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Minireview

The unique properties of glutamate receptor channels

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Rapid excitatory neurotransmission in the central nervous system (CNS) is mediated predominantly by synaptically released L-glutamate which activates cation selective channels with different kinetic and ion conductance properties. Studies with cloned glutamate receptor channels helped delineate the functional properties of channels defined in subunit composition. Moreover, molecular studies have revealed novel genetic mechanisms controlling the expression of important structural channel determinants.

Channel determinant; Ca^{2+} permeability; RNA editing

1. INTRODUCTION

Only since the early sixties has the common metabolite, L-glutamate, been recognized for its role as a ubiquitous excitatory neurotransmitter in the brain. Over the past 5 years ingenious expression cloning studies have been instrumental in isolating receptors [1], channels [2,3] and transporters [4] interacting with glutamate. The structures of these major components of the synaptic excitatory signalling machinery form a world of their own, being at best very distantly related to comparable structures for other prominent amino acid-related transmitters, such as GABA, glycine, and serotonin. Thus, glutamate activates G-protein-coupled, metabotropic receptors that have seven putative transmembrane segments like other 7TM receptors. However, metabotropic glutamate receptors display no sequence similarity to other members of the extensive G-protein-coupled receptor family [1,5]. Similarly, following the molecular characterization of a gene family encoding transporters for neurotransmitters such as GABA, glycine, and biogenic amines [6], the cloning of glutamate transporters [4,7] revealed that these latter are molecularly and mechanistically distinct from previously characterized transporters. Regarding ionotropic glutamate receptors, they, like other neurotransmitter-activated channels, are composed of subunits, giving them a modular design and allowing for functional diversity by select subunit combinations (Fig. 1, Table I). However, these receptors are very distinct in size, sequence, and conserved sequence patterns, from the well-characterized nicotinic acetylcholine/$GABA_A$ receptor superfamily (reviewed in [8]). They may even differ in transmembrane topology and subunit stoichiometry.

Ionotropic glutamate receptors are conveniently categorized into *N*-methyl-D-aspartate (NMDA) receptors, L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors and kainate receptors (Table I). This pharmacological distinction reflects selective agonist properties of exogenous, synthetic compounds on the various glutamate receptor channels [9]. As of today, 16 cDNAs encoding ionotropic glutamate receptor subunits have been cloned (reviewed in [10]). Using heterologous expression systems and biophysical channel analysis, certain rules governing subunit combinations assembling into functional glutamate-activated channels could be established (Table I), and new properties of these channels emerged. Our review summarizes salient molecular and functional features of cloned channels, and speculates on an unusual control mechanism that selectively alters gene-encoded determinants for glutamate-evoked Ca^{2+} permeability.

2. NMDA RECEPTORS

NMDA receptors are uniquely designed to function as molecular coincidence detectors (reviewed in [11]). Under normal synaptic activity these channels contribute little to fast signal transmission owing to a Mg^{2+} ion blocking the NMDA channel pore. In depolarized cells, the Mg^{2+} block is released and the NMDA channel can be fully activated. Hence, NMDA receptor activity is a sensor for synchronous activity states of the pre- and postsynaptic cell. This activity plays a role in conjunc-

