The Glutamate Receptor Ion Channels

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I. Introduction

The ionotropic glutamate receptors are ligand-gated ion channels that mediate the vast majority of excitatory neurotransmission in the brain. The cloning of cDNAs encoding glutamate receptor subunits, which occurred mainly between 1989 and 1992 (Hollmann and Heinemann, 1994), stimulated this field like no other event since the recognition in the early 1980s that the N-methyl-D-aspartate (NMDA)[2] receptor antagonist, D-AP5, has neuroprotective and anticonvulsant properties (reviewed by Choi, 1998; Dingledine et al., 1990), and that calcium entry through glutamate receptor channels plays important roles in development and in forms of synaptic plasticity that may underlie higher order processes such as learning and memory (Maren and Baudry, 1995; Asztely and Gustafsson, 1996). These earlier findings implicated NMDA receptors in a variety of neurologic disorders that include epilepsy, ischemic brain damage, and, more speculatively, neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, Huntington's chorea, and amyotrophic lateral sclerosis.

Glutamate receptors are expressed mainly in the central nervous system, but several potentially important exceptions are worth mentioning. The realization that pancreatic islet cells express glutamate receptors that modulate insulin secretion (Inagaki et al., 1995; Weaver et al., 1996, 1998) and that antagonists of NMDA receptors expressed by osteoclasts and osteoblasts slow bone

resorption (Chenu et al., 1998; Patton et al., 1998) raise the possibilities that antagonists restricted to the periphery might find uses in the treatment of diabetes and osteoporosis. Moreover, there is evidence for the presence of NMDA and non-NMDA receptors in small, unmyelinated sensory nerve terminals in the skin (Ault and Hildebrand, 1993; Carlton et al., 1995). Subcutaneous injection of as little as 300 pmol of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or 30 pmol of MK-801 produced analgesia for a subsequent injection of formalin into the same site. These findings raise the possibility that peripheral glutamate receptors residing on nerve terminals in the skin may be a target for certain forms of pain associated with inflammation. NMDA receptor antagonists can also reduce histamine secretion from mast cells collected from the rat peritoneal cavity (Purcell et al., 1996), and NMDA depolarizes and elevates intracellular Ca^{2+} in mouse taste receptor cells in taste buds (Hayashi et al., 1996). Numerous ionotropic glutamate receptor subunits appear to be expressed by cardiac ganglia, but their functions are unknown (Gill et al., 1998). Thus, the potential therapeutic realm of drugs targeted to glutamate receptors is expanding to include cells (neural and nonneural) in the periphery. Most recently, evidence for a role for ionotropic glutamate receptors expressed by plant cells in light signal transduction has been reported (Lam et al., 1998), suggesting that mammalian receptors may have evolved from a more primitive signaling mechanism.

The cloning of the glutamate receptors in the early 1990s has taken the study of glutamate receptor pharmacology, physiology, and pathophysiology to the molecular level. Several major reviews of the initial fruits of cloning appeared in 1994 (Hollmann and Heinemann, 1994; McBain and Mayer, 1994; Nakanishi and Masu, 1994; Gill, 1994). This review focuses primarily on the functional insights and new pharmacological targets identified by molecular biological approaches since 1994. Although synaptic functions of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have been well understood, until recently the physiological roles of kainate receptors have been elusive. The recent applications of new drugs and genetic

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[2] Abbreviations: NMDA, N-methyl-D-aspartate; PKA, protein kinase A; PKC, protein kinase C; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; UTR, untranslated region; KBP, kainate-binding protein; RT-PCR, reverse transcription-polymerase chain reaction; GABA, γ-aminobutyric acid; CNS, central nervous system; NO, nitric oxide; ABP, AMPA receptor-binding protein; AKAP, A kinase-associated protein; GKAP, guanylate kinase-associated protein; LTP, long-term potentiation; LIVBP, leucine/isoleucine/valine-binding protein; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CRIPT, cysteine-rich interactor of PDZ three; GRIP, glutamate receptor-interacting protein; EPSC, excitatory postsynaptic current; SAPAP, SAP90/PSD-95-associated protein; PSD, postsynaptic density; GK, guanylate kinase; S-SCAM, synaptic scaffolding molecule; NSF, N-ethylmaleimide-sensitive fusion protein; SNAP, synaptic NSF attachment protein; MAP, mitogen-activated protein; chapsyn, channel-associated proteins of synapse; MAP1A, microtubule-associated protein 1A; PEPA, 4-[2(phenylsulfonylamino)ethylthio]-2,6-difluoro-phenoxyacetamide.

knockout technology are finally providing clues to the role of kainate receptors in synaptic transmission (e.g., Clarke et al., 1997; Rodriguez-Moreno and Lerma, 1998). However, to focus this review on the properties of the receptors themselves, we do not provide detailed information on the physiological roles of the various receptors, or their regional distribution, or extensive evaluation of genetically modified mice. Several recent reviews complement this one (Edmonds et al., 1995; Bettler and Mulle, 1995; Steinhauser and Gallo, 1996; Fletcher and Lodge, 1996; Sucher et al., 1996; Ben-Ari et al., 1997; Borges and Dingledine, 1998; Dingledine and McBain, 1998; Ozawa et al., 1998; Myers et al., 1999).

