Glutamate (ionotropic)

Overview: The ionotropic glutamate receptors comprise members of the NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptor classes, named originally according to their preferred, synthetic, agonist (Dingledine et al., 1999; Lodge, 2009). Receptor heterogeneity within each class arises from the homo-oligomeric, or hetero-oligomeric, assembly of distinct subunits into cation-selective tetramers. All glutamate receptor subunits have the membrane topology of an extracellular N-terminus, three transmembrane domains (formed by M1, M3 and M4), a channel lining re-entrant ‘p-loop’ (M2) located between M1 and M3 that enters and exits the membrane at its cytoplasmic surface, and an intracellular C-terminus (see Mayer, 2006). It is beyond the scope of this supplement to discuss the pharmacology of individual ionotropic glutamate receptor isoforms in detail; such information can be gleaned from Dingledine et al. (1999), Jane et al. (2000), Huettner (2003), Cull-Candy and Leszkiewicz (2004), Kew and Kemp (2005), Erreger et al. (2007), Paolotti and Neyton (2007), Chen et al. (2008) and Jane et al. (2009). Agents that discriminate between subunit isoforms are, where appropriate, noted in the tables, and additional compounds that distinguish between receptor isoforms are indicated in the text below.

The classification of glutamate receptor subunits has been recently been re-addressed by NC-IUPHAR (Collingridge et al., 2009). The scheme developed recommends a revised nomenclature for ionotropic glutamate receptor subunits that is adopted here. Alternative appellations that have been used previously (see Lodge, 2009) are indicated in parenthesis to aid transition to the revised nomenclature, but their continued use is not recommended.

