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ACCELERATED COMMUNICATION

Molecular Structure and Pharmacological Characterization of humEAA2, a Novel Human Kainate Receptor Subunit

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SUMMARY

A cDNA encoding a novel human glutamate receptor subunit protein was isolated from a human hippocampal library. This cDNA, termed humEAA2, is most closely related to rat cDNAs for kainate receptor proteins and, when expressed in COS cells, is associated with high affinity kainate receptor binding. The relative potency of compounds in displacing [^{3}H]kainate binding was kainate > quisqualate > domoate > L-glutamate ≫ 6,7-dinitroquinoxaline-2,3-dione > dihydrokainate > 6-cyano-7-nitroquinoxaline-2,3-dione > (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. Homomeric expression of humEAA2 does not appear to elicit ligand-gated channel activity. Nevertheless, the molecular structure and pharmacology of high affinity kainate binding suggest that humEAA2 is a novel subunit protein of a human kainate receptor complex.

Glutamate receptors play an important role in central nervous system physiology and pathology (1). Molecular studies have now demonstrated that these receptors are highly heterogeneous. At least five different guanine nucleotide-binding protein-coupled (metabotropic) glutamate receptors are known to exist, each as a single protein with seven putative membrane-spanning domains (2, 3). Even more complex are the iontropic glutamate receptors, which are heteromeric protein complexes with multiple subunits, each possessing four transmembrane regions and all arranged to form a ligand-gated ion channel (4). Based on molecular structure and selective agonist activation, subunit proteins for iontropic glutamate receptors can be divided into "NMDA," "AMPA," and "kainate" types. An NMDA receptor protein (NMDA-R1) that exhibits homomeric channel activity when activated has recently been cloned from the rat (5). 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 (6–8). Rat GluR5 homomeric receptor channels show very small responses only to glutamate, when their cDNA is expressed in *Xenopus* oocytes (9).

Additionally, GluR6 is a kainate receptor protein identified from rat, which forms homomeric receptor-operated ion channels that are activated by kainate, quisqualate, and glutamate, but not AMPA (10). A structurally related protein, rat KA1, has also been cloned (11). Rat KA1 is a putative kainate receptor subunit that exhibits high affinity [^{3}H]kainate binding but, when expressed in a homomeric manner, does not exhibit any receptor-operated ion channel properties. Nevertheless, the molecular structure and binding pharmacology of rat KA1 suggest that it is a component of the heteromeric receptor complex and might have influence over the ion channel properties of the native receptor.

Here we describe the cloning, molecular structure, and pharmacological characterization of a structurally novel human kainate receptor protein, humEAA2 (to distinguish it from the human gene equivalent of rat GluR1, termed gluH1) (12). humEAA2 is structurally related but not identical to rat KA1 and exhibits [^{3}H]kainate-binding characteristics, suggesting that it is a novel subunit protein of a human kainate receptor complex.

Materials and Methods

Isolation of cDNA for humEAA2. As a first step in the isolation of cDNA for the humEAA2 receptor subunit, the nucleotide sequences of the rat GluR1 receptor subunit (6), chicken kainate-binding protein (13), and frog kainate-binding protein (14) were compared, to identify regions of homology capable of serving as primer-annealing sites for PCR-based amplification. The following oligonucleotide primers, hav-

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; 1*S*,3*R*-ACPD, 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid; PCR, polymerase chain reaction; kb, kilobases; bp, base pairs.

ing nonhybridizing flanks bearing *Hind*III restriction sites, were then synthesized: 5′-GGGGTTTAAGCTTGAGCGTCGTCCTCTT-CCTGGT-3′ and 5′-GGGGTTTAAGCTTGTGAAGAACCACCA-GACGCCG-3′. Using human hippocampal cDNA as template, the primers were used to amplify homologous sequences by the PCR technique. Reaction mixtures contained, in 100 μl, 100 ng of human hippocampal cDNA, 125 pmol of each primer, and 2 units of *Thermus aquaticus* DNA polymerase (in 10 mM Tris·HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, with 0.2 mM of each deoxyribonucleotide). There were then performed 30 PCR cycles of 94° for 1 min, 58° for 1 min, and 72° for 2 min, followed by a final cycle of 72° for 30 min. The PCR product having the expected nucleotide length (239 bp) was then purified from the gel and subcloned for sequencing into phagemid vector pTZ19 (Pharmacia). A comparison of the nucleotide sequence (amplified fragment) with the rat GluR1 revealed only about 60% identity, indicating that a fragment from a novel human gene had been isolated.