II. Gene Families

The three pharmacologically defined classes of ionotropic glutamate receptor were originally named after reasonably selective agonists —NMDA, AMPA, and kainate. It turned out that NMDA, AMPA, and kainate receptor subunits are encoded by at least six gene families as defined by sequence homology: a single family for AMPA receptors, two for kainate, and three for NMDA (Table 1). Sequence similarity and, in some cases, similarity in intron-exon structure (Suchanek et al., 1995) suggests a common evolutionary origin for all of the ionotropic glutamate receptor genes. These genes are scattered over numerous chromosomes (Table 1), although the *GRIA4* and *GRIK4* genes are located near one another on the long arm of chromosome 11 and *GRIK5* and *GRIN2D* may be close together on the long arm of chromosome 19. The protein products of these two pairs of genes do not appear to interact functionally, and it is not known whether these gene pairs are coordinately regulated similar to the gene clusters of the nicotinic acetylcholine receptor (Boulter et al., 1990a).

The δ1 and δ2 genes are distant structural relatives (18–25% amino acid identity) of other glutamate receptor subunits (Lomeli et al., 1993). These orphan subunits do not form functional channels by themselves, nor have they been shown to modify the function of other subunit combinations. However, knockout of the δ2 gene leads to loss of activity-related depression of the parallel fiber-Purkinje cell synapse (Kashiwabuchi et al., 1995), and the mouse Lurcher neurological mutant has recently been shown to be caused by a gain-of-function mutation in δ2, which leads to a large constitutive inward current that may provide a genetic model for excitotoxicity (Zuo et al., 1997). The genetic knockout strategy has recently helped to delineate the potential functions of another protein distantly related to the NMDA receptor subunits, NR3A (previously named NMDA-RL). NR3A coimmunoprecipitates with NR1 and NR2B subunit proteins in homogenates of mouse cerebral cortex (but not with GluR2, GluR6, δ1, or δ2 subunit proteins). Coexpression of NR3A with NR1 and NR2A causes a reduction in both whole-cell currents (Ciabarra et al., 1995) and single-channel conductance (Das et al., 1998), and perhaps a lower Ca^{2+} permeability. Accordingly, NMDA-induced currents in cortical neurons were increased about 3-fold in NR3A knockout mice (Das et al., 1998). These findings strongly suggest that the NR3A subunit may serve a regulatory function in NMDA receptors; in particular for controlling the amplitude and Ca influx through synaptic NMDA receptor channels.

No genetic diseases in humans have yet been linked to mutations in any of the glutamate receptor subunits, although as noted above the mouse Lurcher mutant is caused by a mutation in δ2. Additionally, the genotype at a polymorphic triplet repeat in the 3′ untranslated region (UTR) of human GluR6 appears to have a minor influence on the age of onset of Huntington's disease (Rubenstein et al., 1997). A number of neurological disorders are accompanied by the appearance of antibodies to glutamate receptor subunits (e.g., to GluR3 in Ras-

TABLE 1
Glutamate receptor subunits and their genes

Group	Receptor Family	Subunit	Gene	Chromosome (human)	GenEMBL Accession No.		
					Mouse	Rat	Human
1	AMPA	GluR1	*GRIA1*	5q33	X57497	X17184	I57354
1	AMPA	GluR2	*GRIA2*	4q32-33	X57498	M85035	A46056
1	AMPA	GluR3	*GRIA3*	Xq25-26		M85036	X82068
1	AMPA	GluR4	*GRIA4*	11q22-23		M36421	U16129
2	Kainate	GluR5	*GRIK1*	21q21.1-22.1	X66118	M83560	U16125
2	Kainate	GluR6	*GRIK2*	6q16.3-q21	D10054	Z11715	U16126
2	Kainate	GluR7	*GRIK3*	1p34-p33		M83552	U16127
3	Kainate	KA-1	*GRIK4*	11q22.3		X59996	S67803[a]
3	Kainate	KA-2	*GRIK5*	19q13.2	D10011	Z11581	S40369
4	NMDA	NR1	*GRIN1*	9q34.3	D10028	X63255	X58633
5	NMDA	NR2A	*GRIN2A*	16p13.2	D10217	D13211	U09002
5	NMDA	NR2B	*GRIN2B*	12p12	D10651	M91562	U28861[a]
5	NMDA	NR2C	*GRIN2C*	17q24-q25	D10694	D13212	
5	NMDA	NR2D	*GRIN2D*	19q13.1qter	D12822	D13214	U77783
6	NMDA	NR3A	*GRIN3A*[b]			L34938	
7	Orphan	δ1	*GRID1*		D10171	Z17238	
7	Orphan	δ2	*GRID2*	4q22	D13266	Z17239	

[a] Partial sequence.
[b] Proposed here.

mussen's encephalitis—Rogers et al., 1994; Twyman et al., 1995; Carlson et al., 1997; to GluR2 in nonfamilial olivopontocerebellar degeneration—Gähring et al., 1997; and to several AMPA and kainate receptor subunits in paraneoplastic neurodegenerative syndrome—Gähring et al., 1995), but the role of these antibodies in disease manifestation is unclear (e.g., He et al., 1998).

In addition to these mammalian genes, cDNAs encoding several kainate-binding proteins (KBPs) had been isolated in the early 1990s from frog, chick, and goldfish brain that exhibit weak sequence homology to the mammalian glutamate receptors (for review, Henley, 1994). These proteins have not been found in mammals and do not seem to form functional homo- or heteromeric complexes with other glutamate receptor channels. However, chimeric proteins consisting of the channel-forming domains of KBPs and the ligand recognition domains of GluR1 or GluR6 form functional ion channels (Villmann et al., 1997), suggesting that an undiscovered modulatory subunit may be required to form fully functional KBPs.

III. Receptor Structure

A. Transmembrane Topology

The mechanism by which a receptor protein is threaded through the membrane during synthesis determines which segments face the extracellular and cytoplasmic fluids; this in turn specifies the protein domains that are available for ligand recognition, cytoplasmic modification (phosphorylation etc.), and interactions between the receptor and cytoplasmic proteins. Against initial expectations, glutamate receptors proved to have only three transmembrane domains (M1, M3, and M4) plus a cytoplasm-facing re-entrant membrane loop (M2, Fig. 1). Thus, the N terminus is located extracellularly and the C terminus intracellularly. This was deduced
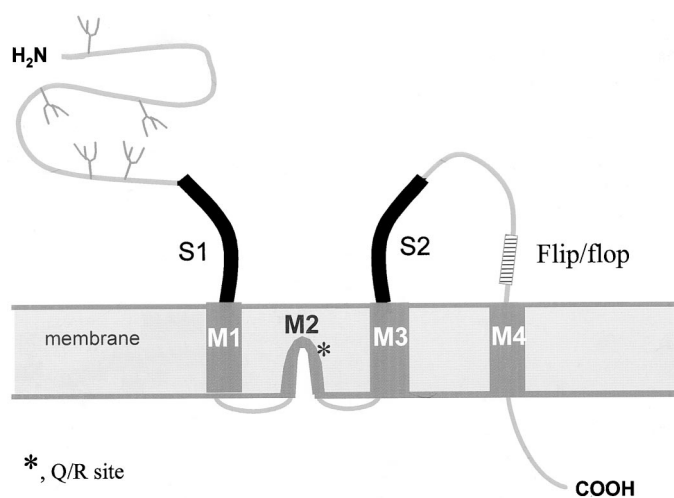

*, Q/R site

FIG 1. Structure of AMPA receptor subunits. The transmembrane topology is shown along with the flip/flop alternatively spliced exon, and the two ligand-binding domains (S1 and S2). Glycosylation sites are shown as trees in the N-terminal region.

first by localization of endogenous and introduced N-glycosylation sites in KBPs (Wo and Oswald, 1994, 1995), GluR1 (Hollmann et al., 1994), GluR3 (Bennett and Dingledine, 1995), and NR1 (Wood et al., 1995), and additionally by analysis of the protease sensitivity of a reporter group fused at different positions to GluR3 (Bennett and Dingledine, 1995). The M2 segment in NMDA receptors is also thought to be a re-entrant loop based on the pattern of accessibility from both sides of the membrane of charged sulfhydryl reagents to cysteines substituted for M2 residues (Kuner et al., 1996), and the same method supports three rather than four transmembrane segments in AMPA receptors (Kuner et al., 1997). The transmembrane topology of glutamate receptors thus appears different from the four-transmembrane model of nicotinic acetylcholine receptors, but similar to that of potassium channels in that a re-entrant loop is present. Residues in this re-entrant second membrane loop control key permeation properties of the ion channel (see below).

B. Subunit Stoichiometry

Early evidence favored a pentameric structure for glutamate receptors based on the size of chemically cross-linked NMDA receptor protein (Brose et al., 1993) or functional analysis of mixtures of native and mutant subunits with different sensitivity to channel blockers (Ferrer-Monteil and Montal, 1996), although velocity sedimentation analysis was consistent with a smaller protein (Blackstone et al., 1992; Wu and Chang, 1994). Premkumar and Auerbach (1997) inferred a pentameric stoichiometry for NMDA receptors consisting of three NR1 and two NR2 subunits. Their conclusion depended on interpretation of the patterns of single-channel conductances observed in mixtures of native and mutant subunits; the asparagine residue in the M2 segment lining the channel (N616) was changed to a glutamine in the mutant. For example, coexpression in *Xenopus* oocytes of NR1(N) with a combination of NR2B (N) and NR2B(Q) produced receptors with three patterns of main and subconductance states, corresponding to those seen with 1N/2N, 1N/2Q, and a third pattern that was considered to reflect 1N/2Q/2N receptors. Because only three different channel types were observed, they concluded that there must be two and only two NR2B subunits in a receptor (i.e., Q/Q, Q/N, and N/N). When a mixture of NR1(N) and NR1(Q) were coexpressed with NR2B(Q), six single-channel current patterns were distinguishable, which by similar logic pointed to three NR1 subunits in a functional receptor. An analogous experimental design by Behe et al. (1995) found fewer single-channel patterns, however, and concluded there are only two copies of NR1, not three as Premkumar and Auerbach (1997) found. Behe et al. (1995) concluded that the most parsimonius model involved a tetrameric protein consisting of two NR1 and two NR2 subunits.

Three more recent studies have been interpreted to favor a tetrameric assembly of subunits similar to that of potassium channels (Doyle et al., 1998) or cyclic nucleotide-gated channels (Liu et al., 1998). Laube et al. (1998) found three components in the dose-response curve for activation of mixtures of wild-type and mutant NMDA receptor subunits [NR1(Q387K) and NR2B(E387A) are described further in Table 2] with either of the two coagonists, glutamate or glycine. Binomial fits to the three receptor populations were most consistent with two NR1 and two NR2 agonist-binding subunits per functional receptor. Binomial analysis of

TABLE 2
Residues important for agonist recognition in rodent glutamate receptors

Subunit	Mutation	GluR2 Equivalent[a]	Ligand Binding[b]	Functional Effect of Mutation
NR1[a, c]	Q387K	E402	β	14,000× ↑ in glycine EC_{50}, 13× ↑ in glutamate EC_{50}
	F390S	Y405	δ	63× ↑ in glycine EC_{50}
	Y392A	M407		12× ↑ in glycine EC_{50}
	F466H	Y450	β*	2,100× ↑ in glycine EC_{50}
	V666A	L650		13× ↑ in glycine EC_{50}
	S669G	G653	β	25× ↑ in glycine EC_{50}
NR1[d]	F735A	L727		16× ↑ in glycine EC_{50}
	F736A	D728		28× ↑ in glycine EC_{50}
NR1[e]	D481N	D447		7× ↑ in glycine EC_{50}
	K483Q	K449	β	130× ↑ in glycine EC_{50}
NR1[f]	D732E	E705	α*	3,700× ↑ in glycine EC_{50}
NR2B[g]	E387A	E402	β	240× ↑ in glutamate EC_{50}
	F390S	Y405	δ	50× ↑ in glutamate EC_{50}
	K459E	K449	β	180× ↑ in glutamate EC_{50}
	H460F	Y450	β*	9× ↑ in glutamate EC_{50}
	R493K	R485	α*	Complete loss of agonist responses
	S486A	P478	β*	43× ↑ in glutamate EC_{50}
	V660A	L650		20× ↑ in glutamate EC_{50}
	S664G	S654	α*	100× ↑ in glutamate EC_{50}
	V709M	M708		30× ↑ in glutamate EC_{50}
NR2A[h]	N463A	D447		6× ↑ in glutamate EC_{50}, no change in gly EC_{50}
	K465E	K449	β	10× ↑ in glutamate EC_{50}, no change in gly EC_{50}
	H466A	Y450	β*	220× ↑ in glutamate EC_{50}, no change in gly EC_{50}
	T665A	T649		7× ↑ in glutamate EC_{50}, no change in gly EC_{50}
	V666A	L650		12× ↑ in glutamate EC_{50}, 1.7× ↑ in gly EC_{50}
	G669A	G653		320× ↑ in glutamate EC_{50}, no change in gly EC_{50}
	T671A	T655	α*	1,000× ↑ in glutamate EC_{50}, no change in gly EC_{50}
GluR1[i]	E398Q	E402	β	8× less sensitive to glutamate desensitization
	Y446F	Y450	β*	4× less sensitive to glutamate desensitization
	L646A	L650		40× less sensitive to glutamate desensitization
	S650V	S654	α*	18× less sensitive to glutamate desensitization
GluR1[j] (mouse)	E398K	E402	β	100,000× ↑ in glutamate EC_{50}
	D443K	D447		5× ↑ in glutamate EC_{50}
	K445E	K449	β	22× ↑ in glutamate EC_{50}
GluR1[k]	K445Q	K449	β	51× ↑ in AMPA EC_{50}, 3× ↑ in glutamate EC_{50}
GluR2[k]	K449E	K449	β	4× ↑ in glutamate EC_{50}
GluR3[l]	L507Y	L483		Abolishes glutamate desensitization
	T504A	T480	α*	134× ↑ glutamate EC_{50}
GluR6[m]	N721S	T686	β	2–3× ↑ glutamate affinity, >16× ↑ AMPA affinity
cKBP[n]	E33V	E402	β	110× ↓ in kainate affinity, no change in glu affinity
	Y36F	Y405	δ	30× ↓ glutamate affinity, 5× ↓ kainate affinity
	Y73I	Y450	β*	90× ↓ glutamate affinity, 10× ↓ kainate affinity
	P100A	P478	α*	No significant change in kainate binding
	T102A	T480	α*	100× ↓ glutamate affinity, 58× ↓ kainate affinity
	R107S	R485	α*	Complete loss of kainate binding
	S266A	G653	β	5–6× ↓ kainate affinity
	S267A	S654	α*	5–6× ↓ kainate affinity
	T268A	T655	α*	Complete loss of kainate binding
	Y299A	T686	β	5–6× ↓ kainate affinity
	E316Q	E705	α*	Complete loss of kainate binding

[a] The designated amino acid residues were mapped onto the rat GluR2 sequence numbered according to Armstrong et al. (1998).
[b] Residues identified in the crystal structure of the ligand-binding domain of GluR2. *, ligand-binding residues, α, residues interacting with all agonists; β, residues predicted to interact only with specific ligands; δ, residues that maintain the shape of the ligand-binding pocket but do not contact ligands. From Armstrong et al. (1998).
[c] Kuryatov et al., 1994 (EC_{50} measured in oocytes expressing NR1/NR2B receptors).
[d] Hirai et al., 1996 (EC_{50} measured in oocytes expressing NR1/NR2B receptors).
[e] Wafford et al., 1995 (EC_{50} measured from oocytes expressing NR1/NR2A receptors).
[f] Williams et al., 1996 (EC_{50} measured in oocytes expressing NR1/NR2B receptors).
[g] Laube et al., 1997 (EC_{50} measured in oocytes expressing NR1/NR2B receptors).
[h] Anson et al., 1998 (measured in oocytes expressing NR1/NR2A receptors).
[i] Mano et al., 1996 (EC_{50} measured in oocytes expressing homomeric GluR1 receptors).
[j] Uchino et al., 1992 (EC_{50} measured in oocytes expressing GluR1 receptors).
[k] Li et al., 1995 (EC_{50} measured in oocytes expressing GluR1 or GluR1/GluR2 receptors).
[l] Stern-Bach et al., 1998 (EC_{50} measured in outside-out patches of HEK293 cells expressing GluR3 receptors).
[m] Swanson et al., 1997b (EC_{50} measured in oocytes expressing homomeric GluR6 receptors).
[n] Paas et al., 1996 (affinity judged by displacement of radiolabeled kainate from HEK 293 membranes).

the functional effect of incorporating increasing amounts of dominant negative NR1 or NR2 subunits into the receptor also supported a tetrameric assembly. Mano and Teichberg (1998) used a similar strategy to conclude that homomeric GluR1 receptors could be tetrameric complexes. Rosenmund et al. (1998) observed that upon agonist binding, activation of single receptor/channels proceeds through a staircase of openings to three different conductance levels of increasing amplitude. To resolve rapid transitions that occur during normal activation, agonist-binding sites were presaturated with the competitive antagonist, NBQX, before agonist application, so that each agonist-binding site was only made available after an antagonist molecule dissociated from the receptor. The authors proposed a model whereby the dissociation of two antagonist molecules and their replacement by two agonist molecules occurred before the first current step appeared. Current levels that were observed subsequently reflected the binding of single agonist molecules to the receptor, suggesting that each receptor contains four functional antagonist/agonist-binding sites, which is consistent with a tetrameric protein.

Thus, the conclusions of six carefully performed functional evaluations of receptor subunit mixtures (Ferrer-Monteil and Montal, 1996; Behe et al., 1995; Premkuhar and Auerbach, 1997; Laube et al. 1998; Mano and Teichberg, 1998; Rosenmund et al., 1998) are exactly split between a tetramer and a pentamer. In attempting to resolve the difference, several issues should be considered. First, each of the studies described above was designed to identify the number of functional binding sites in a receptor and relied on the assumption that each binding site in a receptor behaves independently of the others. It is well known that the agonist-binding sites of at least one other receptor, the muscle nicotinic acetylcholine receptor, show cooperativity, and negative cooperativity between binding of glutamate and glycine to the NMDA receptor is also well established (reviewed by McBain and Mayer, 1994). Second, the data of Rosenmund et al. (1998) suggest that subconductance states of a channel may be related to the number of agonists bound to the receptor. If this is correct, the interpretation of dose-response curves (Laube et al., 1998) and patterns of main and subconductance states (Behe et al., 1995; Premkuhar and Auerbach, 1997) become more complicated. Finally, post-translational processing could increase subunit complexity and potentially lead to overestimation of the number of functional subunits. Rosenmund et al. (1998) and Laube et al. (1998) argue that although their data favor a tetrameric protein, the possibility of a pentameric structure could not be entirely ruled out. Although the functional results are provocative, an unequivocal determination of the number of subunits in a functional glutamate receptor awaits physical methods that probe the structure of the protein itself.

Table 1 lists at least 14 functional glutamate receptor subunits. What pairing rules determine which subunits coassemble? Is subunit stoichiometry invariant as in the case of muscle nicotinic receptors? In the early 1990s many mixing experiments were carried out to search for instances in which coexpression of subunits from one family (NMDA, AMPA, or kainate) might alter the functional properties of receptors in a different family. These attempts were uniformly unsuccessful, and it is now generally accepted that a subunit will only assemble with others within its own family. It seems that the membrane domains may dominate assembly, because chimeras of AMPA and kainate receptors do assemble if the membrane domains are all from the same subunit (Stern-Bach et al., 1994). Functional homomeric receptors can be formed within the AMPA and kainate subunit families but probably not for NMDA receptors. Functional NMDA receptors can be formed by expression of the NR1 subunit by itself in *Xenopus* oocytes but not in mammalian cell lines. However, Soloviev and Barnard (1997) showed that a glutamate receptor subunit, XenU1, is endogenously expressed at very low abundance in *Xenopus* oocytes and can assemble with mammalian NR1 to form functional NMDA receptors. Their finding could explain why expression cloning of NR1 in *Xenopus* oocytes (Moriyoshi et al., 1991) was originally possible and reinforces the current notion that NR1 must partner with one or more NR2 subunits to form functional receptors.

Little is yet known about the exact subunit composition of native glutamate receptors, but immunoprecipitation strategies have shown that NR2A and NR2B subunits can coexist together with NR1 in native NMDA receptors gently solubilized from mammalian brain by sodium deoxycholate at pH 9 (Sheng et al., 1994; Blahos and Wenthold, 1996; Luo et al., 1997; Chazot and Stephenson, 1997); multiple NR1 splice variants can also exist in a receptor assembly (Blahos and Wenthold, 1996). Likewise, NR2D can be immunoprecipitated along with NR1 and either NR2A or NR2B (Dunah et al., 1998). Wenthold et al. (1996), using similar strategies, showed that AMPA receptors immunoprecipitated from the CA1 region of rat hippocampus (primarily pyramidal cells) consisted of two major complexes represented by GluR2 plus either GluR1 or GluR3; very few solubilized receptors appeared to contain both GluR1 and GluR3, but a small fraction of solubilized receptors appeared to be homomeric GluR1. Results from other functional assays appear compatible with heteromultimeric receptors. For example, the glycine dose-response curve of NMDA receptors assembled from NR1-1e, NR2A, and NR2C could not be described as the weighted average of dose-response curves obtained from NR1-1e + NR2A and NR1-1e + NR2C receptors done separately (Wafford et al., 1993). The glycine EC_{50} for the triple subunit combination was intermediate between those for the heterodimeric combinations. All of these results argue

for at least heteroternary NMDA receptors, but do not rule out the presence of some additional binary hetero-mers consisting of NR1 plus a single type of NR2 sub-unit.

It is apparent from the studies described above that multiple subtypes exist within the AMPA and NMDA receptor families based on subunit composition. Further complicating matters, it is now clear that multiple AMPA receptor subtypes coexist within the same neuron. Thus, Zhang et al. (1995) showed that AMPA receptor channels with both low and high calcium permeability could be found within the same retinal ganglion neuron. Washburn et al. (1997) showed that polyamine spider toxins, which selectively block GluR2-lacking AMPA receptors (Iino et al., 1996; Washburn and Dingledine, 1996), removed the inwardly rectifying component of AMPA receptor currents in hippocampal interneurons, suggesting coexpression of GluR2-lacking and -containing receptors in the same cell. Likewise, spermine (Ito et al., 1996) or polyamine spider toxins (Tóth and McBain, 1998; F. Laezza, J. Doherty and R. Dingledine, unpublished) eliminate the inwardly rectifying component of evoked *synaptic* currents in hippocampal interneurons, suggesting that mosaics of GluR2-containing and -lacking receptors are present at individual postsynaptic membranes. Tóth and McBain (1998) have proposed that the expression of calcium-permeable or -impermeable AMPA receptors is determined by the origin of synaptic input onto the dendrites of the target cell. The most direct evidence for multiple AMPA receptor subtypes in a single cell is found in the fusiform cells of the rat dorsal cochlear nucleus. In these cells, postsynaptic AMPA receptors at auditory nerve synapses on basal dendrites contain GluR4 by immuno-histochemistry, whereas receptors in parallel fiber synapses on apical dendrites lack GluR4 (Rubio and Wenthold, 1997). This shows directly that subsynaptic receptor targeting can be guided by the GluR4 subunit.

The studies described above indicate that more than one AMPA receptor subtype can coexist within the same neuron. AMPA receptor diversity is even more extreme, however, because it appears that subunit stoichiometry is not fixed for AMPA receptors as it is for muscle nicotinic receptors. Washburn et al. (1997) studied three GluR2-dependent permeation features of recombinant AMPA receptors composed of different subunits in a variety of ratios, and in native receptors expressed by hippocampal interneurons: rectification, Ca^{2+} permeability and sensitivity to external polyamine block. The shape of AMPA receptor current-voltage curves in individual cells could not be described by an algebraic summation of I-V curves from two populations of receptors, those containing and lacking GluR2 in some fixed but unspecified stoichiometry. Moreover, rectification was much less sensitive to the relative abundance of GluR2 than was Ca^{2+} permeability. Both of these results argue strongly that the number of GluR2 subunits in an AMPA

receptor is not fixed (see also Geiger et al., 1995). Variable AMPA receptor subunit stoichiometry endows excitatory synapses with a much wider range of responses than previously imagined.

C. Ligand-Binding Sites Located in a Hinged Clamshell-like Gorge

A high-resolution crystal structure (approximately 1.9 Å) has recently been obtained for the ligand-binding domain of GluR2 complexed with the agonist kainate (Armstrong et al., 1998). This achievement followed and built upon much effort devoted to model the structure of glutamate receptor subunits, which in turn was made possible by the realization (Nakanishi et al., 1990) that glutamate receptors share weak sequence homology with a large family of bacterial amino acid-binding proteins whose structures had been solved to high resolution. A conserved amino acid-binding pocket (Oh et al., 1993, 1994; Sun et al., 1998) is proposed to exist in all glutamate receptors. This pocket would be formed from two globular domains (S1 and S2) drawn from the sequence adjacent to the M1 domain and the M3-M4 loop, respectively (Fig. 2A). In the bacterial proteins, the two lobes of the binding pocket are in a dynamic equilibrium of open and closed states; binding of ligand stabilizes the closed form of the clamshell structure. Four studies support the idea that the agonist-binding site of glutamate receptors is also a bilobular structure. First, swapping of S1 and S2 domains between GluR3 and GluR6 subunits caused the expected change in agonist pharmacology (Stern-Bach et al., 1994). Second, a soluble (nonmembrane bound) "minireceptor" consisting of these two domains from GluR4 or GluR2 joined by a hydrophilic spacer peptide was able to bind AMPA, glutamate, kainate, quisqualate, and CNQX with the expected affinities (Kuusinen et al., 1995; Arvola and Keinänen, 1996). Similarly, a soluble glycine-binding site with correct pharmacology was preserved in a fusion protein consisting of the S1 and S2 lobes of the NR1 subunit connected by a linker (Ivanovic et al., 1998). Third, inserting the S1 domain from GluR6 into GluR2 decreased the affinity for AMPA and increased kainate affinity (Tygesen et al., 1995). Finally, deletion of the N-terminal 400 amino acids, and the C-terminal 90 amino acids, from GluR6 left a membrane-bound core homomeric receptor that displayed normal [3H]kainate-binding properties (Keinänen et al., 1998). These findings support the concept that individual glutamate receptor subunits, like many other proteins, are constructed in a modular fashion: a pore-forming domain similar to that of potassium channels plus two separate domains that form a ligand-binding site similar to those of the bacterial periplasmic binding proteins (Wo and Oswald, 1995; Paas, 1998). The N-terminal 400 amino acids appear to play no significant role in ligand binding but may be the locus of many modulatory functions in some (e.g., NMDA) receptors. The crystal structure of the ligand-binding domain
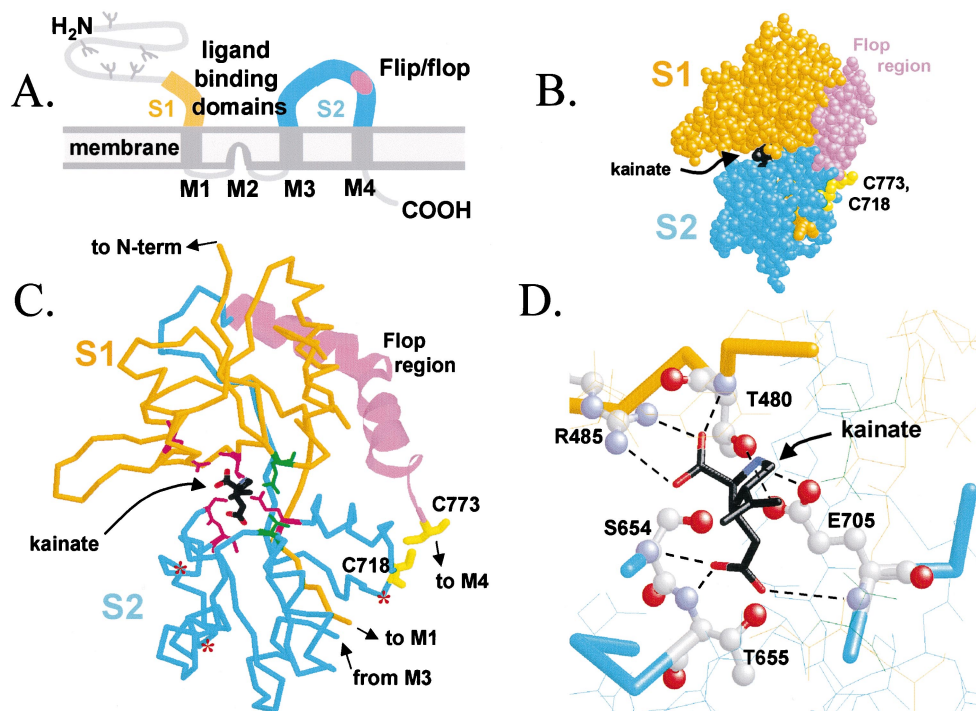
FIG. 2. Crystal structure of the GluR2 subunit and identification of agonist-binding residues. A, schematic of a glutamate receptor subunit with the two domains that contain agonist-binding residues colored in orange (S1) and turquoise (S2). The flip/flop region is indicated in violet. B, space-filled representation of the kainate-bound S1 and S2 domains joined by an 11-residue linker peptide, with coloration the same as in A. The flop domain is helical and located on a solvent-exposed face of the protein. The position of a single-kainate agonist molecule (black) within a deep gorge of the protein is indicated; the two disulfide-bonded cysteines (C718 and C773) are shown in yellow. Red asterisks mark the positions of S662 and S680 (lower left), which in GluR6 are important for PKA, and N721 (adjacent to the yellow C722), which in GluR5 and GluR6 controls agonist sensitivity. C, backbone representation of the subunit, with kainate (black) docked into its binding site. The kainate-binding residues are shown as stick figures in magenta, the two cysteines in yellow, and the flop helix structure in violet. The two green residues (E402 and T686) do not directly bind to kainate but instead interact with each other, helping to hold the clamshell in the closed conformation. D, close-up view of the ligand-binding pocket. The binding residues are in space-filled representation, with atoms colored conventionally (gray = carbon, light blue = nitrogen, red = oxygen). These images were created in rasmol from the pdb file graciously provided by E. Gouaux (Armstrong et al., 1998).

