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Short Sequence-Paper

Human glutamate receptor hGluR3 flip and flop isoforms: cloning and sequencing of the cDNAs and primary structure of the proteins

Vikarna Rampersad, Candace E. Elliott, Stephen L. Nutt, Robert L. Foldes, Rajender K. Kamboj *

Allelix Biopharmaceuticals Inc., 6850 Goreway Drive, Mississauga, Ontario L4V 1V7, Canada

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Abstract

Several cDNA clones encoding the human glutamate receptor subunit GluR3 flip and flop isoforms, were isolated from human hippocampus and fetal brain libraries. DNA sequence analysis revealed overlapping clones permitting the reconstruction of full-length *GluR3-flip* and *GluR3-flop* cDNAs. The *GluR3* cDNAs demonstrated an 94.1–94.7% nucleotide (nt) identity with the corresponding rat cDNAs. The nt sequence of the *GluR3* cDNAs would encode 894 amino acid proteins that have a 99.4% identity with the rat GluR3 isoforms. The human *GluR3* cDNAs predict an additional 6 amino acid in the N-terminal signal peptide as compared to the rat GluR3.

Keywords: Glutamate receptor; AMPA; Kainate; cDNA sequence; Amino acid sequence; RNA splicing; (Human)

The ionotropic glutamate receptors are heteromeric protein complexes with multiple subunits, each possessing transmembrane regions, and all arranged to form a ligand-gated ion channel. These receptors can be classified according to their selective agonists: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), 2-carboxy-4-(1-methylethenyl)-3-pyrrolidinacetic acid (kainate) or *N*-methyl-D-aspartate (NMDA) (see [1] for a recent review). The AMPA and kainate receptors are also refered to as non-NMDA receptors. One approach to understanding the properties of the AMPA receptor involves the cloning and characterization of the genes encoding this receptor.

Recent molecular cloning studies have revealed a number of AMPA receptor subunits that can be classified into four families: GluR1 [2–9], GluR2 [3–6,9–11], GluR3 [3,4,6,10] and GluR4 [4,6,12]. Each of these families include flip and flop isoforms generated by alternative RNA splicing [6,13]. In addition, a carboxy-terminal splice variant has been observed in the case of GluR4 [12]. Further receptor subunit complexity is also generated by RNA editing [14–17]. Expression studies of the cloned proteins have shown that the functional properties of AMPA receptors depend on their subunit composition [1,3,5,6,10,11,15,18,19]. The GluR1–4 subunits and isoforms are differentially expressed in a cell-specific and developmentally-regulated fashion [1, 4,6,12,15,20–25].

The non-NMDA receptors are of medical interest because of their postulated role in human central nervous system physiology and pathology [26]. In addition, several genetic diseases (including the oculocerebral renal syndrome of Lowe) have been mapped to the region of the X chromosome to which GluR3 has been localized [27]. Increasing evidence has emerged that brings into question the relevance of non-human mod-

Abbreviations: aa, amino acid(s); AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; bp, base pair(s); cDNA, DNA complementary to RNA; GluR, glutamate receptor; *GluR*, gene (DNA) encoding GluR; hGluR, human GluR; kainate, 2-carboxy-4-(1-methylethenyl)-3-pyrrolidinacetic acid; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; ORF, open reading frame; PCR, polymerase chain reaction; UTR, untranslated region(s); 50×Denhardt's reagent, 1% (w/v) Ficoll/1% (w/v) polyvinylpyrrolidone/1% (w/v) bovine serum albumin; 1×SSC, 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.6.

* Corresponding author. Fax: +1 (905) 6779595.

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession numbers U10301 and U10302.

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els for the study of the human condition. In particular, it has become evident that amino acid substitutions between homologous proteins (e.g., species homologues) can lead to altered functionality [28–30]. For these reasons, we have continued our studies regarding the molecular cloning and sequence analysis of the human non-NMDA receptor subunits in order to establish a framework for the further study of their pharmacology as it relates to molecular structure, and thereafter for rational drug design.

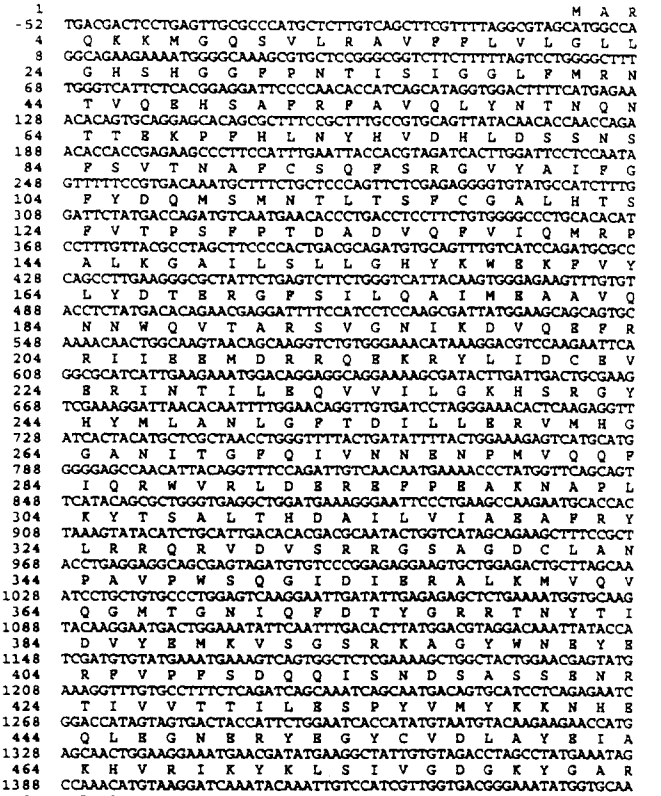

Fig. 1. The nucleotide and deduced amino acid sequence of the h*GluR3-flip* cDNA.

Two h*GluR3* cDNA clones (RKCH221 and RKCH521) were isolated by screening approx. $1 \cdot 10^6$ bacteriophage plaques of a human hippocampal cDNA library (Stratagene Cloning Systems, La Jolla, CA, USA) with a 1.1 kb *Eco*RI fragment constituting the 3′ region of the hum*EAA1* cDNA [31]. The hum*EAA1* *Eco*RI fragment was labeled with [α-^{32}P]dCTP (> 3000 Ci/mmol) using the Amersham Megaprime DNA labeling system. Positive plaques were identified on replica Hybond-N filters (Amersham) under the following low stringency hybridization conditions: 6 × SSC/50% formamide/5 × Denhardt's/0.5% SDS/100 μg/ml denatured salmon sperm DNA. The hybridizations proceeded at 30°C overnight. The filters were washed with 2 × SSC/0.5% SDS at 25°C for 5 min, followed by a 15 min wash at 50°C, and a final wash with 1 × SSC/0.5% SDS for 15 min at 50°C. Three cDNA clones containing the 3′ regions of h*GluR3* were isolated by screening the human hippocampus library (clone RKCSHG132) and a human fetal brain library (Stratagene) (clones RKCSFG34 and RKCSFG241) with a ^{32}P-labeled oligodeoxyribonucleotide (oligo) probe based on the nucleotide sequence of rat *GluR3* [4]: 5′-ACACTCAGAATTACGCTACATACAGAGA-AGGCTACAACGT. This hybridization proceeded at 42°C overnight under the following conditions: 6 × SSC/25% formamide/5 × Denhardt's/0.5% SDS/100 μg/ml denatured salmon sperm DNA. The filters were exposed to Kodak XAR-5 film at −80°C overnight. Positive clones were plaque-purified and excised as phagemids (according to the supplier's specifications), to generate an insert carrying Bluescript-SK variant of the phagemid vector. Nucleotide sequence of denatured, double-stranded templates, was determined by the use of the dideoxy chain-termination reaction using Sequenase (U.S. Biochemical, Cleveland, OH, USA) and a series of strategically situated synthetic oligos. Sequence analysis and comparisons were performed with the GCG (Madison, WI, USA) Analysis Software Package and version 7.3 of the GenBank and European Molecular Biology Laboratory data banks [32].

Four overlapping cDNA clones, designated RKCH521, RKCH221, RKCSHG132 and RKCSFG34 containing cDNA inserts of about 1.6, 2.2, 1.2 and 2.3 kb, respectively, were characterized further. Two overlapping cDNA clones RKCH221 and RKCSFG34 encompassed a full-length h*GluR3-flip* cDNA. Sequence analysis indicated a putative ATG start codon together with 53 nt of 5′ UTR, a 2682 nt open reading frame (ORF), and a 321 nt 3′ UTR including the inframe

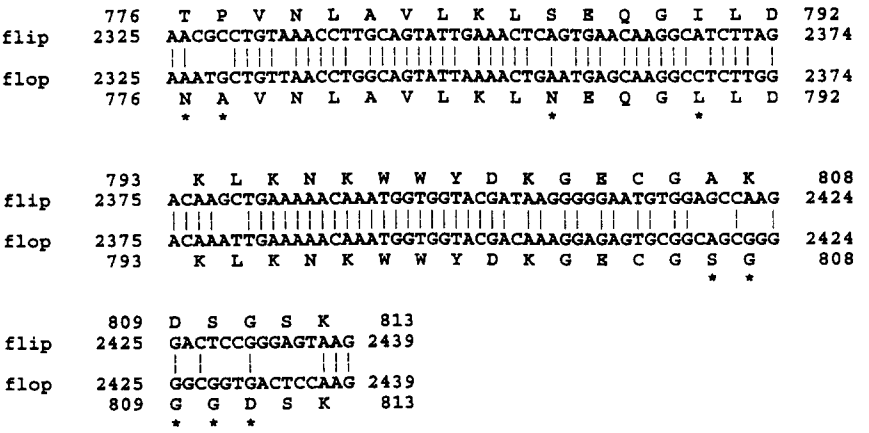

Fig. 2. Comparison of the nucleotide and deduced amino acid sequence of the h*GluR3-flip* and h*GluR3-flop* cDNAs. Amino acid sequence differences are indicated (*).

stop codon (Fig. 1). The remaining two cDNA clones (RKCH521 and RKCSHG132) define the h*GluR3-flop* cDNA and differ from the h*GluR3-flip* cDNA in the region between nt 2327–2436 (Fig. 2). Comparison of the h*GluR3-flip* nt sequence with that of rat *GluR3-flip* [6,10], indicates a 94.1% identity. Comparison of the h*GluR3-flop* nt sequence with that of rat *GluR3-flop* [4] indicates a 94.7% identity. The 894-aa deduced sequence encoded by the h*GluR3* cDNAs share a 99.4% identity (5 aa changes) with the rat homologues. These changes are summarized as follows (human → rat): Gly-154 → Ser; His-155 → Tyr; Ile-173 → Val; Ala-418 → Ser; Leu-525 → Phe. Interestingly all of these changes occur in the predicted extracellular N-terminal regions of the molecule [4]. The nt sequence reported by Boulter et al. [3] differs from those reported here and elsewhere [4,6,10] at 2 aa positions (human → rat[3]): Lys-523 → Asn; Pro-524 → Ala. In addition to these changes, the h*GluR3* nt sequences predict an additional 6 aa in the putative N-terminal signal peptide, as compared to the rat homologues. The amino acid sequence conservation observed between human and rat GluR3 is comparable to that observed for NR1 [33], but greater than that observed for GluR1 [7,8], GluR2 [11], EAA1 [31] and EAA2 [34]. The significance of this higher degree of conservation is as yet unclear.

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