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Perspective

Developing a complete pharmacology for AMPA receptors: A perspective on subtype-selective ligands

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ABSTRACT

AMPA receptors are a family of ligand-gated ion channels that play central roles in rapid neural signaling and in regulation of synaptic strength. Additionally, these receptors are implicated in a number of major psychiatric and neurological diseases. A comprehensive understanding of the roles that AMPA receptors play in the mammalian nervous system has been hampered by the dearth of ligands available to select between individual AMPA receptors subtypes. Here we provide a perspective on opportunities for developing a complete pharmacology for AMPA receptors.

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1. Introduction

AMPA (alpha-amino-3-hydroxy-5-methyl-4-isooxazole-propionic acid) receptors (AMPARs) are a family of ligand-gated ion channels that are activated by glutamate, the principal neurotransmitter in the mammalian central nervous system.[1–3] Several different AMPAR subtypes are expressed throughout the nervous system, on both excitatory and inhibitory neurons. AMPARs expressed on excitatory neurons directly mediate the vast majority of fast, excitatory communication between neurons by ensuring rapid responses to glutamate released into the synaptic cleft. AMPARs expressed on inhibitory neurons indirectly affect this synaptic transmission by modulating the activity of excitatory neurons. In addition to these basal functions, AMPARs play an essential role in synaptic plasticity, the activity-dependent modulation of synaptic transmission that governs the activity of neuronal networks and ultimately, the behavior of the whole animal.[4,5]

Changes in the composition of AMPAR subtypes present at synapses are a key aspect of normal cognitive processes as well as cognitive dysfunction.[4,6–10] For example, 'subtype switching' is an essential mechanism in several forms of synaptic plasticity (e.g., cellular models of memory and addiction).[6,8] In addition, alternate expression patterns of AMPAR subtypes have been proposed to provide a molecular basis for neuropathologies (e.g., schizophrenia, Alzheimer's disease).[9,10] Efforts to delineate the locations and roles that individual AMPAR subtypes play in the nervous system have been hampered, however, by the dearth of subtype-selective ligands for these receptors. Here we discuss the major classes of ligand-binding domains found on AMPARs and provide a perspective on the best opportunity for developing ligands to select between individual subtypes.

Abbreviations: ACPA, (*R*,*S*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isooxazole-propionic acid; aniracetam, 1-[(4-methoxybenzoyl)]-2-pyrrolidinone; ArgTx-636, argiotoxin-636; 2-Bn-Tet-AMPA, (*R*,*S*)-2-amino-3-[3-hydroxy-5-(2-benzyl-2*H*-5-tetrazolyl)4-isoxazolyl]propionic acid; Br-HIBO, (*R*,*S*)-2-amino-3-(4-bromo-3-hydroxy-5-isoxazolyl) propionic acid; Cl-HIBO, (*R*,*S*)-2-amino-3-(4-chloro-3-hydroxy-5-isoxazolyl)propionate; CTZ, cyclothiazide, 6-chloro-3,4-dihydro-3-(5-norbornen-2-yl)-2*H*-1,2,4-benzothiazidiazine-7-sulfonamide-1,1-dioxide; CX-516, 1-(quinoxalin-6-ylcarbonyl)-piperidine; CX-614, 2*H*,3*H*,6a*H*-pyrrolidino[2″,1″-3′2′]1,3-oxazino[6′,5′-5,4] benzo[*e*]1,4-dioxan-10-one; Evans blue, tetrasodium 6,6′-((3,3′-dimethyl-(1,1′-biphenyl-4,4′diyl)bis(azo)bis(4-amino-5-hydroxy-1,3-naphthalenedisulphonate); GYKI-53655, 1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5H-2,3-benzodiazepine; HPP-spermine, *N*-(4-hydroxyphenylpropanyl)-spermine; JSTX-3, Joro spider toxin; LIVBP, leucine-isoleucine-valine-binding protein; LY-404187, *N*-[2-(4′-cyanobiphenyl-4-yl)propyl]propane-2-sulfonamide; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*F*)quinoxaline; NMDA, *N*-methyl-D-aspartate; PEPA, 2-(2,6-difluoro-4-(2-(phenylsulfonamido)ethylthio)phenoxy)acetamide; PhTx-343, philanthotoxin-343; PhTx-74, philanthotoxin-74; Talampanel, (8*R*)-7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7*H*-1,3-dioxolo[4,5-*h*][23]benzodiazepine; TARPs, transmembrane AMPA receptor regulatory proteins; Tezampanel, (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1*H*-tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid.

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2. AMPAR subtypes

Native AMPARs are tetrameric structures consisting of four subunits, GluA1–4 (formerly known as GluR1–4 or GluRA–D[15]).[1–3] In addition, each subunit undergoes alternative splicing in the extracellular domain to produce *flip(i)* and *flop(o)* variants. Seven different principal AMPAR subtypes, consisting of both heteromeric and homomeric assemblies of GluA1–4, are expressed in the mammalian central nervous system.[11–13] Heteromeric receptors, thought to assemble as dimers of dimers,[14] contain two GluA2 subunits and two of either the GluA1, 3, or 4 subunits (i.e., GluA1A2, GluA2A3, GluA2A4) and are the principal subtypes expressed on excitatory neurons.[11,12] Homomeric receptors (i.e., GluA1, GluA2, GluA3, GluA4) are more sparsely expressed in the nervous system, with GluA1 receptors concentrated on inhibitory neurons.[13] The composition of subunits determines a number of key properties of AMPARs including the channel conductance, divalent ion permeability, gating kinetics, trafficking, and pharmacology of the assembled receptor.[15,16] In the following sections the pharmacology of AMPARs with respect to their subtypes is described.

2.1. AMPAR ligand-binding sites

Five different ligand-binding regions have been identified on AMPARs (Fig. 1).[17] Each subunit contains an extracellular amino terminal domain (NTD, also known as the ATD or X-domain) and a ligand-binding domain (LBD). Three additional ligand-binding regions are found at the interface between subunits; specifically, between LBDs, between the extracellular domain and the transmembrane segments, and within the pore. Distinct classes of ligands that target these sites have been developed and can be divided into the following four categories: (1) competitive agonists and antagonists; (2) positive allosteric modulators; (3) negative allosteric modulators; and (4) pore blockers.

Competitive agonists and antagonists exert their influence on AMPARs at the LBD by controlling the gating of the channel.[18,19] Since individual AMPAR subunits each contain a LBD, assembled receptors can accommodate a total of four ligands, with the number and type of bound agonist influencing the conductance level of the channel.[19] Positive allosteric modulators bind at the interface between subunits, with one[20] or two[21] molecules per interface, and potentiate AMPAR currents by slowing the desensitization and/or deactivation of the receptor. Negative allosteric modulators bind at the interface of adjacent subunits between the extracellular LBD and the channel transmembrane domains, inhibiting the conformational change that leads to channel opening.[22] Pore blockers bind within the pore of open receptors, inhibiting the flow of ions through the channel.[23] Each assembled receptor, containing only one pore, binds a single blocker. Thus far, no ligands have been shown to influence the biophysical properties of AMPARs by binding to the NTD.[17,24] Yet, this domain is homologous to bacterial periplasmic binding proteins (i.e., LIVBP), which bind amino acids (i.e., leucine, isoleucine, and valine), and to the NTD on NMDA receptors, for which subtype-selective ligands have been identified.[25,26]

3. Subtype-selective ligands for AMPARs

The importance of AMPARs in synaptic transmission and plasticity has motivated efforts to develop pharmacological tools for controlling the activity of these receptors.[27] Historically, these efforts have primarily and successfully been focused on developing ligands that select between AMPARs and other glutamate-gated ion channels (i.e., NMDA and kainate receptors). However, in recent years the biology of AMPARs has underscored the need for ligands that select between individual AMPAR subtypes.[4,6,8–10] The following sections highlight studies examining the effects of ligands across multiple AMPAR subtypes (summarized in Table 1). This survey is limited to studies of recombinantly expressed wild type receptors that: (1) utilized functional assays (i.e., ionic current recordings and in a few cases Ca^{2+}-flux measurements); (2) evaluated ligand–receptor interactions across multiple subtypes. Since pharmacological data can vary between laboratories (Table 1, see especially entries for (*S*)-glutamate and ArgTx-636), we felt it best to only highlight subtype-selectivity observed within individual studies.

3.1. Competitive agonists and antagonists

The agonists glutamate, AMPA, and kainate, which are widely employed for activating AMPARs, display modest (<5-fold) selectivity among homomeric receptors.[28,29] Banke et al. reported the first subtype-selective agonist, ACPA, an analog of AMPA that is 11-fold more potent at homomeric GluA3 than GluA1 receptors (0.10 ± 0.02 and 1.1 ± 0.3 μM, respectively).[30] Subsequently developed agonists show the opposite selectivity trend, blocking GluA1 and GluA2 more potently than GluA3 and GluA4 receptors. For example, Coquelle et al. reported that Br-HIBO, an isoxazole-based analog of AMPA, is 14-fold selective for GluA1 over GluA3 receptors (EC_{50} = 14 ± 2 and 202 ± 68 μM, respectively).[31] However, Br-HIBO only shows modest selectivity among heteromeric receptors, with five-fold greater efficacy at GluA1A2 over GluA2A3 (EC_{50} = 16 ± 3 and 80 ± 25 μM, respectively). Later, Cl-HIBO was synthesized and shown to have a selectivity of 658- and 1588-fold for GluA1 and GluA2 over GluA3 receptors (EC_{50} = 4.1 ± 1.1, 1.7 ± 0.6, 2700 ± 350 μM, respectively).[32] More recently, 2-Bn-Tet-AMPA, another analog of AMPA, was shown to exhibit a modest six-fold selectivity for GluA4 over GluA1 receptors (EC_{50} = 490 and >3000 μM, respectively).[33] The uracil-derived willardiines are another class of subtype-selective agonists that are generally selective for GluA1 and GluA2 over GluA3 and GluA4 receptors.[34–36] The most selective analog, (*S*)-5-fluorowillardiine is 55-fold more potent at GluA1 than GluA3 receptors (EC_{50} = 0.38 ± 0.07 and 20.9 ± 1.9 μM, respectively). Recently, the selectivity of the willardiines was enhanced by preparing an isomeric 3-hydroxypyridazine 1-oxide derivative, which exhibited 161-fold selectivity for GluA1 over GluA3 receptors (EC_{50} = 0.26 ± 0.06 and 41.6 ± 2.9 μM, respectively).[36] Crystallographic and mutagenesis studies suggest that the selectivity displayed by agonists for GluA1 and GluA2 over GluA3 and GluA4 is due in part to a single amino acid difference in the ligand-binding domain (Y702 in GluA1 and GluA2 versus F in GluA3 and GluA4).[37,38] However, recent structural work reveals a more complex picture in which subtle differences in regions lying outside of the ligand-binding domain influence agonist binding and the subsequent extent of channel opening, shedding new light on the factors governing subtype-selectivity.[39] The recently reported structure of full-length GluA2 AMPAR will undoubtedly provide additional insight into the molecular basis for subtype-selectivity.[40]