To isolate cDNA coding for the entire humEAA2 receptor subunit, a λgt10-based library of human hippocampus cDNA (Clontech) was probed by using a PCR-generated, [α-^{32}P]dCTP-labeled version of the 239-bp amplification product. Of 1×10^6 clones screened, probing identified 60 putative clones under the following hybridization conditions: 6 × standard saline citrate, 50% formamide, 5% Denhardt's solution, 0.5% sodium dodecyl sulfate, 100 μg/ml denatured salmon sperm DNA. Hybridizations were carried out at 37° overnight, and filters were washed with 2 × standard saline citrate/0.5% sodium dodecyl sulfate at 25° for 5 min, followed by a 15-min wash at 50° for 15 min.

After plaque purification, the DNA inserts were subcloned into the pTZ18 vector for sequence analysis. Sequencing revealed one partial clone harboring, internally, a region with a nucleotide sequence similar 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 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 detailed above led initially to the selection of 47 positive clones. For sequencing, pBluescript-SK phagemids carrying the inserts were excised. Sequencing analysis identified two clones sharing a sequence overlap. One clone, carrying a 1.8-kb insert and representing a 5′ region of the open reading frame, was designated pBS/RKLS311. The overlapping clone, carrying a 2.4-kb insert and representing the remaining 3′ region of the open reading frame, was designated pBS/RKLS151. These overlapping clones were used to construct a full-length cDNA containing the entire open reading frame.

Expression of humEAA2 in COS cells. For transient expression in mammalian cells, cDNA coding for humEAA2 was incorporated into the mammalian expression vector pcDNA1 (Invitrogen). This is a multifunctional 4.2-kb plasmid vector designed for cDNA expression in eukaryotic systems and cDNA analysis in prokaryotes. COS-1 cells were transfected with 8 μg of DNA (as pcDNA1/humEAA2) per 10^6 COS cells, by DEAE-mediated DNA transfection, and were treated with chloroquine, according to the procedures described by Sambrook *et al.* (15). Cells were allowed to grow for 3 days in 10% fetal bovine serum-supplemented medium. At the end of the incubation period, dishes were placed on ice and washed with ice-cold phosphate-buffered saline, and the cells were removed by scraping. Cells were harvested by centrifugation at 1000 rpm for 10 min, and the cellular pellet was frozen in liquid nitrogen, for subsequent use in ligand binding assays. Northern blot analysis confirmed expression of receptor-encoding cDNA in COS cells.

[^{3}H]Kainate binding. Frozen transfected COS cells 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° for at least 24 hr. For binding assays, in order to remove any endogenous glutamate while preserving the integrity of the membrane-receptor association, the membrane preparations were dialyzed for 24 hr in >500 volumes of 50 mM Tris·HCl buffer, pH 7.5, at 5°, using Spectrapor 7 dialysis tubing (molecular weight cutoff, 8000).

[^{3}H]Kainate binding experiments (including displacement studies using nonradioactive competitive ligands) were performed by incubating dialyzed membranes (50–100 μg of protein/sample) with [^{3}H]kainate (5 nM), in the same buffer as used for dialysis, in a total volume of 1 ml. L-Glutamate (1 mM) was used to define nonspecific binding. The binding reaction was out carried 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 that had been previously soaked in 0.3% polyethylenimine (16).

Materials. NMDA, AMPA, kainic acid, domoic acid, quisqualic acid, DNQX, CNQX, 1*S*,3*R*-ACPD, and dihydrokainate were purchased from Tocris Neuramin (Essex, England). L-Glutamate (disodium salt) was from Sigma Chemical Co. (St. Louis, MO).