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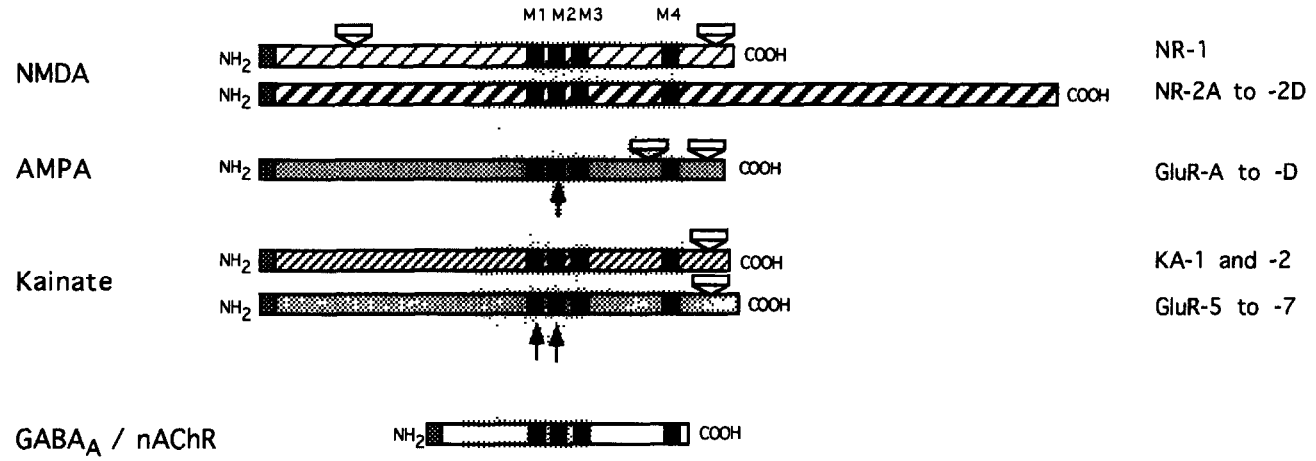

Fig. 1. Schematic representation of ionotropic glutamate receptor subunits in comparison to subunits of other chemically gated ion channels. Subunits for NMDA, AMPA and kainate receptor subtypes of glutamate receptors are at least 900 amino acids in length. The NR2 subunits of NMDA receptors have larger sizes (>1,300 residues) because of extended COOH-termini. All subunits contain four hydrophobic segments (M1 to M4, black boxes) which are involved in determining the transmembrane topology, and which participate in ion channel formation. In AMPA receptor subunit GluR-B and kainate receptor subunits GluR-5 and -6 select residues in M1 and M2 are controlled by RNA editing (arrows). Many subunits occur in several splice forms, with additional or substituted sequences present in the N-terminal (extracellular) region, in the M3 to M4 loop, and in the hydrophilic COOH-terminal part. The stippled area denotes conserved sequences among subunits of different ionotropic glutamate receptor types. The distribution of conserved sequences in these subunits contrasts with the extent of such conserved regions (stippled) in subunits of the superfamily of $GABA_A$ and nicotinic acetylcholine receptors, which differ markedly in size (≈ 450 residues) from glutamate receptor channels.

tion with the characteristically high Ca^{2+} permeability and long activation times of the ion channel. Ca^{2+} influx through NMDA channels is thought to trigger important physiological processes, and is held responsible for certain pathophysiological processes. One prominent example is the activity-dependent enhancement of synaptic efficacy, a process termed long-term potentiation (LTP) and viewed as a synaptic model for certain types of memory [11,12]. A related phenomenon concerns the damage that glutamate excessively released in e.g. ischemic conditions can do to neurons. Most ischemic cell death appears to be wrought by an excess of Ca^{2+} influx through NMDA receptors (reviewed in [13]).

The molecular biology of this receptor indicates that it contains two types of subunits. One subunit (NMDAR1, abbr. NR1) occurs in virtually every neuron [3], and is likely to be a constituent of all NMDA receptor subtypes. In vitro expression of this subunit alone generates (at a reduced efficiency) functional channels that display key properties of NMDA recep-

Table I

Subunits and characteristics of glutamate receptor channels

Subunits	Alternative nomenclature	Receptor type	Heteromeric assembly	Functional characteristics
GluR-A	CluR-1 α1	AMPA	GluR-A to -D;	rapid kinetics, low Ca^{2+} permeability,
GluR-B	GluR-2 α2		most native	small conductance;
GluR-C	GluR-3 α3		receptors contain	glutamate activates desensitizing
GluR-D	GluR-4 α4		GluR-B	current, kainate activates non-desensitizing current
GluR-5	β1	Kainate	GluR-5 to -7	rapid kinetics, glutamate and kainate
GluR-6	β2	(high affinity)	with KA-1, -2	activate rapidly desensitizing current
GluR-7	β3			
KA-1	γ1			
KA-2	γ1			
NR-1	ζ1	NMDA	NR-1 with	slow kinetics, large conductance,
NR-2A	ε1		NR-2 subunits	little desensitization, high Ca^{2+}
NR-2B	ε2			permeability, voltage-dependent
NR-2C	ε3			Mg^{2+} block, glycine as co-agonist
NR-2D	ε4			