Several different classes of competitive antagonists for AMPARs have been developed (e.g., quinoxalinediones, indenoimidazones, and isatine oximes), though only a few studies have characterized the activity of these compounds across multiple AMPAR subtypes or their splice variants. Stein *et al.* demonstrated that NBQX is 3.8-fold more potent at GluA4 than GluA1 (K_B = 102 and 389 nM, respectively), and is 3.3-fold more potent at blocking glutamate-evoked currents from GluA2A4 than GluA1A2 receptors (K_B = 166 and 50.1 nM, respectively) using Schild analysis.[41,42]

3.2. Positive allosteric modulators

Positive allosteric modulators, also referred to as ampakines, potentiate AMPAR-mediated currents by attenuating receptor

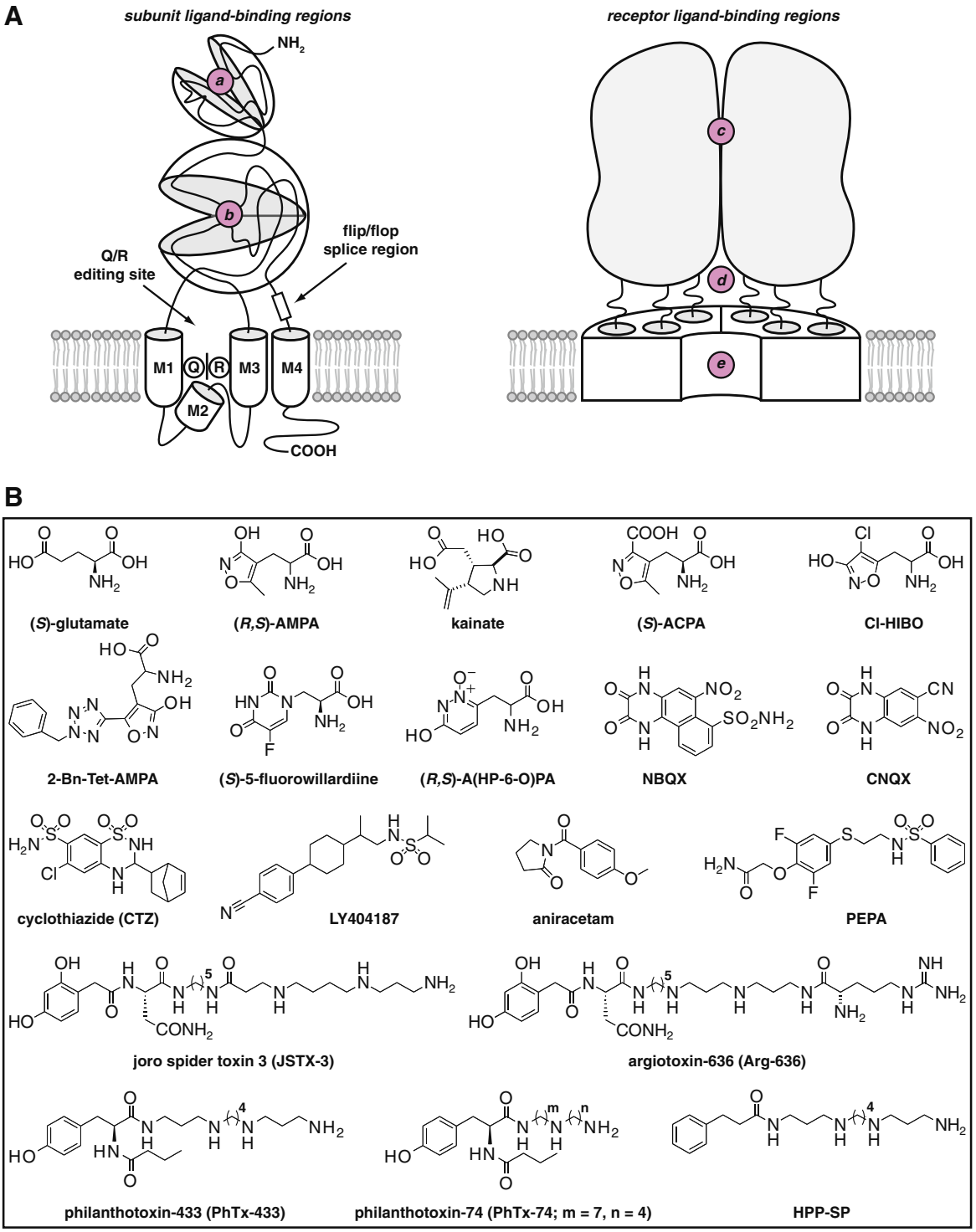

Figure 1. Cartoon depicting the structure of the AMPA receptor, ligand-binding sites found on the receptor (*a*–*e*), and structures of the ligands discussed in the text. (A) Left: Each AMPAR subunit is approximately 900 amino acids in length and consists of a large extracellular domain comprised of the amino terminal domain (*a*: NTD) that influences the assembly of the receptor, the ligand-binding domain (*b*: LBD) that gates the pore of the receptor, three transmembrane segments (1, 3, 4), a reentrant loop (2) that lines the pore of the channel, and a cytoplasmic C-terminal domain that influences receptor trafficking. The Q/R editing site has a profound effect on the physiology and pharmacology of AMPA receptors. The flip/flop splice region influences the desensitization and deactivation of the receptor. Right: Cut open view of the receptor showing two of the four subunits and ligand-binding regions found at the interface between subunits (*c*), the interface between the extracellular domain and the transmembrane segments (*d*), and the channel pore (*e*). (B) Structures of competitive agonists, allosteric modulators, and polyamine toxins shown in Table 1.