NMDA receptors: NMDA receptors assemble as heteromers that may be drawn from GluN1 (GLU N1, NR1, Glu(R)1), GluN2A (GLU N2A, NMDA-R2A, NR2A, GluRe1), GluN2B (GLU N2B, NMDA-R2B, NR2B, GluRe2), GluN2C (GLU N2C, NMDA-R2C, NR2C, GluRe3), GluN2D (GLU N2D, NMDA-R2D, NR2D, GluRe4), GluN3A (GLU N3A, NMDA-R3A) and GluN3B (GLU N3B, NMDA-R3B) subunits. Alternative splicing can generate eight isoforms of GluN1 with differing pharmacological properties. Various splice variants of GluN2B, 2C, 2D and GluN3A have also been reported. Activation of NMDA receptors containing GluN1 and GluN2 subunits requires the binding of two agonists, glutamate to the S1 and S2 regions of the GluN2 subunit and glycine to S1 and S2 regions of the GluN1 subunit (Erreger et al., 2008). In addition to the glutamate and glycine binding sites documented in the table, physiologically important >><> binding within N-terminal domain (NTD) is highly subunit-selective (GluN2A > GluN2B > GluN2C > GluN2D), L-aspartate (GluN2D = GluN2B > GluN2C = GluN2A), D-aspartate (GluN2D > GluN2C = GluN2B > GluN2A), (R5)-(tetrazol-5-yl)glycine (GluN2D > GluN2C = GluN2B > GluN2A), homoquinolinic acid (GluN2B = GluN2A = GluN2D > GluN2C, partial agonist at GluN2A and GluN2C), D-AP5, CGS19755, CGP37849, LY233530, D-CCPene (GluN2A = GluN2B > GluN2C = GluN2D), PPDA (GluN2D = GluN2C > GluN2B > GluN2A, Feng et al., 2004), NVP-AAM077 [GluN2A > GluN2B (human), Auiberson et al., 2002; but weakly selective for rat GluN2A vs. GluN2B, Feng et al., 2004; Frizelle et al., 2006; Neyton and Paolotti, 2006], conantokin-G (GluN2B > GluN2D = GluN2C = GluN2A) glycine (GluN2D > GluN2C > GluN2B > GluN2A), D-serine (GluN2B > GluN2D > GluN2C > GluN2A), (+)-HA966 (partial agonist) 5,7-Dichlorokynurenate, L689560, LY233530, CV196771A, Channel blockers Mg2+ (GluN2A = GluN2B > GluN2C > GluN2D; (–)-MK801, ketamine, phencyclidine, memantine (GluN2C = GluN2D = GluN2B > GluN2A), amantidine, N'-dansyl-spermine (GluN2A = GluN2B > GluN2C > GluN2D)), Probes Glutamate site [H]CPP, [H]GS19755, [H]CGP39653 Glutamate site Glycine, [H]L689560, [H]HMDL105519 Cation channel [H]-MK801 (dizocilpine) Potency orders unreferenced in the table are from Kuner and Schoepfer (1996), Dravid et al. (2007), Erreger et al. (2007), Paolotti and Neyton (2007) and Chen et al. (2008). In addition to the glutamate and glycine binding sites documented in the table, physiologically important inhibitory modulatory sites exist for Mg2+, Zn2+ and protons (Dingledine et al., 1999; Cull-Candy and Leszkiewicz, 2004). Voltage-independent inhibition by Zn2+ binding within N-terminal domain (NTD) is highly subunit-selective (GluN2A > GluN2B = GluN2C > GluN2D). Voltage-dependent blockage of NMDA receptor function is alleviated by polyamines and the inclusion of exon 5 within GluN1 subunit splice variants, whereas the non-competitive antagonists ifenprodil and CP101606 (traxoprodil) increase the fraction of receptors blocked by protons at ambient concentration. Inclusion of exon 5 also abolishes potentiation by polyamines and inhibition by Zn2+ that occurs through binding in the NTD (Traynelis et al., 1998). Ifenprodil, CP101606, haloperidol, felbamate and Ro84304 discriminate between recombinant NMDA receptors assembled from GluN1 and either GluN2A, or GluN2B, subunits by acting as selective, non-competitive, antagonists of hetero-oligomers incorporating GluN2B. LY233530 is a competitive antagonist that also displays selectivity for GluN2B over GluN2A subunit-containing receptors. Similarly, CGP61594 is a photoaffinity label that interacts selectively with receptors incorporating GluN2B versus GluN2A, GluN2D and, to a lesser extent, GluN2C subunits. In addition to influencing the pharmacological profile of the NMDA receptor, the identity of the GluN2 subunit co-assembled with GluN1 is an important determinant of biophysical properties that include sensitivity to block by Mg2+, single-channel conductance and maximal open probability and channel deactivation time (Cull-Candy and Leszkiewicz, 2004; Erreger et al., 2004; Gielen et al., 2009). Incorporation of the GluN3A subunit into tri-heteromers containing GluN1 and GluN2 subunits is associated with decreased single-channel conductance, reduced permeability to Ca2+ and
decreased susceptibility to block by Mg²⁺ (Cavara and Hollmann, 2008). Reduced permeability to Ca²⁺ has also been observed following the inclusion of GluN3B in tri-heteromers. The expression of GluN3A, or GluN3B, with GluN1 alone forms, in Xenopus laevis oocytes, a cation channel with unique properties that include activation by glycine (but not NMDA), lack of permeation by Ca²⁺ and resistance to blockade by Mg²⁺ and NMDA receptor antagonists (Chatterton et al., 2002). The function of heteromers composed of GluN1 and GluN3A is enhanced by Zn²⁺, or glycine site antagonists, binding to the GluN1 subunit (Madry et al., 2008). Zn²⁺ also directly activates such complexes. The co-expression of GluN1, GluN3A and GluN3B appears to be required to form glycine-activated receptors in mammalian cell hosts (Smothers and Woodward, 2007).

