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CONTRIBUTIONS OF DIFFERENT KAINATE RECEPTOR SUBUNITS TO THE PROPERTIES OF RECOMBINANT HOMOMERIC AND HETEROMERIC RECEPTORS

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Abstract—The tetrameric kainate receptors can be assembled from a combination of five different subunit subtypes. While GluK1–3 subunits can form homomeric receptors, GluK4 and GluK5 require a heteromeric partner to assemble, traffic to the membrane surface, and produce a functional channel. Previous studies have shown that incorporation of a GluK4 or GluK5 subunit changes both receptor pharmacology and channel kinetics. We directly compared the functional characteristics of recombinant receptors containing either GluK4 or GluK5 in combination with the GluK1 or GluK2 subunit. In addition, we took advantage of mutations within the agonist binding sites of GluK1, GluK2, or GluK5 to isolate the response of the wild-type partner within the heteromeric receptor. Our results suggest that GluK1 and GluK2 differ primarily in their pharmacological properties, but that GluK4 and GluK5 have distinct functional characteristics. In particular, while binding of agonist to only the GluK5 subunit appears to activate the channel to a non-desensitizing state, binding to GluK4 does produce some desensitization. This suggests that GluK4 and GluK5 differ fundamentally in their contribution to receptor desensitization. In addition, mutation of the agonist binding site of GluK5 results in a heteromeric receptor with a glutamate sensitivity similar to homomeric GluK1 or GluK2 receptors, but which requires higher agonist concentrations to produce desensitization. This suggests that onset of desensitization in heteromeric receptors is determined more by the number of subunits bound to agonist than by the identity of those subunits. The distinct, concentration-dependent properties observed with heteromeric receptors in response to glutamate or kainate are consistent with a model in which either subunit can activate the channel, but in which occupancy of both subunits within a dimer is needed to allow desensitization of GluK2/K5 receptors.

Key words: kainate receptors, mutagenesis, patch-clamp, glutamate.

INTRODUCTION

Kainate receptors are cation-permeable, ligand-gated channels activated by the excitatory neurotransmitter glutamate. The ionotropic glutamate receptors, which include AMPA, NMDA, and kainate receptors, are tetrameric in structure, and each of the homologous subunits contains three full transmembrane domains and a ligand binding site (Traynelis et al., 2010; Kumar and Mayer, 2013). Kainate receptors are located both pre- and post-synaptically, where they regulate neurotransmitter release and mediate excitatory neurotransmission (Contractor et al., 2011; Lerma and Marques, 2013; Sihra and Rodriguez-Moreno, 2013). Although the post-synaptic current produced by kainate receptors is relatively small in amplitude compared to that of the AMPA receptors, it is characterized by a slow deactivation rate, allowing synaptic integration and temporal summation in response to repetitive stimulation (Castillo et al., 1997; Frerking et al., 1998; Frerking and Ohliger-Frerking, 2002).

Kainate receptors are assembled from a combination of five different subunits (GluK1–GluK5). The GluK1–3 subunits (formerly GluR5–7) are able to produce functional homomeric receptors in heterologous expression systems. These homomeric receptors exhibit relatively low sensitivity to activation by glutamate, and are characterized by rapid and complete desensitization in response to even sub-maximal glutamate levels (Sommer et al., 1992; Heckmann et al., 1996; Schiffer et al., 1997; Paternain et al., 1998). GluK1 subunits are highly expressed in the developing brain (Bahn et al., 1994) and may play an important role in neuronal maturation (Lauri et al., 2005, 2006; Segerstrale et al., 2010; Carta et al., 2014). In the adult, these subunits are predominantly expressed in interneurons, and contribute to both pre- and post-synaptic kainate receptor populations (Carta et al., 2014). GluK2-containing receptors appear to be responsible for most of the kainate receptor mediated post-synaptic response at the mossy fiber-CA3 synapse in the hippocampus, which represents the best characterized of the kainate receptor populations (Carta et al., 2014). The GluK2 subunit is also widely

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Abbreviations: EDTA, Ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle medium; BES, N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid.

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expressed in other brain regions, and in combination with GluK5 is considered to be the most common kainate receptor isoform (Perrais et al., 2010). The GluK3 subunit is likely to contribute primarily to presynaptic kainate receptors, forming heteromeric complexes with GluK2 subunits (Pinheiro et al., 2007). The GluK3-containing receptors have distinct functional characteristics, with a unique pharmacological profile and exceptionally low sensitivity to activation by glutamate, with an EC_{50} in the mM range (Schiffer et al., 1997; Pinheiro et al., 2007).