Results

The nucleotide sequence analysis of the cloned cDNA revealed an open reading frame encoding 980 amino acid residues (Fig. 1). An analysis of the deduced amino acid sequence of humEAA2 shows that the amino terminus has a stretch of hydrophobic amino acids, serving as a leader sequence. The first 18 amino acids are likely to be cleaved off to form the mature protein, which is predicted to start with a glutamine residue at the amino terminus (17). The predicted mature protein consists of 962 amino acids and has a calculated molecular weight of 107,176. 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 to TM4). The locations of these transmembrane domains in the humEAA2 protein sequence are similar to those proposed for various subunits of AMPA/kainate/NMDA receptors (8) (Fig. 2). Based on this assignment, humEAA2 consists of a 527-amino acid amino-terminal extracellular domain, followed by a region containing four putative transmembrane domains (TM1 spanning residues 528–547, inclusive, TM2 spanning residues 572–590, TM3 spanning residues 601–619, and TM4 spanning residues 786–806) and, finally, an extracellular carboxyl-terminal domain of 156 amino acid residues.

The predicted humEAA2 polypeptide shares significant amino acid identity with rat glutamate receptor subunits (GluR1, 34.7%; GluR2, 34.8%; GluR3, 34.8%; GluR4, 33.8%; GluR5, 43.4%; GluR6, 45.2%; KA1, 67.6%; and NMDAR1, 25.2%) (see Refs. 5–11). Sequence conservation is most striking within the region encompassed by the transmembrane domains, where various rat AMPA/kainate receptor subunits share >52% sequence identity with the humEAA2 receptor subunit. This would predict that the humEAA2 polypeptide is a glutamate-gated ion channel receptor subunit. The extracellular amino-terminal and carboxyl-terminal domains of humEAA2 exhibit only low sequence identity with any of the other published glutamate receptor subunits. The humEAA2 subunit has 10 potential *N*-glycosylation sites within the proposed amino-terminal extracellular domain.

In vitro transcribed RNA from humEAA2 cDNA was injected into *Xenopus* oocytes, to test whether this subunit can form a homo-oligomeric ion channel. We did not record any responses to the application of glutamate receptor agonists in a large number of oocytes tested.[1] Nevertheless, after transient expres-

[1] H. Sudan and P. N. R. Usherwood, unpublished observations.

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-54                                                    -1
ATGCCGGCTGAGCTGCTGCTGCTGCTGATTGTTGCCTTCGCCAGCCCCAGCTGCCAGGTGCTCTCATCACTGCGCATGGCTGCAATCCTGGATGATCAGACAGTGTGT      54
MetProAlaGluLeuLeuLeuLeuLeuIleValAlaPheAlaSerProSerCysGlnValLeuSerSerLeuArgMetAlaAlaIleLeuAspAspGlnThrValCys      18
-18                                                   -1   1

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GlyArgGlyGluArgLeuAlaLeuAlaLeuAlaArgGluGlnIleAsnGlyIleIleGluValProAlaLysAlaArgValGluValAspIlePheGluLeuGlnArg      54

GACAGCCAGTACGAGACCACGGACACCATGTGTCAGATCTTACCCAAAGGGGTTGTGTCTGTCCTTGGGCCCTCCTCTAGCCCAGCATCTGCCTCCACCGTGAGCCAT     270
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IleCysGlyGluLysGluIleProHisIleLysValGlyProGluGluThrProArgLeuGlnTyrLeuArgPheAlaSerValSerLeuTyrProSerAsnGluAsp     126

GTCAGCTTGGCGGTCTCCCGAATCCTCAAGTCCTTCAACTACCCCTCGGCCAGCCTCATCTGCGCCAAGGCTGAGTGCCTGCTGCGATTGGAGGAACTGGTGCGTGGC     486
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AspAlaAsnAlaSerIleSerHisLeuIleLeuArgLysAlaSerGluLeuGlyMetThrSerAlaPheTyrLysTyrIleLeuThrThrMetAspPheProIleLeu     234

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HisLeuAspGlyIleValGluAspSerSerAsnIleLeuGlyPheSerMetPheAsnThrSerHisProPheTyrProGluPheValArgSerLeuAsnMetSerTrp     270

AGGGAGAACTGTGAAGCCAGCACCTACCTGGGCCCTGCGCTGTCAGCCGCCCTGATGTTTGACGCCGTGCACGTGGTGGTGAGCGCTGTCCGAGAGCTGAACCGCAGC     918
ArgGluAsnCysGluAlaSerThrTyrLeuGlyProAlaLeuSerAlaAlaLeuMetPheAspAlaValHisValValValSerAlaValArgGluLeuAsnArgSer     306

CAGGAGATCGGTGTGAAGCCTCTGGCCTGTACATCGGCCAACATTTGGCCCCACGGGACCAGCCTCATGAACTACCTGCGCATGGTAGAGTATGATGGGCTGACCGGG     1026
GlnGluIleGlyValLysProLeuAlaCysThrSerAlaAsnIleTrpProHisGlyThrSerLeuMetAsnTyrLeuArgMetValGluTyrAspGlyLeuThrGly     342

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CTGGCCATGAATGCCACCACCCTGGACATCAACCTGTCGCAGACACTGGCCAACAAGACCCTGGTGGTCACAACCATCCTGGAGAACCCATACGTCATGCGCCGGCCC     1242
LeuAlaMetAsnAlaThrThrLeuAspIleAsnLeuSerGlnThrLeuAlaAsnLysThrLeuValValThrThrIleLeuGluAsnProTyrValMetArgArgPro     414

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AspGlyLeuTyrGlyAlaProGluProAsnGlySerTrpThrGlyMetValGlyGluLeuIleAsnArgLysAlaAspLeuAlaValAlaAlaPheThrIleThrAla     486

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CCATGCCTGCGGGCACGCCCCCACATCCTGGAGAACCAGTACACGCTGGGCAACAGCCTGTGGTTTCCCGTGGGGGGCTTCATGCAGCAGGGCTCGGAGATCATGCCC     1782
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GAGGTGCCTGTGGAGTCGGCCGATGACCTGGCAGATCAGACCAACATCGAGTATGGCACCATCCACGCCGGCTCCACCATGACCTTCTTCCAGAATTCACGGTACCAA     1998
GluValProValGluSerAlaAspAspLeuAlaAspGlnThrAsnIleGluTyrGlyThrIleHisAlaGlySerThrMetThrPhePheGlnAsnSerArgTyrGln     666

ACGTACCAGCGCATGTGGAACTACATGCAGTCGAAGCAGCCCAGCGTGTTCGTCAAGAGCACAGAAGAGGGCATTGCCGCCGTCCTCAACTCCCGCTACGCCTTCCTG     2106
ThrTyrGlnArgMetTrpAsnTyrMetGlnSerLysGlnProSerValPheValLysSerThrGluGluGlyIleAlaAlaValLeuAsnSerArgTyrAlaPheLeu     702

CTCGAGTCCACCATGAACGAATACCACCGGCGCCTCAACTGCAACCTCACCCAGATCGGGGGACTCCTCGACACCAAGGGCTACGGCATTGGCATGCCGCTGGGCTCC     2214
LeuGluSerThrMetAsnGluTyrHisArgArgLeuAsnCysAsnLeuThrGlnIleGlyGlyLeuLeuAspThrLysGlyTyrGlyIleGlyMetProLeuGlySer     738

CCGTTCCGGGATGAGATCACACTGGCCATCCTGCAGCTTCAGGAGAACAACCGGCTGGAGATCCTGAAGCGCAAGTGGTGGGAGGGGGGCCGGTGCCCCAAGGAGGAG     2322
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GACCATCGAGCTAAAGGTTTGGGCATGGAGAACATTGGTGGCATTTTTATCGTGCTCATCTGTGGCCTCATCATTGCTGTCTTCGTGGCGGTCATGGAATTCATATGG     2430
AspHisArgAlaLysGlyLeuGlyMetGluAsnIleGlyGlyIlePheIleValLeuIleCysGlyLeuIleIleAlaValPheValAlaValMetGluPheIleTrp     810

TCCACACGGAGGTCAGCTGAGTCCGAGGAGGTGTCGGTGTGCCAGGAGATGCTGCAGGAGCTGCGCCACGCCGTTTCTTGCCGCAAGACGTCGCGTTCCCGCCGGCGC     2538
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CCCCGGGAGCTGGCGGAGCACGAGTGA        2889
ProArgGluLeuAlaGluHisGluEnd        962
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Fig. 1. Nucleotide sequence of the cDNA encoding the humEAA2 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 −54 encode a putative signal peptide. Numbers of the nucleotides and amino acid residues are given to the *right* of each line.