desensitization and/or deactivation and have been pursued for their potential as cognition enhancing or 'nootropic' drugs. Several distinct chemical classes of these compounds have been reported, including the benzamides (e.g., aniracetam, and CX-614), the benzothiadiazines (e.g., cyclothiazide), and a broad collection of arylsulfonamides (e.g., LY-404187 and PEPA).[27,43] Positive allosteric modulators typically show low to moderate (<10-fold) selectivity among AMPAR subtypes.[44–50] However, they show significant selectivity between flip and flop splice variants of these AMPARs. For example, cyclothiazide (CTZ) (EC_{50} = 1 μM at $GluA1_i$), which

Table 1
Table showing the relative activity of several agonists, antagonists, allosteric modulators, and pore blockers across multiple AMPAR subtypes

Ligand	Receptor (Activity)	Conditions	Ref
Agonists			
(S)-Glutamate	$GluA1_o$ (6.4 ± 1.1 μM); $GluA3_o$ (1.3 ± 0.3 μM)	*a*	30
(S)-Glutamate	$GluA1_o$ (22 ± 4 μM); $GluA2_o$(Q) (6.2 ± 1.0 μM); $GluA3_o$ (35 ± 15 μM); $GluA4_o$ (8.1 ± 2.3 μM) $GluA1_oA2_o$ (18 ± 3 μM); $GluA2_oA3_o$ (11 ± 2 μM); $GluA2_oA4_o$ (3.5 ± 1.5 μM)	*a*	31
(S)-Glutamate	$GluA1_iA2_i$ (6.16 ± 0.55 μM); $GluA2_iA4_i$ (32.3 ± 4.3 μM)	*a* *	42
(S)-Glutamate	$GluA1_i$ (2.26 ± 0.38 μM); $GluA2_o$ (2.19 ± 0.21 μM); $GluA3_o$ (3.66 ± 0.05 μM); $GluA4_o$ (2.75 ± 0.06 μM)	*a,* *	79
(S)-Glutamate	$GluA1_i$ (51 ± 4 μM); $GluA2(Q)_i$ (190 ± 21 μM); $GluA3_i$ (52 ± 5 μM); $GluA4_i$ (20 ± 4 μM)	*b,c,q*	29
(R,S)-AMPA	$GluA1_o$ (8.7 ± 1.3 μM); $GluA3_o$ (1.4 ± 0.2 μM)	*a*	30
(R,S)-AMPA	$GluA1_iA2_i$ (3.31 ± 0.2 μM); $GluA2_iA4_i$ (5.01 ± 0.56 μM)	*a*	42
(R,S)-AMPA	$GluA1_i$ (53 ± 8 μM); $GluA2(Q)_i$ (67 ± 12 μM); $GluA3_i$ (37 ± 5 μM); $GluA4_i$ (16 ± 5 μM)	*b,c,q*	29
Kainate	$GluA1_iA2_i$ (57.5 ± 5.2 μM); $GluA2_iA4_i$ (64.6 ± 8.1 μM)	*a*	42
Kainate	$GluA1_i$ (7.51 ± 0.98 μM); $GluA2_o$ (10.57 ± 1.00 μM); $GluA3_o$ (26.61 ± 2.27 μM); $GluA4_o$ (29.06 ± 3.75 μM)	*a,* *	79
Kainate	$GluA1_o$ (27 ± 3 μM); $R3_o$ (31 ± 3 μM)	*a*	30
Kainate	$GluA1_i$ (23 ± 5 μM); $GmA2(Q)_i$ (380 ± 18 μM); $GluA3_i$ (40 ± 2 μM); $GluA4_i$ (47 ± 5 μM)	*b,c,q*	29
Kainate	$GluA1_i$ (73 ± 12 μM); $GluA1_o$ (60 ± 12 μM)	*a*	45
(R,S)-ACPA	$GluA1_o$ (1.1 ± 0.3 μM); $GluA3_o$ (0.10 ± .02 μM)	*a*	30
(S)-ACPA	$GluA1_o$ (0.27 ± 0.01 μM); $GluA3_o$ (1.6 ± 0.2 μM); $GluA4_o$ (1.7 ± 0.5 μM)	*a*	100
Br-HIBO	$GluA1_o$ (14 ± 2 μM); $GluA2_o$(Q) (5.4 ± 1.3 μM); $GluA3_o$ (202 ± 68 μM);$GluA4_o$ (39 ± 4 μM) $GluA1_oA2_o$ (16 ± 3 μM); $GluA2_oA3_o$ (80 ± 25 μM); $GluA2_oA4_o$ (26 ± 10 μM)	*a*	31
Cl-HIBO	$GluA1_i$ (4.7 ± 1.1 μM); $GluA2_o$(Q) (1.7 ± 0.6 μM); $GluA3_i$ (2700 ± 350 μM); $GluA4_i$ (1300 ± 130 μM)	*a*	32
2-Bn-Tet-AMPA	$GluA1_i$ (>3000 μM); $GluA4_i$ (490 μM)	*a,q*	33
(S)-F-Willardiine	$GluA1_i$ (0.382 ± 0.066 μM); $GluA2_i$(Q) (0.463 ± 0.037 μM); $GluA3_i$ (20.9 ± 1.9 μM); $GluA4_i$ (11.9 ± 1.8 μM)	*a*	36
(R,S)-A(HP-4-O)PA	$GluA1_i$ (0.258 ± 0.058 μM); $GluA3_i$ (41.6 ± 2.9 μM)	*a,u*	36
Antagonists			
NBQX	$GluA1_i$ (389 nM); $GluA4_i$ (102 nM); $GluA1_iA2_i$ (166 nM); $GluA2_iA4_i$ (50.1 nM)	*a,r*	42
NBQX	$GluA1_i$ (2.0 ± 0.2 μM); $GluA2(Q)_i$ (0.59 ± 0.26 μM); $GluA3_i$ (0.63 ± 0.18 μM); $GluA4_i$ (1.6 ± 0.3 μM)	*b,c,s*	29
CNQX	$GluA1_iA2_i$ (589 nM); $GluA2_iA4_i$ (501 nM)	*a,r*	42
Allosteric Modulators			
Cyclothiazide	$GluA1_i$ (103 ± 30); $GluA1_o$ (22 ± 4); $GluA3_i$ (69 ± 19); $GluA4_i$ (171 ± 67); $GluA1_iA2_i$ (137 ± 37); $GluA1_oA2_o$ (61 ± 13)	*a,d*	44
Cyclothiazide	$GluA4_i$ (5.9 ± 1.3 μM); $GluA1_iA2_i$ (6.6 ± 3.3 μM); $GluA1_oA2_o$ (>76 μM); $GluA2_iA4_i$ (5.0 ± 0.3 μM) $GluA2_oA4_o$ (>91 μM)	*a,i*	47
Cyclothiazide	$GluA1_i$ (10 μM); $GluA1_o$ (29 μM)	*a,j* *	86
Cyclothiazide	$GluA1_i$ (2 μM); $GluA1_o$ (10 μM)	*a,j,* *	86
Cyclothiazide	$GluA1_i$ (19.80 ± 4.73 μM); $GluA2(Q)_i$ (2.24 ± 0.05 μM); $GluA2(Q)_o$ (> 100 μM); $GluA3_i$ (13.7 ± 4.24 μM); $GluA4_i$ (3.91 ± 0.19 μM)	*b,c,m*	49
Cyclothiazide	$GluA1_i$ (5.31 ± 0.97); $GluA1_o$ (1.34 ± 0.13); $GluA3_i$ (3.59 ± 0.22); $GluA3_o$ (5.59 ± 0.23); $GluA1_iA2_o$ (19.92 ± 1.54); $GluA1_oA2_i$ (11.57 ± 0.85); $GluA1_oA2_o$ (3.53 ± °0.09); $GluA2_oA3_i$ (6.83 ± 0.71); $GluA2_iA3_o$ (7.18 ± 0.99); $GluA2_oA3_o$ (10.51 ± 2.28)	*a,g*	101
LY404187	$GluA1_i$ (5.65 ± 4.11 μM); $GluA2(Q)_i$ (0.15 ± 0.03 μM); $GluA2(Q)_o$ (1.44 ± 0.03 μM); $GluA3_i$ (1.66 ± 0.25 μM); $GluA4_i$ (0.21 ± 0.02 μM)	*b,c,m*	49
Aniracetam	$GluA1_oA2_o$ (17 ± 3.5); $GluA1_iA2_i$ (8.1 ± 2.2) $GluA2_oA4_o$ (77 ± 16); $GluA2_iA4_i$ (7.5 ± 1.7)	*a,e*	47
PEPA	$GluA1_i$ (5 ± 1); $GluA1_o$ (18 ± 3); $GluA3_i$ (41 ± 4); $GluA3_o$ (131 ± 26); $GluA4_i$ (11 ± 1); $GluA4_o$ (75 ± 6); $GluA1_iA2_i$ (20 ± 2); $GluA1_oA2_o$ (124 ± 20); $GluA2_iA3_i$ (28 ± 3); $GluA2_oA3_o$ (138 ± 30); $GluA2_iA4_i$ (10 ± 1); $GluA2_oA4_o$ (74 ± 11); $GluA1_i$ (1.90 ± 0.17); $GluA1_o$ (2.9 ± 0.24); $GluA3_i$ (2.51 ± 0.09); $GluA3_o$ (11.14 ± 1.17);	*b,f*	50
PEPA	$GluA1_i$ (1.90 ± 0.17); $GluA1_o$ (2.9 ± 0.24); $GluA3_i$ (2.51 ± 0.09); $GluA3_o$ (11.14 ± 1.17); $GluA1_iA2_o$ (20.08 ± 1.93); $GluA1_oA2_i$ (9.49 ± 0.54); $GluA1_oA2_o$ (7.44 ± 0.47); $GluA2_oA3_i$ (4.78 ± 0.35); $GluA2_iA3_o$ (8.85 ± 1.26); $GluA2_oA3_o$ (21.07 ± 6.35)	*a,h*	101
GYKI-53655	$GluA_i$ (28 μM); $GluA2_i$ (100 μM)	*a,p,* *	57
Pore Blockers			
JSTX-3	$GluA1_o$ (0.04 μM); $GluA3_o$ (0.03 μM)	*a,k*	62
JSTX-3	$GluA1_o$ (88 ± 9); $GluA3_o$ (96 ± 3); $GluA4_o$ (82 ± 12); $GluA1_oA2_o$ (0); $GluA2_oA3_o$ (0)	*a,n*	62
Argiotoxin-636	$GluA1_i$ (0.35 μM); $GluA3_i$ (0.23 μM); $GluA4_i$ (0.43 μM); $GluA1_iA2_i$ (5.5 μM)	*a,l*	63
Argiotoxin-636	$GluA1_o$ (3.4 μM); $GluA1_oA2_o$ (300 μM)	*a,l*	62
HPP-Spermine	GluA1 (0.46 μM); GluA3 (0.064 μM); GluA4 (0.32 μM)	*a,k*	68
Philanthotoxin-343	$GluA1_o$ (2.8 μM); $GluA1_oA2_o$ (270 μM)	*a,l*	64
Philanthotoxin-74	$GluA1_i$ (100 ± 0); $GluA2_i$ (2.1 ± 0.5); $GluA3_i$ (100 ± 0); $GluA1_iA2_i$ (84.3 ± 1.4); $GluA2_iA3_i$ (2.6 ± 0.5)	*a,o,* *	67
Philanthotoxin-74	$GluA1_i$ (0.168 ± 0.021 μM); $GluA1_iA2_i$ (1.6 ± 0.5 μM)	*a,m,t*	66

Structures of the ligands are shown in Figure 1. All data are taken from functional assays of recombinantly expressed wild type AMPARs (see reference column) and are reported as IC_{50} and EC_{50} unless otherwise noted (see conditions column). The following refers to the conditions for these experiments: *a*: recombinant expression in Xenopus oocytes; *b*: recombinant expression in HEK293 cells; *c*: Ca^{+2} flux assay; *d*: fold increase in peak response to 300 μM glutamate ± 100 μM cyclothiazide; *e*: fold increase in peak response to 300 μM glutamate ± 5 mM aniracetam; *f*: fold increase in peak response to 3000 μM glutamate ± 100 μM PEPA; *g*: fold increase in peak response to 10 μM AMPA ± 100 μM cyclothiazide; *h*: fold increase in peak response to 10 μM AMPA ± 100 μM PEPA; *i*: EC_{50} in the presence of 300 μM glutamate; *j*: EC_{50} in the presence of 10 μM glutamate; *k*: agonist = 300 μM kainate; *l*: agonist = 100 μM kainate; *m*: agonist = 100 μM glutamate; *n*: percent inhibition with 0.5 μM JSTX-3 in the presence of 300 μM kainate; *o*: percent inhibition with 100 μM PhTx-74 in the presence of 100 μM glutamate; *p*: measurements carried out in the presence of 200 μM glutamate and 500 μM trichlormethiazide; *q*: experiments carried out with 100 μM cyclothiazide; *r*: values reported as K_B and quanitifed using Schild analysis; *s*: values reported as K_B and quantified using the Cheng–Prusoff method; *t*: values reported as K_i and quantified using the Hill–Lanqmuir equation; *u*: A(HP-6-O)PA stands for 2-amino-3-(3-hydroxy-pyridiazin-6-yl 1-oxide)propionic acid; *: AMPARs were coexpressed with the γ2 TARP.

slows desensitization, is more efficacious at *flip* isoforms, potentiating glutamate-evoked $GluA1_i$ currents 103-fold compared to 22-fold for $GluA1_o$ currents at a concentration of 100 μM.[44] In another report, Miu et al. demonstrated that LY-404187 is approximately 10-fold more potentat blocking desensitization at $GluA2_i$ compared to $GluA2_o$ receptors (EC_{50} = 15 ± 0.03 and 1.44 ± 0.03 μM, respectively).[49]

Other classes of compounds have the opposite selectivity, potentiating flop over flip isoforms. For example, Partin et al. demonstrated that aniracetam (EC_{50} >1 mM), which slows deactivation, potentiated $GluA1_oA2_o$ currents twofold more than $GluRA_iA2_i$ currents, and $GluA2_oA4_o$ currents 10-fold more than $GluA2_iA4_i$ currents at a concentration of 5 mM.[51] PEPA (EC_{50} = 50 μM at $GluA3_i$), another molecule that slows deactivation, potentiated AMPAR currents among flop isoforms 3- to 7-fold more than flip isoforms at a concentration of 100 μM.[50] Intriguingly, PEPA also showed some selectivity among the principal receptor subtypes, potentiating $GluA3_o$ ~7-fold more than $GluA1_o$ receptors, and among heteromeric receptor subtypes, potentiating $GluA1_oA2_o$ and $GluA2_oA3_o$ ~2-fold more than $GluA2_oA4_o$ receptors.[50]

3.3. Negative allosteric modulators

Negative allosteric modulators are non-competitive antagonists of AMPARs. These ligands are comprised of several distinct chemical classes, including the 2,3-benzodiazepines (e.g., GYKI-53655, LY300164), quinazolinones (e.g., CP-465,022), arylphthalazines (e.g., SYM-2207), and tetrahydrosioquinolines. The latter two series are structural derivatives of 2,3-benzodiazepines.[27,52,53] Although many of the negative allosteric modulators are potent AMPAR antagonists, few have been examined for subtype-selectivity. Those that have been tested (GYKI-52466, GYKI-53655, LY300164) demonstrated only slight (<4-fold) selectivity among receptor subtypes or splice variants.[54–57] AMPAR residues important for the binding of GYKI-53655 and CP-465,022 have been identified in mutagenesis studies.[22]

3.4. Pore blockers

Polyamine toxins are a class of low molecular mass compounds originally identified as natural products found in the venoms of wasps and spiders.[58–61] Though these compounds appear to have evolved for paralyzing prey by binding to glutamate-gated ion channels expressed at the insect neuromuscular junction, they have also proven to be potent inhibitors of several ligand-gated ion channels in the mammalian central nervous system, including AMPA, NMDA, kainate, and nicotinic acetylcholine receptors. The structural motif common among these molecules is a polyamine tail linked to an aromatic head-group. Considerable evidence suggests that polyamine toxins bind within the pore of open channels, with inhibition typically being both use- and voltage-dependent.