tors. Of the other subunit, currently four variants (NR2A to D) have been molecularly cloned, each encoded by its own gene with a unique expression pattern in the brain [14–16]. These subunits fail to form functional homomeric channels but impart on NMDA receptors containing them particular subtype-specific properties, including different strengths of Mg^{2+} blockade, and pharmacological differences (e.g. differential glycine sensitivity). There are also kinetic differences since upon removal of agonist, whole-cell currents decay with distinct, slow time courses. It is believed that the subtype-specific, slow offset kinetics of NMDA currents have physiological impact with respect to the amount of Ca^{2+} flux through the channel, after termination of presynaptic glutamate release. Additional molecular diversity of NMDA receptors emerges from the fact that the common channel subunit NR1 can exist in different forms generated by alternative splicing [17]. Functional differences for these forms need determining.

Although NR1 and NR2 subunits have little sequence similarity (18% identity) they share an important molecular determinant in the form of an asparagine residue, located in what appears to be a channel forming region (Fig. 1). Site-directed mutagenesis combined with biophysical measurements of recombinantly expressed heteromeric receptor channels reveals that this asparagine residue determines the two most distinctive properties of NMDA receptors; high Ca^{2+} permeability and voltage-dependent Mg^{2+} block. These properties are also evident in homomeric NR1 receptors [3]. However, in heteromeric NR1–NR2 receptor channels, substituting the asparagine of NR1 affects predominantly the Ca^{2+} permeability of the channel, whereas a substitution of the asparagine in NR2 subunits changes the Mg^{2+} block [18].

3. AMPA RECEPTORS

These prominent glutamate receptor channels in brain are distinguished from other ionotropic glutamate receptors by possessing a high-affinity binding site for [^{3}H]AMPA. AMPA receptor channels are primarily responsible for fast excitatory neurotransmission by glutamate, and co-exist with NMDA receptors in many synapses (reviewed in [19]). The hallmarks of the AMPA channel are fast kinetics [20,21] and low permeability to divalent cations [21,22].

In molecular terms, AMPA receptors assemble from a pool of four subunits (GluR-A to -D) which share a high degree of sequence identity (70%) [23,24]. Exactly how many subunits enter functional receptor channels has yet to be determined. Co-expression in heterologous systems of two subunits generates channels with properties much like their neuronal counterparts [21], but only if one subunit is GluR-B. GluR-B dominates the gating, ion conductance and permeability properties of AMPA receptors [25,26]. GluR-B-containing AMPA receptors are characterized by linear current–voltage (*I-V*) relations and low Ca^{2+} permeability like native channels, whereas AMPA receptors lacking GluR-B are Ca^{2+} permeable and possess more complex, doubly rectifying *I-V* relations. The functional dominance of GluR-B may be reflected in the fact that its mRNA is found in most neurons. Hence, the expression level of the GluR-B gene determines to a major degree the low divalent permeability of glutamate-gated AMPA receptors in neural cells. This notion has been confirmed by a study of Bergmann glial cells which express AMPA receptor channels lacking GluR-B. These cells show considerable glutamate-activated Ca^{2+} entry and glutamate-evoked whole-cell currents with a doubly rectifying *I-V* relationship [27,28].

The structure mediating dominance of GluR-B in AMPA receptor channels is a positively charged amino acid residue (arginine, R) in a putative channel-forming region of this subunit [29,30]. The other three AMPA receptor subunits carry an uncharged glutamine (Q) residue in the homologous position. Interestingly, this position (Q/R site) is occupied by asparagine in the homologous site of NMDA receptor channels [3,14]. As detailed above, this asparagine in NMDA receptor subunits is essential for high Ca^{2+} permeability and for voltage-dependent Mg^{2+} block. Indeed, AMPA receptors engineered to contain an asparagine in their Q/R site have high Ca^{2+} permeability and low Mg^{2+} permeability, reminiscent of NMDA receptors [30,31]. This finding highlights the functionally homologous design of ionotropic glutamate receptors in their channel-forming region. The charge and size of a single amino acid side chain in a critical channel site determine the particular ion conductance and permeability properties that characterize the different glutamate-activated channels (Table I).