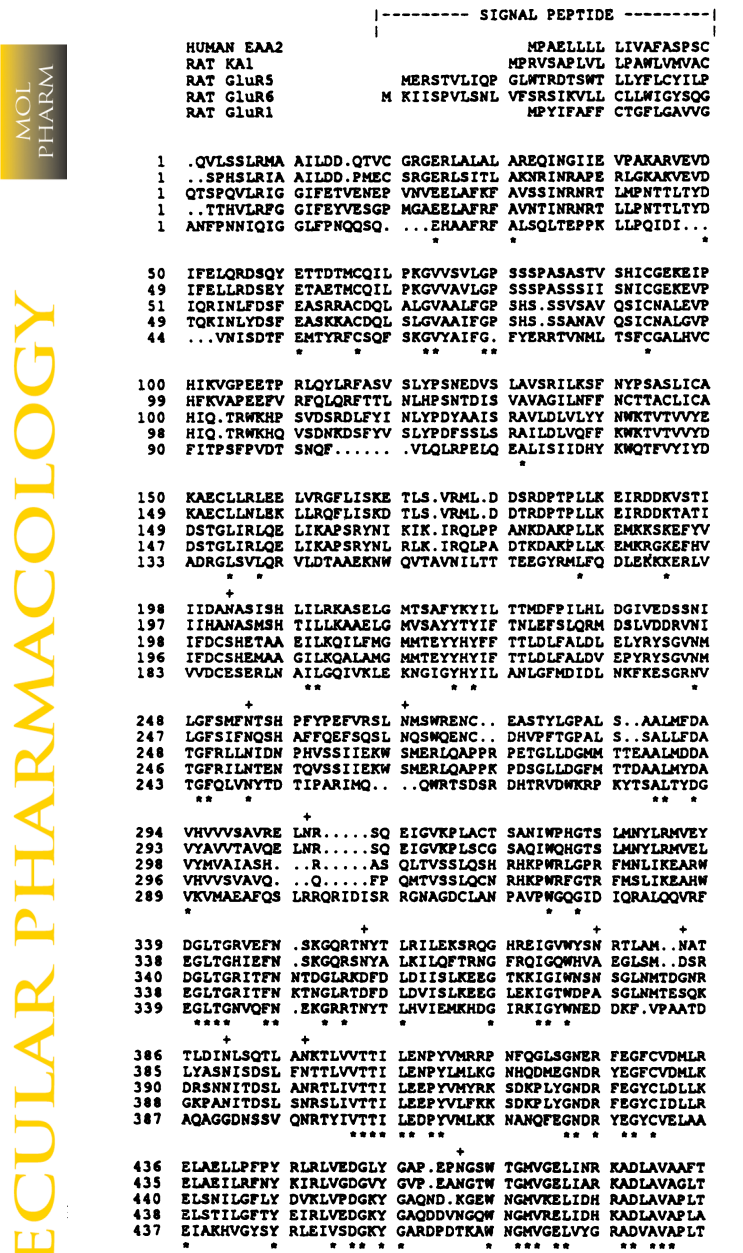

Fig. 2. Alignment of the deduced amino acid sequence of the humEAA2 receptor subunit with four published rat glutamate receptor subunits. The sequences of rat GluR1, rat GluR5, rat GluR6, rat KA1, and the humEAA2 polypeptide were aligned with the aid of the computer program Pileup (a sequence analysis software package by Genetics Computer Group, Inc.) (25). *Dotted lines*, gaps introduced for better alignment. *Asterisks*, positions at which the identical amino acid is found in all five polypeptide sequences. All polypeptide sequences are numbered from the proposed mature amino terminus. The predicted signal peptide sequences and transmembrane regions TM1 to TM4 are marked. *Crosses*, potential *N*-linked glycosylation sites in humEAA2; *filled circles*, calmodulin-dependent protein kinase type II consensus phosphorylation sites (26) in the predicted intracellular domains (between TM1 and TM2 and between TM3 and TM4).

sion of humEAA2 in COS cells, the binding of selective excitatory amino acid ligands to washed and dialyzed membranes 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 2.6 ± 0.7 nM and a B_{max} of 384 ± 157 fmol/mg of protein. Fig. 3 shows a representative saturation curve and Scatchard plot from these experiments. When the NMDA receptor ligand [^{3}H]CGS 19755 (10 nM) (18) or the AMPA receptor ligand [^{3}H]AMPA (5 nM) (19) was used, no specific binding was observed (data not shown). Kainate was the most potent displacer of [^{3}H]kainate (5 nM) 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 CNQX and DNQX exhibited affinity constants for this receptor in the micromolar range. NMDA, as well as the selective metabotropic (guanine nucleotide-binding protein-coupled) excitatory amino acid agonist 1*S*,3*R*-ACPD, did not affect [^{3}H]kainate binding at up to 100 μM (Fig. 4; Table 1).