Initial studies evaluating the effects of naturally occurring polyamine toxins across multiple AMPAR subtypes demonstrated that these compounds generally are selective for a subset of AMPARs—namely, channels lacking the GluA2 subunit (i.e., GluA1, GluA3, GluA4). For example, the natural product joro spider toxin (JSTX-3) blocks nearly all of the current from GluA1, GluA3 and GluA4 homomers, while having no effect on the heteromeric receptors tested (i.e., GluA1A2 and GluA2A3) at a concentration of 0.5 μM.[62] Similarly, argiotoxin-636 (ArgTx-636) inhibits GluA1, GluA3, and GluA4 homomers with nearly equal potency (IC_{50} = 0.35, 0.23, and 0.43 μM, respectively), and is more than 10-fold less potent at GluA1A2 heteromers (IC_{50} = 5.5 μM).[63] A subsequent study with Arg-636 estimated that the difference in selectivity between GluA1 homomers and GluA1A2 heteromers is closer to 100-fold (IC_{50} = 3.4 and 300 μM, respectively).[64] This study also showed that the natural product philanthotoxin-343 (PhTx-343), a synthetic analog of the wasp toxin philanthotoxin-433, inhibits GluA1 homomers 100 times more potently than GluA1A2 heteromers (IC_{50} = 2.8 and 270 μM, respectively).[64]

Mutagenesis studies suggested that a single pore-lining residue accounts for the general selectivity among polyamine toxins for GluA2-lacking receptors.[15,16,62,65] In particular, edited GluA2 subunits carry a positively charged arginine (R) at the Q/R site (Fig. 1), which is thought to repel positively charged polyamines, rather than the uncharged glutamine (Q) present in the other subunits. Mutation of the GluA2 subunit (GluA2R586Q) yields receptors that are potently inhibited by polyamine toxins.[62] Along with a similar selectivity observed among endogenous polyamines (e.g., spermine, spermindine), these studies led to a widely held assumption that GluA2-containing receptors are insensitive to polyamine toxins. However, more recent studies demonstrate that polyamine toxins can be developed to inhibit GluA2-containing receptors (see below).

In the ensuing years, the naturally occurring polyamine toxins served as templates for designing numerous synthetic analogs, a number of which block GluA2-containing receptors. For example, Kromann et al. reported several analogs of PhTx-433 that block GluA1A2 receptors at low micromolar concentrations.[66] Nilsen and England demonstrated that PhTx-74 is a selective inhibitor among GluA2-containing receptors, preferentially blocking GluA1A2 receptors over GluA2A3 receptors.[67] Intriguingly, replacing the C-terminal domain on GluA3 with that from GluA1 resulted in chimeric GluA2A3 receptors that were partially blocked by PhTx-74, demonstrating that the Q/R site is not the sole determinant of polyamine toxin sensitivity.[67] Among GluA2-lacking receptors, Washburn and Dingledine found that the IC_{50} for the synthetic polyamine toxin HPP-spermine was 5–6-fold lower at GluA3 homomers than GluA1 and GluA4 homomers (IC_{50} = 64, 460, and 320 nM, respectively).[68]

3.5. Miscellaneous subtype-selective ligands

Other subtype-selective ligands, which have yet to be assigned a clear binding site within the AMPAR, have also been identified. Evans Blue, an organic dye derived from 1,3-napthalene disulfonic acid, selectively modulates the desensitization of GluA1, GluA1A2, and GluA2A3, but not GluA3 receptors.[69–71] Recently, a *Conus* snail polypeptide, con-ikot–ikot, was identified that blocks AMPAR desensitization, showing a greater enhancement for the flop versus flip isoform of GluA1 receptors.[72] This toxin appears to bind to a site that is distinct from the CTZ binding site.

4. AMPAR auxiliary subunits

In recent years (following most of the compound characterization presented in this review), the remarkable discovery was made that most if not all AMPARs in the mammalian central nervous system are associated with auxiliary subunits called transmembrane AMPAR regulatory proteins (TARPs), of which there are six isoforms enriched in the brain (γ2, γ3, γ4, γ5, γ7, γ8) and cornichon homologs (CNIHs), of which there are two isoforms (CNIH-2, CNIH-3). Considerable evidence suggests that TARPs and CNIHs have profound effects on the trafficking and biophysical properties of AMPARs.[73–78] Furthermore, a number of studies have demonstrated that TARPs alter the activity of several pharmacological probes for AMPARs. These studies, the vast majority of which examined the effect of the γ2 TARP on the GluA1 AMPAR subtype, revealed that TARPs reduce the IC_{50} for glutamate and kainate (see * entries in Table 1),[57,79–84] increase the IC_{50} for CNQX and covert this competitive antagonist into a partial agonist,[57,84,85] reduce

the EC_{50} and IC_{50} for allosteric modulators (e.g., cyclothiazide, GYKI-53655, CP-465,022),[57,82,86] and alter the efficacy of pore blockers (e.g., spermine, NASPM).[84,87] While informative, the aforementioned studies do not directly demonstrate that auxiliary subunits alter the subtype-selectivity of pharmacological ligands among AMPAR subtypes. In fact, we are aware of only a few studies that shed some light onto this possibility.[84,86,88–90] Tomita et al. showed that whereas the EC_{50} for cyclothiazide is 2.9-fold higher at $GluA1_o$ compared to $GluA1_i$ receptors in the absence of TARPs, it is five-fold higher, respectively, when these receptors are co-expressed with the γ2 TARP (see * entries in Table 1).[86] Kott et al. demonstrated that the particular TARP subtype with which the AMPAR is associated also affects the activity of pharmacological probes by showing that the TARP-associated shift in IC_{50} for CNQX differs by a factor of 11.3 between the γ2 and γ4 TARP.[84] The association of AMPARs with auxiliary subunits greatly expands the population of AMPARs present in the CNS and these studies provide evidence that both the presence and type of the associated auxiliary subunit can impact the subtype-selectivity of an AMPAR ligand. Thus, studies that systematically evaluate of the impact of auxiliary subunits on the subtype-selectivity of AMPAR ligands will represent an important undertaking.