The presence of the dominant arginine residue in the critical Q/R site of GluR-B cannot be deduced from sequence analysis of the GluR-B gene. In fact, judging from the gene sequence, a glutamine residue should occupy the Q/R site of GluR-B. The vast majority of GluR-B mRNA molecules, however, contain an arginine codon, as predicted from cDNA analysis. Indeed, 99.99% of all GluR-B subunits in brain carry an arginine residue in the Q/R site, consistent with the properties of native AMPA receptor channels. The discrepancy between gene and cDNA has been resolved by postulating that a single nucleotide in GluR-B RNA is modified, thereby generating the CGG codon for arginine from a genomic CAG codon for glutamine. This curious process of converting a glutamine to an arginine codon (possibly by deamidation of adenosine) constitutes the first example of RNA editing in the brain [32]. The physiological consequences of a defect in this unusual genetic control mechanism remain speculative. AMPA receptor channels with increased Ca^{2+} permeability should be a

direct consequence, possibly followed by delayed cell death for populations of neurons unable to cope with an increased Ca^{2+} load.

4. KAINATE RECEPTORS

Kainate is a potent neurotoxin that has agonist properties on some subtypes of glutamate receptor channels. It activates in AMPA receptors a non-desensitizing current. It activates desensitizing currents in a different glutamate receptor that displays high-affinity binding of [^{3}H]kainate. The in vivo role of the latter receptor channel, designated high-affinity kainate receptor, is unresolved. Subunits for such receptors have been cloned and shown to be expressed widely in the CNS. However, no evidence for desensitizing current components evoked by kainate in patches from somata of central neurons has been forthcoming. It is now thought that these channels may be located exclusively in dendrites, and immunocytochemical evidence supports this [33,34].

Two subunit families have been identified by molecular cloning from which high-affinity kainate binding sites can be reconstituted in heterologous expression systems (Fig. 1, Table I). Subunits of one family (GluR-5,-6,-7) form functional heteromeric channels when combined with members of the other subunit family (KA-1,-2) but can also form homomeric channels. Homomeric channel formation has not been seen for KA-1 nor for KA-2, and these two subunits do not form heteromeric channels when combined ([35]; reviewed in [36]).

The two subunits GluR-5 and -6 are affected by site-specific RNA editing, similar to GluR-B. Analysis reveals that between 30 and 70% of RNA encoding these subunits is edited in the Q/R site [32]. For GluR-6, two additional edited sites have been identified that are located in another membrane-spanning region (M1). GluR-6 has the distinction of occuring in eight molecular variants with respect to edited positions alone [37]. Several of these variants, when analyzed in a homomeric configuration by recombinant expression, show differences in Ca^{2+}/Cs^{+} reversal potentials of glutamate-activated whole-cell currents [37]. Thus, there is a distinct possibility that brain cells can regulate the RNA editing of those nucleotides which code for structures mediating or preventing glutamate-evoked Ca^{2+} influx.

5. OUTLOOK

Studies with defined, recombinantly expressed ionotropic glutamate receptors have revealed functional properties that are not readily obtained from studying such receptors in native brain membranes. One reason for this is that, in native membranes, different channel types and their subtypes co-exist, and pharmacological means for their dissection may not be always available. Alternatively, modeling excitatory synaptic responses from functional properties of recombinantly expressed single receptors requires knowledge of the receptor populations and their true subunit composition in the postsynaptic membrane. Hence, matching results from recombinant expression studies with those obtained from native membranes is obligatory. Research efforts will be directed towards elucidating regulatory mechanisms underlying channel expression in select cell populations of the brain. In particular, the involvement of different molecular types of glutamate receptor channels in the various phenomena of synaptic plasticity needs investigating. Such studies may also uncover a physiological role for RNA editing.

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