**AMPAs and kainate receptors:** AMPA receptors assemble as homomers, or heteromers, that may be drawn from GluA1 (GLU A1, GluR1, GluRA), GluA2 (GLU A2, GluR2, GluRB, GluR-B, GluR-K2), GluA3 (GLU A3, GluR3, GluRC, GluR-C, GluR-K3) or GluA4 (GLU A4, GluR4, GluR-D) subunits. Transmembrane AMPA receptor regulatory proteins (TARPs) of class I (i.e. y2, y3, y4 and y8) act, with variable stoichiometry, as auxiliary subunits to AMPA receptors and influence their trafficking, single-channel conductance and gating (reviewed by Ziff, 2007; Esteban, 2008; Milstein and Nicoll, 2008). The nomenclature of kainate receptor subunits has been revised to provide a logical numerical sequence that harmonizes with their gene names (Collingridge et al., 2009). Functional kainate receptors can be expressed as homomers of GluK1 (GLUK1, GluK5, GluR-S, EA3A), GluK2 (GLUK2, GluB6, GluK-6, EA4X) or GluK3 (GLUK3, GluR7, GluR-7, EAAX) subunits. GluK1–3 subunits are also capable of assembling into heterotetramers (see Lerma, 2003; Pinheiro and Mulle, 2006). Two additional kainate receptor subunits, GluK4 (GLUK4, KA1, KA-1, EA1A) and GluK5 (GLUK5, KA2, KA-2, EA2A), when expressed individually, form high-affinity binding sites for kainate, but lack function, probably due to retention within the endoplasmic reticulum (reviewed by Huettner, 2003 and Jane et al., 2009). GluK4 and GluK5 can form heteromers when co-expressed with GluK1–3 subunits (Lerma, 2003). Kainate receptors may also exhibit ‘metabotropic’ functions (Rodriguez-Morino and Shiha, 2007). RNA encoding the GluA2 subunit undergoes extensive RNA editing in which the codon encoding a p-loop glutamine residue (Q) is converted to one encoding arginine (R). This Q/R site strongly influences the biophysical properties of the receptor. The class II TARP y5 interacts selectively with AMPA receptors containing edited GluA2 subunits to uniquely modify their biophysical properties (Kato et al., 2008). Recombinant AMPA receptors lacking RNA edited GluA2 subunits are: (i) permeable to Ca²⁺; (ii) blocked by intracellular polyamines at depolarized potentials causing inward rectification (the latter being reduced by TARPs); (iii) blocked by extracellular argiotoxin and Joro spider toxins; and (iv) demonstrate higher channel conductances than receptors containing the edited form of GluA2 (Seeburg and Hartner, 2003; Isaac et al., 2007). GluK1 and GluK2, but not other kainate receptor subunits, are similarly edited and broadly similar functional characteristics apply to kainate receptors lacking either an RNA edited GluK1, or GluK2, subunit (Lerma, 2003).

Native AMPA and kainate receptors displaying differential channel conductances, Ca²⁺ permeabilities and sensitivity to block by intracellular polyamines have been identified (Cull-Candy et al., 2006; Isaac et al., 2007; Liu and Zukin, 2007). GluA1–4 can exist as two variants generated by alternative splicing (termed ‘flip’ and ‘flop’) that differ in their desensitization kinetics and their desensitization in the presence of cyclothiazide that stabilizes the non-desensitized state. TARPs also stabilize the non-desensitized conformation of AMPA receptors and facilitate the action of cyclothiazide (Milstein and Nicoll, 2008). Splice variants of GluK1–3 also exist, but their functional significance is unknown (Lerma, 2003).

