrane suspensions, and samples were incubated on ice in a 1-ml volume per sample for 1 h. Protein content per tube was ~150 μg. Reactions were terminated by rapid filtration through Whatman GF/B filters.

composed of 936 amino acid residues. The human EAA1 subunit has eight potential *N*-glycosylation sites within the proposed amino-terminal extracellular domain, which are also present in rat KA1. The human EAA1 subunit has one potential *N*-glycosylation site within the proposed carboxy-terminal extracellular domain, and this site is not present in rat KA1. The proposed intracellular domains between TM1 and TM2 and between TM3 and TM4 contain consensus phosphorylation sites for Ca^{2+}-calmodulin-dependent protein kinase type II and protein kinase C (Kemp and Pearson, 1990). These enzymes have been suggested to play an important role in the induction and maintenance of long-term potentiation (Kennedy, 1989). The conservation of some cysteine residues and some putative *N*-glycosylation and phosphorylation sites in human EAA1 indicates structural features similar to the previously described glutamate receptor subunits (Fig. 2).

Human EAA1-specific mRNA was transcribed in vitro and injected into *Xenopus* oocytes, to test whether this subunit can form a homooligomeric ion channel. We did not record any responses to the application of glutamate receptor agonists in a large number of oocytes tested. We did not detect any indication of ion channel activity when humEAA1 RNA was analyzed in combination with RNA encoding the humEAA2 subunit (H. Sudan and P. N. R. Usherwood, personal communication).

CHO cell lines stably expressing humEAA1 were established as described in Materials and Methods. This is the first report describing successful establishment of stable cell lines expressing a glutamate receptor subunit. The binding of selective EAA ligands to washed and dialyzed membranes prepared from the CHO cells stably expressing humEAA1 was examined, and high-affinity binding of [^{3}H]kainate was found. In saturation analysis experiments (three experiments), [^{3}H]kainate bound with a K_D of 2.3 ± 0.37 n*M* and a B_{max} of 20.8 ± 4.0 fmol/mg of protein. Figure 3 shows a representative saturation curve and Scatchard plot from these experiments. When the NMDA receptor ligand [^{3}H]CGS-19755 (10 n*M*) (Murphy et al., 1988) or the AMPA receptor ligand [^{3}H]AMPA (5 n*M*) (Honoré et al., 1982) was used, no specific binding was observed (data not shown). Kainate was the most potent displacer of [^{3}H]kainate (5 n*M*) binding, followed by quisqualate, domoate, and then L-glutamate (Fig. 4). The K_i values for these compounds were in the nanomolar range (Table 1). AMPA, dihydrokainate, and the quinoxalinedione AMPA receptor antagonists, DNQX and CNQX, exhibited affinity constants for this receptor in the micromolar range. NMDA, as well as the selective metabotropic (G protein-coupled) EAA agonist 1*S*,3*R*-ACPD, did not affect [^{3}H]kainate binding at up to 100 μ*M* (Fig. 4 and Table 1). The relative potency of compounds in displacing [^{3}H]kainate binding was kainate > quisqualate > domoate > L-glutamate ⩾ AMPA > dihydrokainate > DNQX > CNQX (Fig. 4). This pharmacological profile is similar to that of the cloned humEAA2 (Kamboj et al., 1992) receptor subunit profile, which was kainate > quisqualate > domoate > L-glutamate ⩾ DNQX > dihydrokainate > CNQX > AMPA; however, humEAA1 has considerably lower affinities for dihydrokainate, DNQX, and CNQX.

DISCUSSION

We have isolated a new member of the human EAA receptor gene family, humEAA1, that has a nanomolar affinity for kainate. Human EAA1 has ~35% amino acid sequence identity with the cloned rat AMPA (GluR1–GluR4) receptor subunits and ~37% amino acid sequence identity with the cloned chicken kainate binding protein (Gregor et al., 1989) and frog kainate binding protein (Wada et al., 1989). It has higher sequence identity with the cloned rat kainate [GluR5–GluR7 (42–44%)] receptor subunits and has even higher sequence identity with the cloned rat, mouse, and human high-affinity kainate (KA1, 94%; γ2, KA2, and humEAA2, 69%) receptor subunits. The human EAA1 receptor subunit appears to be a human homologue of rat KA1. It differs most strikingly from the rat KA1 (Werner et al., 1991) receptor subunit in the proposed carboxy-terminal extracellular domain. A stretch of 40 amino acid residues at the extreme carboxy-terminus, starting at residue Cys^{896}, has no significant identity with the corresponding region in rat KA1. There are only five of 40 amino acid residues that are identical in this region. The functional significance of this difference is not known at present. Also, the proposed mature rat KA1 protein is composed of 935 amino acid residues, whereas the

predicted mature humEAA1 protein is composed of 936 amino acid residues. The deduced amino acid sequence of human EAA1 is entirely consistent with the proposed structure of subunits of ligand-gated ion channels, which is based on four membrane-spanning α-helices following an extracellular amino-terminal domain. Sequence conservation between humEAA1 and other glutamate receptor subunits is most striking within the region encompassed by the transmembrane domains, and also there is conservation of some cysteine residues and some putative *N*-glycosylation and phosphorylation sites in humEAA1; this would predict that the humEAA1 polypeptide is a glutamate-gated ion channel receptor subunit.