4.1. Defining 'subtype-selective'

In developing subtype-selective ligands for AMPARs, it is important to consider the degree of selectivity that is required for a ligand to be useful. Ideally, there should be at least one concentration of the ligand that fully activates or antagonizes one receptor subtype, while having no effect on the other receptor subtypes. In terms of receptors recombinantly expressed in a heterologous system (e.g., Xenopus ooctyes), this translates to a difference in the EC_{50}/IC_{50} of >10-fold (assuming a Hill coefficient of one) between subtypes.[91] However, it is important to recognize that the affinity of a ligand may be significantly influenced by the experimental conditions and subtype-selectivity observed in a heterologous system may not translate to receptors natively expressed in neurons. For example, the IC_{50} of a competitive antagonist depends on the concentration of the agonist (e.g., glutamate),[41,91] and the affinity of voltage-dependent ligands (e.g., polyamine toxins) varies with membrane potential.[92] Therefore, it is important to demonstrate subtype-selectivity under physiologically relevant conditions. The utility of the subtype-selective NMDA receptor antagonists ifenprodil and NVP-AAM077 is illustrative of this point.[91,93]

5. Outlook for rationally developing subtype-selective ligands

A wide variety of ligands have been developed for AMPARs and have contributed to our understanding of the mechanisms involved in the activation, desensitization, and blockade of these receptors. The development of subtype-selective ligands for AMPARs has lagged behind, though encouraging efforts suggest that it is possible to advance the pharmacology of AMPARs in this direction. Several competitive agonists are selective among homomeric receptors (i.e., GluA1 and GluA2 over GluA3 and GluA4), though none are selective for a single subtype and they offer little selectivity among heteromeric AMPARs. Positive allosteric modulators often provide selectivity between flip and flop isoforms, but demonstrate little selectivity among the seven principal AMPAR subtypes. Pore blockers are generally selective for GluA2-lacking over GluA2-containing receptors, but only two ligands show selectivity among receptors within these two groups (HPP-SP among GluA2-lacking receptors, PhTx-74 among GluA2-containing receptors).

The lack of subtype-selective ligands may be due to several factors. First, much of the effort in developing ligands for AMPARs has been aimed at distinguishing them from the other families of glutamate receptors (i.e. NMDA and kainate receptors). Thus, many AMPAR ligands have not been evaluated in terms of their efficacy across multiple AMPAR subtypes. Second, the diversity of biologically relevant AMPARs to screen against is vast. In addition to the seven principal subtypes, and their *flip(i)* and *flop*(*o*) isoforms, recent studies reveal that native AMPARs prefer to assemble with auxiliary subunits (i.e., TARPs, cornichons).[76,78] Third, certain classes of ligands are inherently non-selective owing to the characteristics of their binding sites. For example, competitive agonists binding to the LBD and showing selectivity among homomeric receptors, cross-react with heteromeric receptors comprised of two different LBDs (e.g., a GluA1-selective competitive agonist will activate both GluA1 homomers and GluA1A2 heteromers).

Based on these factors, we offer a few points to take into consideration in developing subtype-selective ligands. First, testing existing classes of ligands across all seven principal AMPAR subtypes may uncover structural templates useful for developing subtype-selective ligands. Second, evaluating both existing and newly developed ligands against AMPARs co-expressed with their auxiliary subunits may prove productive. These receptor complexes represent the biologically relevant targets and the auxiliary subunits have been shown to alter the pharmacology of AMPARs (see above). Third, developing ligands that target the quarternary structure of the receptor, and thus make contacts with the entire assembly rather than single subunits, may be advantageous. From this perspective, the channel pore and the subunit interfaces represent promising ligand-binding regions for achieving subtype-selectivity.

The rational design of a selective ligand for a protein-binding site remains one of the most challenging areas of chemistry,[94] and developing perfectly selective ligands for each AMPAR subtype may be an unachievable goal. For basic science applications, tailoring ones efforts toward a specific biological question can obviate the need for perfect subtype-selectivity. For example, since excitatory neurons primarily express heteromeric AMPARs (i.e., GluA1A2, GluA2A3, GluA2A4), developing ligands that select among these subtypes will provide invaluable tools for studying excitatory synaptic transmission and plasticity. In terms of clinical applications, recent advances in drug discovery suggest that ligands displaying perfect selectivity may exhibit lower clinical efficacy than multi-target drugs.[95] Indeed, several AMPAR ligands that are not subtype-selective are currently undergoing clinical trials (e.g., the positive allosteric modulator CX-516 for Alzheimer's disease;[96] the negative allosteric modulator Talampanel for epilepsy;[97] the competitive antagonist Tezampanel for migrane[98]). Whether these ligands will be more effective than ligands displaying improved selectivity among AMPAR subtypes remains to be seen.[99] Certainly the ongoing development of potent and selective AMPAR ligands will continue to expand the possibilities for manipulating and monitoring the activity of these channels in the nervous system.

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