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>AMPA</th>
<th>Kainate</th>
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<tbody>
<tr>
<td>Ensembl gene family ID</td>
<td>ENSF00000000118</td>
<td>ENSF00000000118</td>
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<tr>
<td>Selective agonists</td>
<td>AMPA, (S)-5-fluorowillardiine</td>
<td>ATPA, (S)-4-AHC, 8-deoxy-neodys hernine, (S)-4-iodowillardiine, LY404187, selective for receptors containing a GluK1 subunit, (2S,4R)-4-methylglutamate (SYM2081), dys hernine, domoic acid (inactive at GluK3), kainate (low potency at GluK3)</td>
</tr>
<tr>
<td>Selective antagonists</td>
<td>NBQX, ATPO, LY293558, GYK153655/LY300168 (active isomer GYK153784/LY303070)</td>
<td>UBPS302, UPB310, ACET, LY382884, LY466195 (all selective for receptors containing a GluK1 subunit), NS3763 (non-selective, GluK1-selective), MSVIII-19 (GluK1-selective), 2,4-epi-neodys hernine (GluK1- and GluK2-selective)</td>
</tr>
<tr>
<td>Positive modulators</td>
<td>Pyrrolidines (piraceta m, aniracetam), benzothiazidines (cyclothiazide, S18986), benzylpiperidines [CX-516 (BPD-12), CX-546], benzothiadiazides (cyclothiazide, S18986), 2,4-benzophysophonanilides (LY392098, LY404187 and LY530430)</td>
<td>Concanavalin A (GluK1 and GluK2, not GluK3)</td>
</tr>
<tr>
<td>Channel blockers</td>
<td>Intracellular polyamines, extracellular argiotoxin, extracellular Joro spider toxins, (selective for channels lacking GluA2)</td>
<td>Intracellular polyamines (subtype-selective)</td>
</tr>
<tr>
<td>Probes</td>
<td>[³H]AMPA, [³H]CNQX</td>
<td>[³H]Kainate, <a href="2S,4R">³H</a>-4-methylglutamate</td>
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All AMPA receptors are additionally activated by kainate (and domoate) with relatively low potency (EC50 ~ 100 μM). Inclusion of TARPs within the receptor complex increases the potency and maximal effect of kainate (Milstein and Nicoll, 2008). AMPA is weak partial agonist at GluK1 and at heteromeric assembles of GluK1/GluK2, GluK1/GluK5 and GluK2/GluK5 (Jane et al., 2009). Quinoxalinediones such as CNQX and NBQX show limited selectivity between AMPA and kainate receptor subunits. LY293558 also has kainate (GluK1) receptor activity as has GYK153655 (GluK3 and GluK2/GluK3) (Jane et al., 2009). ATPO is a potent competitive antagonist of AMPA receptors, has a weaker antagonist action at kainate receptors comprising GluK1 subunits, but is devoid of activity at kainate receptors formed from GluK2 or GluK2/GluK5 subunits. The pharmacological activity of ATPO resides with the (S)-enantiomer. ACET and UPB310 may block GluK3, in addition to GluK1 (Perrais et al., 2009), (2S,4R)-4-methylglutamate (SYM2081) is equipotent in activating (and desensitizing) GluK1 and GluK2 receptor isoforms and, via the induction of desensitization at low concentrations, has been used as a functional antagonist of kainate receptors. Both (2S,4R)-4-methylglutamate and LY339434 have agonist activity at NMDA receptors. (2S,4R)-4-methylglutamate is also an inhibitor of the glutamate transporters EAAT1 and EAAT2.

**Delta subunits:** GluD1 (GluRδ1) and GluD2 (GluRδ2) comprise, on the basis of sequence homology, an ‘orphan’ class of ionotropic glutamate receptor subunit. They do not form a functional receptor when expressed solely, or in combination with other ionotropic glutamate
receptor subunits, in transfected cells (Yuzaki, 2003). However, GluD2 subunits bind D-serine and glycine and GluD2 subunits carrying the mutation A654T form a spontaneously open channel that is closed by D-serine (Naur et al., 2007).