We did not detect ion channel activity when humEAA1 RNA was injected into *Xenopus* oocytes either singly or in combination with RNA encoding the humEAA2 subunit. The electrophysiological results suggest that either the expression of human EAA1 in *Xenopus* is poor or that humEAA1 encodes a subunit that requires at least one additional subunit, other than humEAA2, to form a fully functional receptor-activated ion channel. Absence of homooligomeric channel activity has been reported for other subunits of the EAA receptor family, such as the kainate binding subunit protein from chick brain (Gregor et al., 1989) or frog brain (Wada et al., 1989), rat GluR7 (Bettler et al., 1992), rat KA1 (Werner et al., 1991), rat KA2 (Herb et al., 1992), mouse γ2 (Sakimura et al., 1992), and humEAA2 (Kamboj et al., 1992). However, the rat KA2 subunit has been shown to interact with rat GluR5 and GluR6 subunits to produce new properties, such as AMPA-sensitive channels, including the formation of agonist-sensitive channels (Herb et al., 1992). The functional data that was shown for mouse γ2 (enhanced kainate-induced currents when coexpressed with mouse GluR6) also strengthen the contention that these proteins are subunits of heteromeric kainate ionotropic EAA receptor complexes (Sakimura et al., 1992).

However, humEAA1 has pharmacological characteristics that strongly suggest it is a subunit protein for a kainate-type of ionotropic EAA receptor. Human EAA1 receptor protein expressed in the stable cell line exhibited high-affinity [^{3}H]kainate binding with a K_D of 2.3 ± 0.37 n*M*. This value is in close agreement with K_D values for the high-affinity [^{3}H]kainate binding sites found in brain membranes (Monaghan et al., 1989) and also with [^{3}H]kainate binding site on cells expressing rat KA1 (Werner et al., 1991). Although two high-affinity [^{3}H]kainate binding sites (K_D of 5 and 50 n*M*) have been identified using brain tissues (Monaghan et al., 1989), we observed only one [^{3}H]-kainate binding site on cells expressing humEAA1. The rank order of displacement affinities was kainate > quisqualate > domoate > L-glutamate ≫ AMPA > dihydrokainate > DNQX > CNQX ≫ NMDA = 1*S*,3*R*-ACPD. This is similar to that of the rank order of potency that was reported for the recently described humEAA2 (Kamboj et al., 1992) receptor subunit, which was kainate > quisqualate > domoate > L-glutamate ≫ DNQX > dihydrokainate > CNQX > AMPA ≫ NMDA = 1*S*,3*R*-ACPD, but humEAA1 has considerably lower affinities for dihydrokainate, DNQX, and CNQX. The humEAA1 pharmacological profile is also similar to the rank order of potency that was reported for the recently described rat KA1 receptor (Werner et al., 1991). Like humEAA1, rat KA1 also exhibits high-affinity [^{3}H]kainate binding but does not appear to support channel activity when expressed as a homomeric receptor complex. Furthermore, the pharmacology of [^{3}H]kainate binding displacement was also similar to that observed when channel activity was studied using the rat GluR6 receptor clone (Egebjerg et al., 1991). Agonist potency at the rat GluR6 receptor was kainate > quisqualate > L-glutamate ≫ AMPA, and the quinoxalinedione AMPA antagonist CNQX had a K_i of 4 μM. [^{3}H]Kainate binding of humEAA1 exhibited the same rank order of agonist potency; however, it has a considerably lower K_i value for CNQX (17.2 μM). The affinity of CNQX for the human EAA1 was about fourfold lower than that for the rat GluR6 receptor protein but was ~33-fold lower than that for cloned AMPA receptors. For example, the K_i value for CNQX using the rat GluR1 receptor was 0.519 μM (Dawson et al., 1990). This is consistent with previous evidence that quinoxalinedione AMPA antagonists can block the electrophysiological effects of kainate and are selective but not specific for AMPA receptors (Honoré et al., 1988). The pharmacological profile of humEAA1 differs from that of the rat GluR6 and rat GluR7 subunits. Human EAA1 has a 30–40-fold higher affinity for kainate than the rat GluR6 and rat GluR7 (Bettler et al., 1992). Human EAA1 has a lower affinity for domoate than kainate, whereas rat GluR6 and rat GluR7 have higher affinities for domoate than kainate (Bettler et al., 1992).

HumEAA1 has the characteristics of a subunit of a human high-affinity kainate receptor, based on its molecular structure and binding pharmacology. The successful cloning of the humEAA1 cDNA should therefore lead to a better understanding of the molecular nature of the high-affinity kainate receptors and their role in normal and diseased human CNS. Because drug–receptor interactions should not be extrapolated from animal to human species without verification (Oksenberg et al., 1992), the availability of a human high-affinity kainate receptor subunit would allow for drug screening and drug design of receptor-selective drugs based on human receptors.

Acknowledgment: We thank Professor Eric A. Barnard (Royal Free Hospital, London) for his advice and helpful discussion during the course of this work. We wish to thank C. Elliott, R. Fantaske, M. Deverill, and G. Peskleway for their excellent technical assistance with cDNA cloning,

DNA sequencing, and cell culture. We also thank Dr. R. Foldes for discussion and critical reading of the manuscript.

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