The GluK4–5 subunits (formerly KA1 and KA2) are distinct both structurally and functionally from the GluK1–3 subunits. They are obligate heteromers, and must assemble with GluK1–3 subunits to produce functional surface receptors which contain two of each subunit type (Gallyas et al., 2003; Ren et al., 2003; Perrais et al., 2010; Reiner et al., 2012). A high-affinity interaction between the amino-terminal domains provides a mechanism for preferential assembly of heteromeric receptors (Kumar et al., 2011) and in neurons, it is likely that most post-synaptic kainate receptors are heteromers, containing GluK1 or GluK2 along with either a GluK4 or GluK5 subunit (Petralia et al., 1994; Darstein et al., 2003; Fernandes et al., 2009). While GluK5 is widely expressed throughout the brain, GluK4 is expressed primarily in the hippocampus (Bahn et al., 1994). Incorporation of a GluK4 or GluK5 subunit changes the functional and pharmacological properties of recombinant receptors, increasing sensitivity to glutamate, allowing activation by AMPA, slowing deactivation, and altering the concentration-dependence of desensitization (Sakimura et al., 1992; Herb et al., 1992; Pinheiro and Mulle, 2006; Barberis et al., 2008; Mott et al., 2010; Fisher and Mott, 2011, 2013).

Each subunit within the tetrameric receptor contains an agonist binding site, and thus has the potential to contribute to channel activation and gating. Previous studies demonstrated that, for heteromeric receptors, glutamate binding to the higher affinity GluK4 or GluK5 subunits is able to activate the channel, with higher concentrations required to produce desensitization through the lower affinity GluK2 partner (Mott et al., 2010; Fisher and Mott, 2011). Some studies indicated that binding of just one subunit is sufficient to produce desensitization of AMPA receptors (Robert and Howe, 2003) and that partially bound states of homomeric kainate receptors are more likely to desensitize than to open (Barberis et al., 2008; Perrais et al., 2009a) although others suggested that partly desensitized conducting states could contribute to the current response (Bowie and Lange, 2002). In addition, work with subunit-selective agonists or antagonists (Swanson et al., 2002; Fisher and Mott, 2011; Pinheiro et al., 2013; Fisher, 2014) and tethered ligands (Reiner and Isacoff, 2014) suggests that partial occupancy of the binding sites in homomeric or heteromeric receptors may be sufficient for activation of the receptors but is not necessarily sufficient for complete desensitization. Therefore, the relationship between subunit occupancy and channel gating may be dependent upon the subunit composition of the kainate receptor.

Studies with genetically modified mouse models lacking one or more of the kainate subunits indicate that different receptor isoforms have distinct roles in regulating neuronal function and behavioral responses (Mulle et al., 1998; Contractor et al., 2003; Ruiz et al., 2005; Fisahn et al., 2005; Pinheiro et al., 2007; Fernandes et al., 2009; Catches et al., 2012; Lowry et al., 2013). To determine the role of subunit composition in the response of kainate receptors to glutamate, we characterized the properties of recombinant homomeric and heteromeric receptors containing combinations of the GluK1, GluK2, GluK4 and GluK5 subunits. In addition, to determine the contribution of each subunit to receptor activation and desensitization, we examined the impact of binding site mutations that reduced agonist sensitivity. Our results show that each kainate receptor subunit has distinct pharmacological properties, and are consistent with a model in which occupancy of both subunits within a dimer pair may be necessary for complete desensitization of the receptor.

EXPERIMENTAL PROCEDURES

Cell culture and transfection of mammalian cells

Full-length cDNAs for rat kainate receptor subunits GluK1a(Q) (obtained from Dr. C. Mulle, Univ. Bordeaux, France), GluK2(Q), GluK4 and GluK5 (all obtained from Dr. S. Heinemann, Salk Institute, San Diego, CA, USA) in mammalian expression vectors were transfected into the human embryonic kidney cell line HEK-293T (GenHunter, Nashville, TN, USA). Point mutations were generated using the QuikChange procedure and products (Agilent Technologies, Santa Clara, CA, USA). Residue numbering includes the signal sequence. Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA) and mutations were verified by DNA sequencing (University of South Carolina Environmental Genomics core, Columbia, SC, USA). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were passaged by a 2-min. incubation with 0.025% trypsin/0.01% EDTA solution in phosphate-buffered saline (10 mM Na_2HPO_4, 150 mM NaCl, pH = 7.3).

The cells were transiently transfected using calcium phosphate precipitation. A total of 5 μg of cDNA was transfected into the cells. For homomeric receptors, 4 μg of GluK1 or GluK2 was transfected. To produce heteromeric receptors, plasmids were added to the cells in a 1:3 ratio (GluK1 or 2:GluK4 or 5), previously shown to optimize formation of a homogeneous population (Barberis et al., 2008). To allow identification of positively transfected cells, 1 μg of the plasmid pHook™-1 (Invitrogen Life Technologies, Grand Island, NY, USA) which encodes a surface antibody, was also included (Chesnut et al., 1996). The cells were incubated at 3% CO_2 for 4–6 h with the DNA/calcium phosphate mixture and then treated with a 15% glycerol solution in BES-buffered saline (25 mM BES (N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic