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Molecular Diversity of Glutamate Receptors and Implications for Brain Function

Shigetada Nakanishi

The glutamate receptors mediate excitatory neurotransmission in the brain and are important in memory acquisition, learning, and some neurodegenerative disorders. This receptor family is classified in three groups: the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)–kainate, and metabotropic receptors. Recent molecular studies have shown that many receptor subtypes exist in all three groups of the receptors and exhibit heterogeneity in function and expression patterns. This article reviews the molecular and functional diversity of the glutamate receptors and discusses their implications for integrative brain function.

Signal transmission at neuronal synapses is mediated by a variety of receptors that specify neurotransmitter interactions and transmit information into target cells. The vast majority of synapses in the central nervous system (CNS) uses glutamate as a neurotransmitter to produce rapid neuronal excitation (*1*). Glutamate neurotransmission also participates in neuronal plasticity and neurotoxicity. Neuronal plasticity elicited by glutamate is exemplified by long-term potentiation (LTP) in the hippocampus and long-term depression (LTD) in the cerebellum, which are long-lasting, use-dependent enhancement and depression in synaptic efficacy, respectively (*2*, *3*). Because memory is postulated to be encoded in the brain through long-lasting changes in synaptic efficacy produced by the prior use of synapses, LTP and LTD are believed to be fundamental processes that underlie information storage in the brain. Excess glutamate neurotransmission, on the contrary, triggers neuronal degeneration and neuronal cell death (*4*). For example, during brain ischemia and hypoglycemia, massive stimulation of glutamate receptors by high concentrations of extracellular glutamate causes deterioration of neuronal activity and finally leads to neuronal cell death. Gluta-

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mate neurotoxicity may also underlie slowly progressive degenerative diseases such as Huntington's disease and Alzheimer's disease (*4*). Thus, glutamate neurotransmission plays an essential role in brain function and dysfunction.

Glutamate receptors can be categorized into two groups termed ionotropic and metabotropic receptors on the basis of pharmacological, electrophysiological, and biochemical studies (*1*). The ionotropic receptors contain integral, cation-specific ion channels, whereas the metabotropic receptors are coupled to G proteins and modulate the production of intracellular messengers (*1, 5*). The ionotropic receptors can be subdivided into *N*-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)–kainate receptors according to their selective agonists (*1*). The utilization of recombinant DNA techniques has greatly advanced our understanding of the structures, properties, and expression of the glutamate receptors. This article deals with recent progress in the molecular and genetic studies of the glutamate receptors and discusses some new aspects explored in these areas of research.

Complementary DNA Cloning of Glutamate Receptors

Conventional molecular cloning has required the purification and partial sequencing of a receptor protein. However, the receptor purification has been hindered in many cases because receptors are tightly embedded in the membrane and are present in the cell in low concentrations and because there is no appropriate high-affinity ligand. To circumvent these problems, we, in collaboration with Kuno, developed a new cloning approach for receptors and ion channels on the basis of the original finding of Barnard and coworkers (*6*). *Xenopus laevis* oocytes faithfully and efficiently express foreign receptors and ion channels after injection of mammalian messenger RNA (mRNA). The expression of these proteins can be identified by electrophysiological measurements of the activity of an expressed channel per se or that of an oocyte channel linked to a foreign receptor via oocyte second messengers. This finding was extended by us (*7*) and Julius *et al.* (*8*) for receptor cloning (Fig. 1). A complementary DNA (cDNA) library is made in an RNA expression vector from tissue polyadenylated [poly(A)$^+$] RNA, and an mRNA mixture is synthesized in vitro and assayed after injection in oocytes. The expression of a receptor is tested by the measurement of electrophysiological responses to ligand application. When a positive response is observed, the library is subdivided serially until a single positive clone can be identified. With the use of this strategy, the cDNA clone for the AMPA-kainate receptor and those for the NMDA and metabotropic receptors were successfully isolated in Heinemann's laboratory and in our laboratory, respectively (*9–11*).

The Structure of Ligand-Gated Ion Channels

Because the NMDA and AMPA-kainate receptors show a fundamental similarity to other members of ligand-gated ion channels [nicotinic acetylcholine (nAChR), γ-aminobutyric acid ($GABA_A$), and glycine receptors], I will briefly summarize the structural characteristics of the nAChR, which has been most thoroughly explored at the molecular level, owing largely to the extensive studies of Numa and co-workers (*12*). The nAChR is a pentameric transmembrane protein composed of four kinds of homologous subunits. These subunits are, for example, assembled in the molar stoichiometry of $\alpha_2\beta\gamma\delta$ in the muscle form and arranged pseudosymmetrically around a central, cation-permeating pore. It is generally assumed that the four transmembrane segments (TMI through TMIV) of the five subunits form membrane-spanning α helices in which the TMII segments with polar side chains line the channel pore (*12*). Three clusters of negatively charged and glutamine residues are adjacent to the TMII segments of the constituent subunits of nAChR. These amino acids are thought to form three (cytoplasmic, intermediate, and extracellular) anionic rings that determine the channel properties characteristic of the nAChR (*13*). It is also predicted that a narrow central ring of the channel pore is formed by uncharged polar residues (threonine and serine) close to the cytoplasmic side of the membrane (*13*).

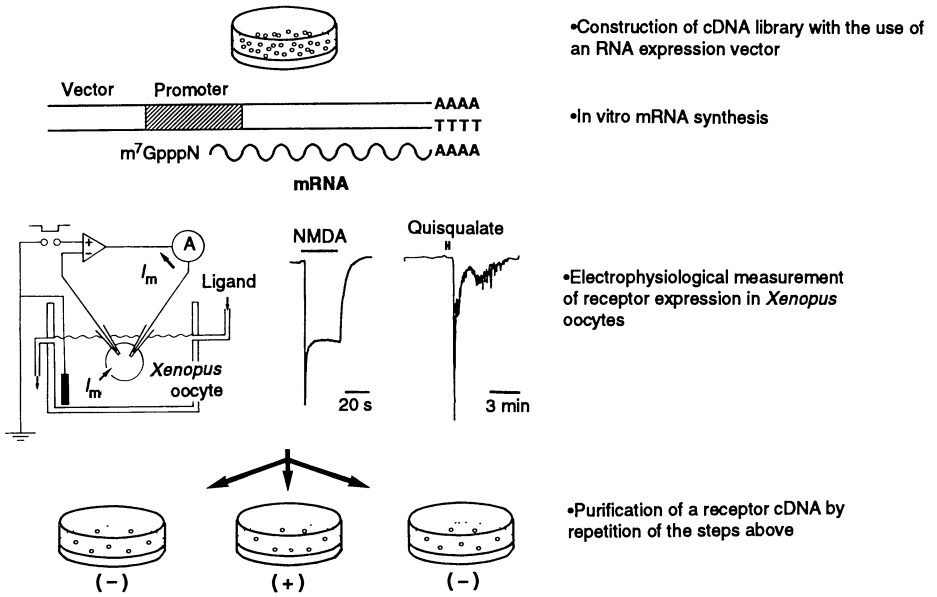

Fig. 1. Strategy for molecular cloning of a receptor cDNA clone by combination of an oocyte expression system with electrophysiology. I_m, membrane current; *A*, current monitor.

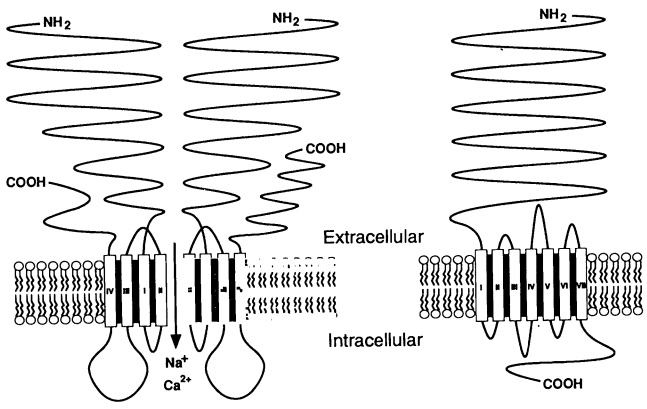

Fig. 2. Transmembrane models of the ionotropic receptors (left) and metabotropic receptors (right). The TMII segments of the ionotropic receptors are postulated to line an ion channel pore in heteromeric assemblies of the receptor subunits.

AMPA-Kainate Receptors

Since Hollmann and co-workers first reported a cDNA clone for a functional AMPA-kainate receptor subunit (*9*), a growing family of related subunits that form functional AMPA-kainate receptor-channel complexes has been identified by crosshybridization and polymerase chain reaction (PCR)–mediated DNA amplification techniques (*14*). These subunits can be subclassified into three groups according to their sequence similarity and agonist selectivity (*14*). The one consists of four subunits (GluR1 through GluR4, also termed GluR-A through GluR-D) and shows high affinities toward AMPA, whereas the other two groups represent two different kinds of the kainate-selective subunits (GluR5 through GluR7 and KA-1 and KA-2) (*14*). All subunits have approximately 900 amino acid residues, share a common transmembrane topology and channel architecture with other members of ligand-gated ion channels, and contain four putative membrane-spanning regions following a large extracellular NH_2-terminal domain (Fig. 2). Because the molecular properties of the AMPA-kainate receptors have already been reviewed (*15*), only the recent advances in this field of research are described herein.

Expression analysis in *Xenopus* oocytes and in mammalian cells indicated that the AMPA-kainate receptor subunits form heteromeric oligomers that mutually differ in electrophysiological properties and ionic selectivity (*14, 16*). Various combinations of different subunits among the GluR1 through GluR4 subgroups alter both the current-voltage (*I-V*) relation and the Ca^{2+} permeability (*16*). The homomeric GluR2 channel and the heteromeric assemblies with this subunit show near-linear *I-V* relations and only weak Ca^{2+} permeability. When GluR2 is removed from the heteromeric formation, such channels display doubly rectifying *I-V* relations and a substantial Ca^{2+} permeability. GluR2 thus dominantly determines the channel conductance and Ca^{2+} permeation. GluR2 contains an arginine residue within the TMII segment, whereas the other three subunits carry a glutamine residue at the corresponding position (*14*) (Fig. 3). Site-directed mutagenesis indicated that the disparate channel properties are indeed determined by the presence of arginine or glutamine at the TMII segment (*16*). The AMPA-kainate receptors that bear resemblance to receptors that lack GluR2 were electrophysiologically identified in a small population of hippocampal neuronal cells, retinal bipolar cells, and cerebellar Bergmann glial cells (*17*). Thus, although the AMPA-kainate receptors have been thought to selectively conduct monovalent cations, glutamate may trigger Ca^{2+}-dependent intracellular events by activating the Ca^{2+}-permeable AMPA-kainate receptors in certain neuronal and glial cells.

More surprising is the finding by Sommer *et al.* (*18*), who reported that a glutamine codon (CAG) is encoded at the Gln-Arg position in the genomic sequences for all subunits, even though an arginine codon (CGG) is found in cDNA sequences of the GluR2, -5, and -6 subunits. This adenosine-to-guanosine alteration was explained by an RNA editing mechanism, the enzyme-directed, posttranscriptional modification of RNA transcripts, although a different mechanism such as a nucleotide exchange in the genomic DNA was also formally discussed (*18*). The editing efficiency varies among the AMPA-kainate receptor subunits and also during neuronal development (*18*). GluR2 exists in the edited form, whereas the GluR5 and -6 subunits occur in both edited and unedited forms. Because the channel conductance and the Ca^{2+} permeability are governed by the presence or absence of an edited subunit, the physiological relevance of this editing mechanism is extremely intriguing.

The molecular complexity of the AMPA-kainate receptors is further increased by alternative splicing in the sequence between the TMIII and TMIV segments (*19*). This alternative region spans 38 amino acid residues encoded on adjacent exons. The two alternative splice molecules, designated flip and flop, impart distinct kinetics and amplitudes of agonist-induced responses (*19*). Thus, the AMPA-kainate receptors are now known to be more diverse than previously believed, not only in their molecular entities but also in their functional properties.

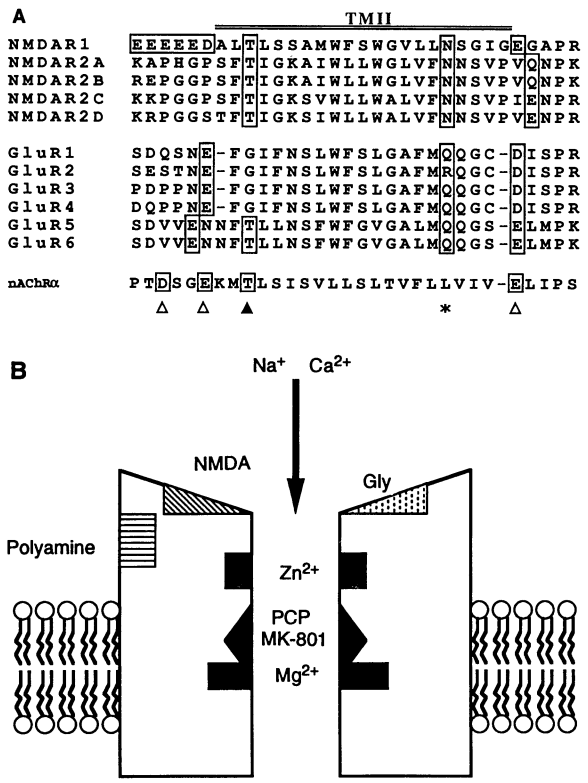

Fig. 3. Comparison of the amino acid sequences of the putative TMII segments (**A**) and multifunctional properties of the NMDA receptor-channel complex (**B**). In (A), white and black triangles and the asterisk indicate the positions of three anionic rings and a central ring of the nAChR and that of the Gln (Q)-Arg (R) editing of the AMPA-kainate receptors, respectively; in GluR5 and GluR6, both edited (R) and unedited (Q) forms are present. Boxes indicate the amino acids discussed in the text. In (B), the locations of agonist and antagonist binding sites are schematically shown. PCP, phencyclidine.

NMDA Receptors

The NMDA receptor-channel complex has several characteristic features (*1*). The integral channel of this receptor is highly permeable to Ca^{2+} as well as to Na^+ and K^+, and the resultant increase of intracellular Ca^{2+} is thought to be responsible for evoking both neuronal plasticity and neurotoxicity (*1, 2*). This receptor is distinguished from other glutamate receptors by the action of a number of selective agonists and antagonists and also by its unique properties, including modulation by glycine, a voltage-dependent channel block by Mg^{2+}, polyamine activation, and Zn^{2+} inhibition (*1*). The NMDA receptor has been extensively characterized by protein purification and other biochemical techniques, but some contradictory observations have been reported (*20*). The receptor-channel complex purified by affinity chromatography consists of several subunits with different molecular masses (33 to 67 kD), and all of these subunits seem to be required for NMDA receptor activity in reconstituted liposomes (*20*). However, the sizes of these subunits are much smaller than those determined by other biochemical methods such

as radiation inactivation analysis and photoaffinity labeling (*20*).

We examined the identity of the cloned receptor (NMDAR1) by characterizing electrophysiological and pharmacological properties in *Xenopus* oocytes (*10*). The results of this analysis indicated that NMDAR1 possesses the properties characteristic of the NMDA receptor, including Ca^{2+} permeability, voltage-dependent block by Mg^{2+}, and glycine enhancement (*10*). The profile of agonists and antagonists of NMDAR1 is also in good agreement with that reported for NMDA receptors in neuronal cells (*10*). Glutamate, ibotenate, quisqualate, and homocysteate are all effective agonists, whereas the antagonists include D-(−)-2-amino-5-phosphonovalerate, 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate, CGS19755 (competitive antagonists), 7-chlorokynurenate (a noncompetitive antagonist acting at a glycine binding site), MK-801 (a channel blocker), and Zn^{2+}. The single polypeptide encoded by the cloned cDNA thus has the potential to form a receptor-channel complex characteristic of the NMDA receptor (*10*) (Fig. 3).

The above characterization indicated that NMDAR1 forms a homo-oligomeric structure to produce a functional receptor-channel complex. However, the current amplitude obtained with NMDAR1 in *Xenopus* oocytes was much smaller than that observed with brain poly(A)$^+$ RNA, which suggests the existence of additional NMDA receptor subunits that form a heteromeric oligomer (*10*). The laboratories of Mishina and Seeburg as well as our laboratory showed the presence of at least four additional NMDA receptor subunits (NMDAR2A through NMDAR2D; NMDAR2A through NMDAR2C are also termed ε1 through ε3) by PCR and crosshybridization techniques (*21–23*) (Table 1). The cDNA cloning of a polypeptide of a putative NMDA receptor characterized by protein purification has also been reported by Michaelis and co-workers (*24*). This protein (516 amino acid residues), however, has no homology to any known glutamate receptors. Although it has a glutamate binding activity, expression in it of the other characteristics of NMDA receptors remains to be demonstrated (*24*).

The individual NMDAR2 subunits, in contrast to NMDAR1, evoke no appreciable electrophysiological response after agonist application (*21–23*). However, combined expression of NMDAR1 with the NMDAR2 subunits markedly potentiates responses to NMDA or glutamate (*21–23*). Thus, NMDAR1 serves as a fundamental subunit necessary for the NMDA receptor-channel complex and forms a heteromeric configuration with different members of the

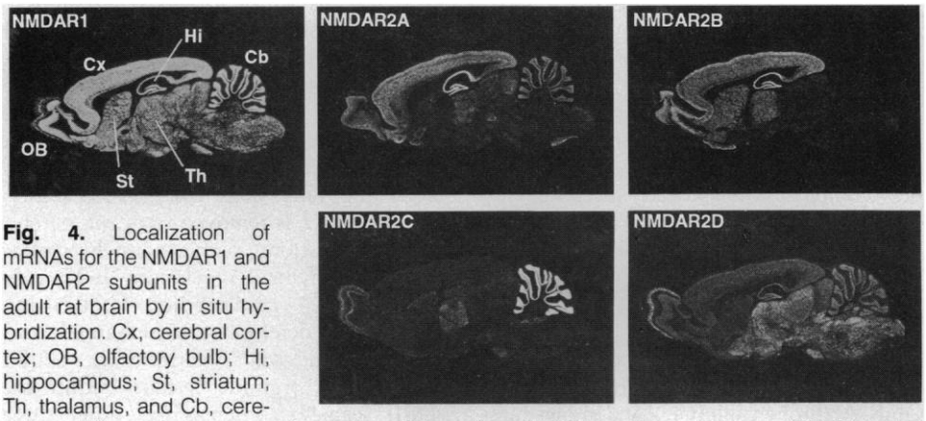

Fig. 4. Localization of mRNAs for the NMDAR1 and NMDAR2 subunits in the adult rat brain by in situ hybridization. Cx, cerebral cortex; OB, olfactory bulb; Hi, hippocampus; St, striatum; Th, thalamus, and Cb, cerebellum. This analysis was performed in collaboration with Shigemoto and Mizuno (*23*). [Adapted from T. Ishii *et al.* (*23*)]

Table 1. The NMDA receptor family.

Subunit	Properties
NMDAR1	Seven isoforms generated by alternative splicing; amino acid residues of NMDAR1A through NMDAR1G: 938 (main isoform), 959, 901, 922, 885, 922, and 906.
	Essential for the activity of the NMDA receptor-channel complex, including agonist and antagonist selectivity, Ca^{2+} permeability, Mg^{2+} blockade, glycine modulation, polyamine activation, and Zn^{2+} inhibition.
	Expressed in almost all neuronal cells.
NMDAR2	Four subtypes encoded by different genes and showing about 15% sequence homology with NMDAR1; residues of NMDAR2A through NMDAR2D: 1464, 1482, 1250, and 1323.
	Co-expression with NMDAR1 potentiates NMDA receptor activity and produces functional heterogeneity, depending on the subunit configurations.
	Expressed more restrictedly.

NMDAR2 subunits. The combination of NMDAR1 with different subunits shows functional variability in electrophysiological and pharmacological properties (*21–23*). The affinity for agonists, the effectiveness of antagonists, the kinetics of responses, the sensitivity to Mg^{2+} block, and the stimulatory effect of glycine are different, depending on the subunit composition (*21–23*).

NMDAR1 shares an overall similarity in both protein sequence and hydrophobicity profile to the AMPA-kainate receptors (*10*). NMDAR1 is thus thought to comprise four transmembrane domains preceded by a large extracellular domain (Fig. 2). The amino acid sequences of NMDAR1 are more than 99% identical among the rat, mouse, and human species (*25*). The NMDAR2A through NMDAR2D subunits show only about 15% amino acid homology with NMDAR1 (*21–23*) but possess structural characteristics similar to those of NMDAR1. The NMDAR1 and NMDAR2 subunits all contain many consensus phosphorylation sites for Ca^{2+}-calmodulin–dependent protein kinase and protein kinase C at the cytoplasmic domains (*10, 23*). These protein kinases play a crucial role in the induction and maintenance of LTP (*2*). The NMDAR2 subunits have a peculiar structural feature in comparison to that of other ligand-gated ion channels, for the NMDAR2 subunits contain a large COOH-terminal extension after the TMIV segment (*21–23*). This discussion of the structures of both the AMPA-kainate receptors and the NMDA receptors, however, is made mainly on the basis of their similarity to other ligand-gated ion channels in structure, and additional structural and biochemical evidence is necessary for the determination of the precise topology of these receptors.

In NMDAR1, six additional isoforms are generated by alternative splicing (*26*). These isoforms have an insertion at the NH_2-terminal region or deletions at two different COOH-terminal regions, or a combination of an insertion and deletions. No functional difference was observed by homomeric expression of these isoforms (*26*), but the possible difference that results from a heteromeric formation with the NMDAR2 subunits remains to be determined.

Under the assumed transmembrane model of NMDAR1 and four NMDAR2 subunits (*10, 21–23*), negatively charged residues and glutamine residues are present

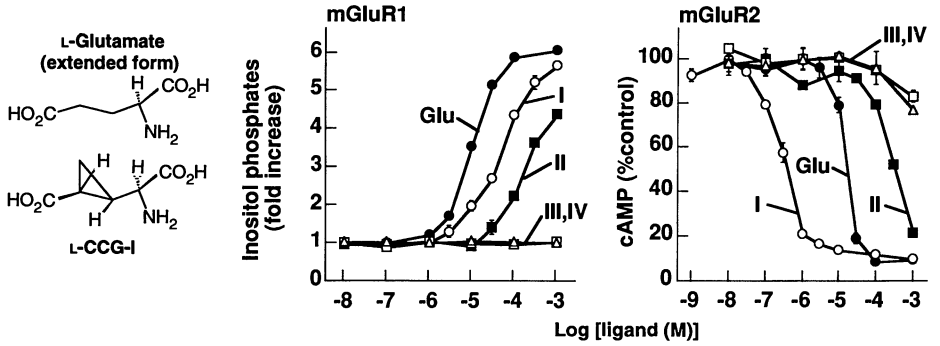

Fig. 5. Dose-response curves of four L-CCG isomers for stimulation of inositol phosphate formation in mGluR1-expressing cells and for inhibition of the forskolin-stimulated cAMP formation in mGluR2-expressing cells. Glu, glutamate; I through IV, L-CCG-I through L-CCG-IV. [Adapted from Y. Hayashi *et al.* (*37*)]

Table 2. The mGluR family. For the mRNA expressions of individual subtypes, only characteristic sites are described; high expression (+++) or no appreciable expression (−) of mRNA is indicated.

Receptor	Amino acids	Effector	Agonist selectivity and characteristic expression sites
			Group 1
mGluR1α	1199	IP_3/Ca^{2+} ↑	Quisqualate > glutamate ≧ ibotenate > tACPD. CA2, CA3, Purkinje cells (+++)
mGluR1β	906	IP_3/Ca^{2+} ↑	An alternative form of mGluR1α
mGluR5	1171	IP_3/Ca^{2+} ↑	Quisqualate > glutamate ≧ ibotenate > tACPD. CA1, CA2, CA3 (+++), Purkinje cells (−)
			Group 2
mGluR2	872	cAMP ↓	Glutamate ≃ tACPD > ibotenate > quisqualate. Cerebellar Golgi cells (+++)
mGluR3	879	cAMP ↓	Glutamate ≃ tACPD > ibotenate > quisqualate. Expressed in both neuronal and glial cells
			Group 3
mGluR4	912	cAMP ↓	AP4 > glutamate >> tACPD. Quisqualate (no agonist activity)
mGluR6	871	cAMP ↓	Retina (+++). Agonist selectivity (?)

at the putative extracellular side of the TMII segments (Fig. 3), and these residues most likely form an extracellular anionic ring that determines cation selectivity. NMDAR1 possesses a stretch of glutamate residues at the putative intracellular side near the TMII segment and an aspartate residue at the position that corresponds to the intermediate ring of the nAChR. No such negatively charged residues are present in the NMDAR2 subunits. All five receptor subunits contain a threonine residue at the position that corresponds to the central ring of the nAChR.

All NMDA receptor subunits possess an asparagine residue at the position equivalent to the Gln-Arg editing site of the AMPA-kainate receptors. The importance of the asparagine residue of NMDAR1 in the regulation of Ca^{2+} permeability and channel blockade was shown by electrophysiological characterization of mutated receptors in which the asparagine was replaced with either glutamine or arginine (*27*). These substitutions reduced or abolished Ca^{2+} permeability and inhibition by Mg^{2+}, Zn^{2+}, an NMDA antagonist, MK-801, and an antidepressant, desipramine (*27*). It is thus most likely that an asparagine ring is formed at a central part of the channel pore of the NMDA receptor and provides a constriction that controls the permeability of Ca^{2+} and channel blockades by Mg^{2+} and other cationic channel blockers.

The NMDAR1 mRNA is expressed ubiquitously in almost all neuronal cells throughout the brain regions (*10*). In contrast, the mRNAs for different (Fig. 4) NMDAR2 subunits show overlapping but different expression patterns in the rat brain (*22, 23*). For example, the NMDAR2A mRNA is prominently expressed in the cerebral cortex and hippocampus, whereas the NMDAR2B mRNA is distributed in the forebrain. The NMDAR2C and NMDAR2D mRNAs predominate in the cerebellum and in the diencephalic/lower brain stem regions, respectively. Thus, the anatomical and functional differences of the NMDAR2 subunits could provide the molecular basis for the generation of the heterogeneity in the physiological and pharmacological properties of the NMDA receptors proposed to occur in different neuronal cells and brain regions (*28*).

Our understanding of the mechanisms that underlie the induction of LTP in the hippocampus has been greatly advanced (*1, 2*). Under weak neuronal stimulation, only slight activation of NMDA receptors occurs because the NMDA receptor channels are blocked by Mg^{2+}. When strong stimulation (tetanus) is transmitted, the depolarization evoked through the activation of the AMPA-kainate receptors reduces Mg^{2+} blockade of the NMDA receptor channels and allows Ca^{2+} to permeate. This Ca^{2+} entry then triggers the sequence of events that result in the enhancement of synaptic efficacy. The cloned NMDA receptors fulfill the properties necessary for the proposed mechanisms for the induction of LTP. However, the functional heterogeneity of different heteromeric assemblies of the NMDA receptor subunits is also obvious, and the mechanisms that underlie all forms of LTP may not be the same (*2*).

Metabotropic Glutamate Receptors

Molecular cloning by cross-hybridization and PCR techniques has revealed the existence of at least six subtypes of metabotropic glutamate receptors (mGluRs) (mGluR1 through mGluR6) (*11, 29–31*) (Table 2). The six mGluRs are considerably larger than the other members of the G protein–coupled receptor family but show a common structural architecture with a large extracellular NH_2-terminal domain that precedes seven transmembrane segments (*29, 30*) (Fig. 2). However, no sequence homology is observed with any other members of the G protein–coupled receptor family (*30*). The amino acid sequences of the six mGluRs are highly conserved not only in the seven transmembrane segments but also in their preceding extracellular domains. Among the six receptors, cysteine residues are remarkably conserved in the extracellular NH_2-termini, which suggests the importance of the cysteine residues in the structural formation of mGluRs. The mGluRs thus represent a novel subfamily of the G protein–coupled receptor family. The six receptor subtypes can be subdivided into three subgroups according to their sequence similarities: mGluR1 and mGluR5, mGluR2 and mGluR3, and mGluR4 and mGluR6. A smaller alternative splice form of mGluR1 (mGluR1β) is also generated as a result of the insertion of a small DNA segment that contains a termination codon in the region that follows the transmembrane segments (*29*).