**Abbreviations:** (S)-4-AHC; (S)-6-amino-3-(3-hydroxy-7,8-dihydro-6H-cyclopenta[d]isoxazo[4,5]-4-yl)propionic acid; ACET, (S)-1-(2-amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione; AMPA, (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; APTAS, (R,S)-α-amino-3-(3-hydroxy-5-t-butylisoxo[4,5]-4-yl)propionic acid; ATPO, (R,S)-α-amino-3-(3-[5-t-butyl-3-(phosphonothioethyl)-4-isoxazolyl]propionic acid; CGP37849, (R,S)-α-amino-4-methyl-5-phosphono-3-pentenonic acid; CGP39653, (R,S)-α-amino-4-propyl-5-phosphono-3-pentenonic acid; CGS19755, (+)-cis-4-phosphonomethylpyridine-2-carboxylic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CP101606, (15S,2S)-1-4-hydroxyphenyl-2-(4-hydroxy-4-phenylpiperidino)-1-propanol; CPP, (R,S)-2-(carboxyethyl)-4-(4-carboxyethyl)-1-phosphono-3-pentenoic acid; CX-516, 1-(quinoxalin-6-yl-carbonyl)piperidine; CX-546, 1-(4-benzodioxan-6-ylcarbonylpiperidine); D-AP5, (R)-5-amino-2-phosphono pentanoate; D-CPPene, (R,E)-3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonic acid; GI96771A, E,4,6-dichloro-3-(2-oxo-1-phenylpyrrolidin-3-yldenemethyl)-1H-indole-2-carboxylic acid; GIYK31655, (+)-1-(4-aminoethyl)-3-methylcarboxamidobenzamoyl-4-methyl-3,4-dihydro-7,8-(methyleneoxido)-5H-2,3-benzodiazepine, also known as LY330168; GIYK31784, (+)-1-(4-aminoethyl)-3-methylcarboxamidobenzamoyl-4-methyl-3,4-dihydro-7,8-(methyleneoxido)-5H-2,3-benzodiazepine, also known as LY330070; H69660, trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonylaminol-1,2,3,4-tetrahydroquinoline; I701324, 7-chloro-4-hydroxy-3-(3-phenoxyl)phenyl-2(Hquinolone); LY233053, (+)-cis-4-(2H-tetrazol-5-yl)methylpyridine-2-carboxylic acid; LY233536, (+)-(6-(1H-tetrazol-5-ylmethyl)decahydrosaquinoline-3-carboxylic acid; LY293558, (3S,4αR,6αR,8αR)-6-([2-(1H-tetrazol-5-yl)ethyl]decahydropseudoquinoline-3-carboxylic acid; LY339434, (2S,4R,6-E)-2-amino-4-carboxy-7-(2-naphthyl)pyrroli dine-3-carboxylic acid; LY382884, (3S,4αR,6αS,8αS)-6-([4-(2-carboxyphenny1)ethyl]decahydropseudoquinoline-3-carboxylic acid; LY392009, propene-2-sulfonic acid 2-[4-thiophen-3-yl-phenoxy]propyl amide; LY404187, propene-2-sulfonic acid 2-[4-(cyano phenyl)4-propyl]amide; LY466195, (3S,4αR,6αS,8αR)-6-((S)-2-carboxy-4,4-difluoro-1-pyrroli dinyl)methyl]decahydro-3-isoquinolinedicarboxylic acid; LY503430, (R,E)-1-[1-fluoro-1-methyl(2-propyl-2-sulfonylethoxy)methyl]phenyl-4-carboxylic acid methylamide; M1105519, (E)-3-(2-phenyl-2-carboxyethyl)-4,6-dichloro-1H-indole-2-carboxylic acid; MSVIII-19, (2S,3αR,7αR)-2-(2S,3αR,7αR)-2-(3-amino-1-hydroxypyrrolidin-2-one)-3-[(2-carboxyethyl)2-carboxymethyl]-5-methylpyrimidine-2,4-dione; NVP-AAM077, (R)-[S)-1,4-bromophenylethylamino]-2,3-dioxo-1,2,4,3,4,3,4,3,4,4,4,4,4-tetradhydroxyquinoxalin-5-yl]methylphosphonic acid; PPDA, (29'S,3R*)-[1-(phenanthren-2-carboxyethyl)piperazine-2,3-dicarboxylic acid; RO-843040, 4-(3-[4-(4-fluorophenyl)-3,6-dihydro-2H-pyridin-1-yl]-2-hydroxypropoxypenamide; S10986, (S)-2,3-dihydro-[4,3j]-cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide; UBP302, (S)-1-(2-amino-carboxyethyl)-3-(2-carboxybenzyl)4-methylpyrimidine-2,4-dione; UBP310, (S)-1-(2-amino-carboxyethyl)-3-(2-carboxybenzyl)-4-methylpyrimidinum-2,4-dione

Further Reading


References