Discussion

We have isolated a new member of the excitatory amino acid receptor gene family, humEAA2, that has nanomolar affinity

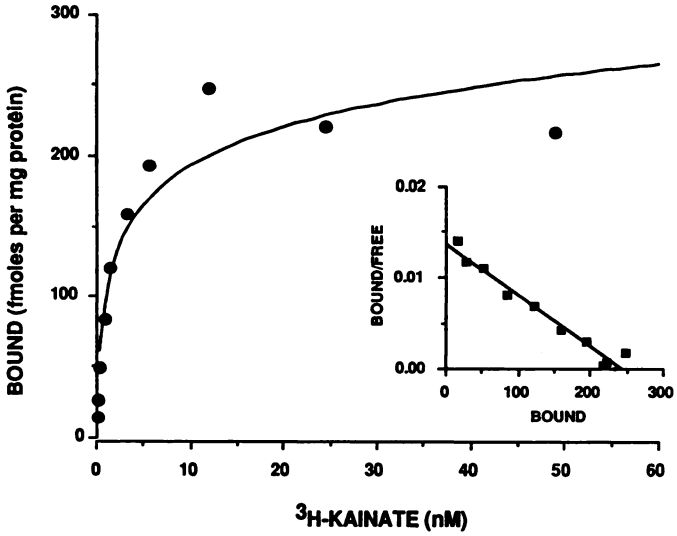

Fig. 3. Representative saturation and Scatchard (*inset*) plots of [^{3}H]kainate binding to humEAA2-transfected COS cell membranes. Dialyzed membranes were incubated with [^{3}H]kainate in the absence (total binding) or presence (nonspecific binding) of 1 mM L-glutamate, in a 1-ml volume, at 0° for 1 hr. Bound ligand was separated from free ligand by rapid filtration. Data are expressed as specific [^{3}H]kainate bound (total minus nonspecific).

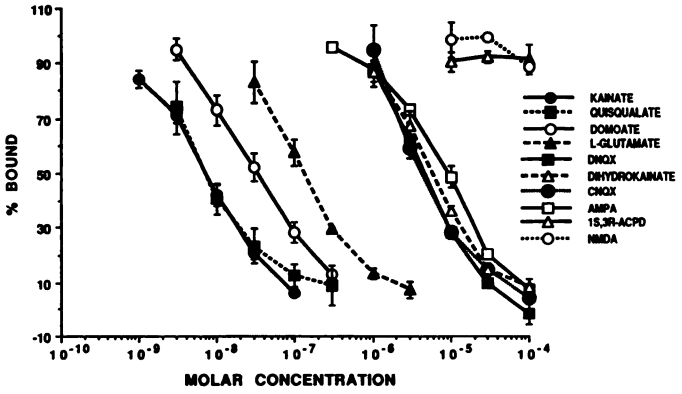

Fig. 4. Displacement curves of [^{3}H]kainate binding to humEAA2-transfected COS cell membranes by excitatory amino acid analogues. Dialyzed humEAA2-transfected COS cell membranes were incubated with [^{3}H]kainate (5 nM). Nonspecific binding was determined with 1 mM L-glutamate. Data are expressed as percentage of specific [^{3}H]kainate binding (mean ± standard error) from three experiments, performed in triplicate.

for kainate. humEAA2 has about 34% amino acid sequence identity with the cloned rat AMPA (GluR1, GluR2, GluR3, and GluR4) receptor subunits and has higher sequence identity with the cloned rat kainate (GluR6, 45%; KA1, 68%) receptor subunits. The deduced amino acid sequence of humEAA2 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 humEAA2 and other glutamate receptor subunits is most striking within the region encompassed by the transmembrane domains; this would predict that the humEAA2 polypeptide is a glutamate-gated ion channel receptor subunit.