Many of the agonists for mGluRs cross-react with the NMDA and AMPA-kainate

receptors. We investigated the properties of individual receptors by DNA transfection and stable expression in Chinese hamster ovary cells (*29, 30, 32*) (Table 2). Consistent with their high sequence homology, mGluR1 and mGluR5 stimulate inositol trisphosphate (IP_3) formation and intracellular Ca^{2+} mobilization (*30, 32*). These receptors also induce arachidonic acid release. The agonist selectivity is virtually identical between these two receptors (*30, 32*). The two receptors are, however, slightly different in their pertussis toxin (PTX) sensitivity (*30*). In contrast, the other mGluR subtypes inhibit the forskolin-stimulated accumulation of intracellular adenosine 3′,5′-monophosphate (cAMP) in an agonist-dependent manner (*29, 33*). However, the agonist selectivity is different between the two subgroups of the receptors. In mGluR2 and mGluR3, L-glutamate and *trans*-1-aminocyclopentane-1,3-dicarboxylate (tACPD) effectively inhibit the cAMP accumulation (*29, 33*). In mGluR4, 2-amino-4-phosphonobutyrate (AP4) is the most potent agonist with a potency one order of magnitude greater than L-glutamate (*33*). Quisqualate, which is the most potent agonist for mGluR1 and mGluR5, is only a weak or even ineffective agonist for both mGluR2-mGluR3 and mGluR4 subgroups. mGluR2, mGluR3, and mGluR4 are all sensitive to PTX (*29, 33*), which indicates that these receptors are coupled to a PTX-sensitive inhibitory G (G_i) protein.

The mRNAs for different subtypes of the mGluR family show overlapping but different expression patterns in the rat brain (*11, 29, 30, 33, 34*). Particularly interesting is that the receptor subtypes of the same subgroup, although very similar in their signal transduction and agonist selectivity, are differentially expressed in the CNS (Table 2). It was also recently found that the mGluR1 mRNA is autoregulated by activation of this receptor in cultured cerebellar granule cells (*35*). Further investigations of the regulation of individual mGluR mRNAs will provide much insight into the function of this receptor family in long-term processes such as LTP and LTD.

The development of more selective and potent agonists for mGluR subtypes is indispensable for mGluR research. Ohfune and co-workers chemically synthesized eight isomers of the conformationally restricted glutamate analog, 2-(carboxycyclopropyl)glycine (CCG), including their L- and D-forms (*36*), and Shinozaki and co-workers reported that two of the extended L-isomers, L-CCG-I and L-CCG-II, potently activate the IP_3-coupled mGluR in *Xenopus* oocytes injected with rat brain poly(A)$^+$ RNA (*36*). We, in collaboration with Ohfune, investigated the agonist potencies and selectivities of eight CCG isomers for mGluRs by examining their effects on the signal transduction of the representative subtypes mGluR1, mGluR2, and mGluR4 (*37*). L-CCG-I and L-CCG-II in fact effectively stimulate phosphatidylinositol hydrolysis in mGluR1-expressing cells (Fig. 5). L-CCG-I is also active at mGluR4 (*37*). Remarkably, L-CCG-I is more effective for mGluR2. The potency of L-CCG-I with the effective dose for half-maximal response (EC_{50}) of 3×10^{-7} M is more than one order higher than that of glutamate, and the mGluR2 is fully activated at the concentration of 10^{-6} M, where virtually no effects on mGluR1 and mGluR4 are produced. This compound has no effect on the NMDA and AMPA-kainate receptors (*36*). Thus, L-CCG-I is very useful for distinguishing the functions not only of the metabotropic and ionotropic receptors but also of the different subtypes of the mGluRs.

The physiological role of mGluRs is not well understood, but recent evidence has indicated many potential regulatory functions of mGluRs in the CNS (*38*). For example, activation of mGluRs regulates neuronal excitability through suppression of K^+ currents in hippocampal synapses (*38*). In cerebellar Purkinje cells, LTD seems to require the functional cooperation of both the AMPA-kainate receptors and mGluRs (*39*). In these cells, which express the mGluR1/mGluR5 subgroup, an intracellular increase of Ca^{2+} may be responsible for the functions of mGluRs. Our data, however, have also indicated that other mGluR subtypes are linked to the inhibitory cAMP cascade. The receptors linked to this inhibitory cAMP cascade are involved in many cases in the suppression, rather than in the augmenting, of neurotransmission.

Thus, although glutamate is believed to be an excitatory neurotransmitter, this neurotransmitter may also mediate suppression of neurotransmission in certain synapses. In fact, Yamamoto and co-workers already reported that glutamate acts to suppress activity in cerebellar Golgi cells, where mGluR2 is prominently expressed (*40*). Furthermore, AP4 and tACPD, which are potent agonists for mGluR4 and mGluR2/mGluR3, respectively, suppress synaptic transmission by inhibition of glutamate release at a presynaptic site (*41*). These receptors thus appear to represent presynaptic "autoreceptors." Furthermore, AP4 selectively hyperpolarizes retinal bipolar cells by lowering intracellular guanosine 3′,5′-monophosphate (cGMP) levels through the activation of G protein–coupled phosphodiesterase (*42*). This receptor has not been characterized yet. However, because mGluR6 is highly homologous to the AP4-selective mGluR4 and is enriched in the retina, the possible coupling of mGluR6 to the cGMP cascade may be interesting. It is thus already clear that different subtypes of mGluRs have different roles in glutamate neurotransmission through coupling to intracellular signal transduction mechanisms.

Conclusion

Molecular cloning studies have demonstrated the existence of the vast majority of the AMPA-kainate, NMDA, and metabotropic receptors in the CNS. It is thus conceivable that glutamate neurotransmission in different synapses is mediated through distinct receptors and combinations of different receptors. The AMPA-kainate receptors evoke fast, voltage-independent synaptic responses and in turn promote the activation of voltage-dependent NMDA receptors. The mGluR subtypes exert long-lasting actions through the activation and inhibition of intracellular signals.

An obvious and important question is thus which receptor subtypes and what sorts of cooperative functions of different receptor subtypes are involved in the physiological functions of glutamate transmission in the CNS. The cellular localization (presynaptic or postsynaptic) of individual receptors is also important for an understanding of each receptor function. Equally important are investigations of intracellular mechanisms that underlie receptor function (for example, receptor and channel modulation, protein phosphorylation, and gene expression). The mechanisms that underlie glutamate neurotoxicity also remain to be elucidated. In this context, certain mGluR subtypes seem to be involved in attenuating glutamate release at a presynaptic site, and selective and potent agonists of mGluRs may thus be useful in investigations of glutamate neurotoxicity as well as for therapeutic purposes. The relevance and mechanisms of glutamate neurotoxicity in slowly progressive neurodegenerative diseases have not yet been settled. Because the molecular nature of the NMDA and metabotropic receptors has become elucidated only recently, a number of questions remain to be answered. Further studies of the glutamate receptors will be undoubtedly interesting and important for an understanding of integrative functions of glutamate neurotransmission in the CNS.

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Multistep Carcinogenesis: A 1992 Perspective

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Cancer is the leading cause of death in Japan. Recent changes in cancer incidence patterns may reflect the trend toward a more Western diet and life-style. Among the dietary factors that contribute to carcinogenesis are the heterocyclic amines, a group of mutagenic compounds present in cooked meat and fish. Carcinogenesis is a multistep process in which cells accumulate multiple genetic alterations as they progress to a more malignant phenotype. Recognition of the growing number of interacting factors that contribute to carcinogenesis may force reconsideration of current methods of risk assessment.

Since 1981, cancer has been the major cause of death in Japan, followed by cardiovascular disease (*1*). The Japanese Ministry of Health and Welfare recorded 223,604 cancer-related deaths in 1991 (*2*). The incidence of gastric cancer has been declining, whereas cancers of the colon, pancreas, and breast have been increasing in incidence (*3*). These changes in organ site distribution may be due to recent changes in the nation's dietary habits, including reduced salt intake and increased fat intake (*4*). A similar link between diet and cancer incidence patterns has been demonstrated previously in studies of Japanese emigrants to Western countries (*5*). The incidence of lung cancer is rising steadily in Japan because the anti-smoking movement is still at an early stage (*3*).

As with the other modern sciences, the opening of the gates of Japan to foreign countries at the end of the Edo era (1603–1867) facilitated progress in cancer research and clinical practice; this progress followed the introduction of knowledge and state-of-the-art technology, first from Germany and later from the United States, especially after World War II. Since that time, international research efforts have led to vast improvements in cancer detection and treatment. These improvements are reflected in the improved survival rates of patients treated at the National Cancer Center in Tokyo. Thirty years ago, only 37% of the patients treated at the Center survived 5 years or more, whereas this figure is now estimated to be about 50% (*6*). Indeed, we have become accustomed to seeing cancer patients successfully return to their normal daily activities after treatment.

Despite this tremendous progress, many problems in cancer detection and treatment remain. Certain groups of cancers are still almost impossible to diagnose early enough to permit effective treatment. Among these are cancers of the pancreas and gallbladder, small-cell lung cancer, scirrhous-type gastric cancer, and ovarian cancer. Prostate cancers also pose a particular problem because, without sophisticated molecular analyses, it is difficult to distinguish between occult disease and disease that will progress and therefore require aggressive treatment. Another, more recently recognized problem is the occurrence of second primary cancers among patients who had previously been cured of their first cancers by medical intervention. Many of these second cancers are neither metastases nor recurrences of the earlier disease but rather are examples of "multiple primary neoplasia." The development of these second tumors is not necessarily treatment-related since they are observed in patients who have never received chemo- or radiotherapy. About 8% of the patients at the National Cancer Center in Tokyo are now being treated for such tumors, as compared with less than 2% in 1962 (*7*). As discussed below, these cancers may originate from "primed" cells that harbor some, but not all, of the genetic alterations required for cancer development. New genetic alterations—and potentially a new malignancy—may arise from exposure of these primed cells to environmental and endogenously produced carcinogenic factors.

In this article, I consider several aspects of current research on the causes and treatment of cancer in Japan. First, I describe studies on diet and cancer, particularly

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