The electrophysiological results suggest either that the expression of humEAA2 in *Xenopus* is poor or that humEAA2 encodes a subunit that requires at least one additional subunit

TABLE 1

Affinities of excitatory amino acid analogues for [^{3}H]kainate binding to humEAA2 receptors in transfected COS cells

Values shown are means ± standard errors of three separate experiments, each performed in triplicate. [^{3}H]Kainate binding was conducted with a radioligand concentration of 5 nM. Reactions were initiated by the addition of dialyzed membrane suspensions, and samples were incubated on ice, in 1-ml volume/sample, for 1 hr. Protein content per tube was ~50 μg. Reactions were terminated by rapid filtration through 0.3% polyethylenimine-soaked filters.

Compound	K_i	Hill coefficient
	nM	
Kainate	2.41 ± 0.62	0.911 ± 0.069
Quisqualate	10.1 ± 5.85	0.782 ± 0.184
Domoate	12.9 ± 2.32	0.898 ± 0.109
L-Glutamate	51.3 ± 32.6	1.006 ± 0.082
DNQX	1,600 ± 101	1.160 ± 0.040
CNQX	1,810 ± 254	1.019 ± 0.179
Dihydrokainate	2,340 ± 230	1.107 ± 0.121
AMPA	3,010 ± 137	0.992 ± 0.064
1*S*,3*R*-ACPD	>100,000	
NMDA	>100,000	

to form a fully functional receptor-activated ion channel. Absence of homo-oligomeric channel activity has been reported for other subunits of the excitatory amino acid receptor family, such as the kainate-binding subunit protein from chick brain (13), frog brain (14), and rat brain (11). Rat GluR2 and GluR5 homo-oligomeric glutamate receptors show very small responses to agonists when expressed in *Xenopus* oocytes (7, 9). Alternatively, it is possible that humEAA2 subunit forms ion channels that desensitize very fast. Such glutamate receptor ion channels have been reported in C-fibers of the dorsal root ganglia (20). It is also possible that humEAA2 expressed in the *Xenopus* oocyte system does not undergo post-translational modifications as it would in neuronal cells and, thus, is not completely activated upon agonist application. Finally, there are potential sites for calmodulin-dependent protein kinase type II in the putative intracellular domains of the humEAA2 protein (Fig. 2), and there is evidence that phosphorylation, too, can modulate the activity of glutamate receptors (21).

However, the novel protein demonstrated here has pharmacological characteristics that strongly suggest that it is a subunit protein for the kainate type of iontropic excitatory amino acid receptor. humEAA2 receptor protein exhibited high affinity (nanomolar) [^{3}H]kainate binding. The rank order of displacement affinities was kainate > quisqualate > domoate > L-glutamate ≫ AMPA ≫ NMDA = 1*S*,3*R*-ACPD. This is similar to the rank order of potency that was reported for the recently described rat KA1 receptor (11). Like humEAA2, 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 from humEAA2-transfected cells was also similar to what was observed when channel activity was studied by using the rat GluR6 receptor clone (10). Agonist potency at the rat GluR6 receptor was kainate > quisqualate > L-glutamate ≫ AMPA, and the quinoxalinedione AMPA antagonist CNQX had a K_i value of 4 μM. humEAA2 [^{3}H]kainate binding exhibited the same rank order of agonist potency and a similar K_i value for CNQX (1.8 μM). The affinity of CNQX for humEAA2 was similar to that for the rat GluR6 receptor protein but was considerably lower than that for cloned AMPA receptors. For example, the K_i value for CNQX using rat GluR1 receptor was 0.519 μM (22). 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 (23).

humEAA2 has the characteristics of a novel subunit of a human high affinity kainate receptor, based on its molecular structure and binding pharmacology. The successful cloning of the humEAA2 cDNA should, therefore, lead to a better understanding of the molecular nature of the kainate receptors and their role in normal and diseased human central nervous system.

After the completion of this manuscript, a paper appeared describing the cloning of a novel subunit of the mouse glutamate receptor cDNA, designated as γ2 (24). The sequences from humEAA2 and mouse γ2 share 91% identity at the nucleotide level and 98% homology at the amino acid level. The proposed mature mouse γ2 protein is composed of 961 amino acid residues, whereas the predicted humEAA2 mature protein is composed of 962 amino acid residues. The additional amino acid residue is a serine at residue 883 of the humEAA2 mature protein subunit. The functional data that were shown for mouse γ2 protein (enhanced kainate-induced currents when coexpressed with mouse GluR6) strengthen the contention that humEAA2 is a subunit protein of heteromeric kainate iontropic excitatory amino acid receptor complexes.

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