of GluR2 confirmed the bilobular structure (Fig. 2, A and B) and revealed additional details about the ligand-binding site.

Figure 2B shows a spacefill model of the GluR2 structure, with color-coded S1 and S2 lobes folding around the kainate molecule (shown partly buried in black). An immediate conclusion from inspection is that the ligand-binding pocket appears to be entirely contained within a single subunit rather than being at the interface between two subunits. The flip/flop domain (violet) is an α-helical structure on the side opposite the ligand-binding gorge. The subunit backbone is shown in Fig. 2C, where the residues that make contact with the kainate molecule are shown in magenta and kainate itself in black. The two residues in green (E402 and T686) do not participate directly in kainate binding but instead interact with one another to help hold the S1 and S2 lobes together. The two conserved cysteines that play a role in redox modulation of NMDA receptors are disulfide-bonded and shown in yellow. Figure 2D shows an enlarged view of the ligand-binding pocket, with electrostatic or polar interactions indicated by dotted lines. It is visually clear that kainate, binding deep within the S1 to S2 cleft, stabilizes the closed form of the clamshell structure by simultaneously bonding with residues in both S1 and S2 lobes.

How does ligand binding lead to channel opening? One can speculate that closure of the S1 and S2 lobes places a torque on the receptor that is transmitted to the channel region. The resulting mechanical force could increase the likelihood that the channel structure itself undergoes a conformational change to the open state. Whether desensitization is caused by a time-dependent relaxation of the "molecular spring" that connects the S1 and S2 lobes to the membrane, to partial unbinding of the agonist molecule that releases one of the lobes, to further closure of the cleft as residues in the outer reaches of the two lobes interact when the nearly closed clamshell breathes, or to some other event, remains for future work.

The ability of different agonists to either bind or activate glutamate receptors has now been assessed in well over 100 mutants in an effort to identify residues important for agonist binding. Residues that have been shown to influence agonist potency by more than a fewfold in kainate, AMPA, and NMDA receptors are identified in Table 2. These residues have been mapped onto the sequence of the GluR2 subunit so their positions can be

compared. It is immediately apparent that several amino acids appear in homologous positions in the different subunits. An example is R485, the guanidinium group of which interacts with a carboxyl group of kainate in GluR2 (Fig. 2D). This arginine is also present in NR2B and cKBP and, when mutated to a lysine or serine, abolishes agonist responses (Table 2). Other examples are found by perusing Table 2. It is remarkable that, with one exception, the correspondence is excellent between the predictions made by functional evaluation of point mutations and the direct structural identification of residues in or near the binding pocket. That exception is Pro478. In GluR2, the carbonyl group of P478 is positioned near T480 and appears to bind to the nitrogen of kainate (not shown in Fig. 2D), but mutation of the homologous proline in chick KBP had no effect on agonist binding (Table 2).

Certain residues do not participate directly in agonist recognition but instead serve allosteric roles. For example, E402 in GluR2 has its homologs in the NR1, NR2B, GluR1, and chick KBP; in all of these subunits mutation of the E402 homolog has mild-to-substantial effects on the potency of the respective agonists (Table 2). E402 does not directly contact the kainate molecule in GluR2 but instead interacts with T686 of the opposite lobe to shape the binding pocket (Fig. 2C). Other examples of important modulatory or allosteric residues are N721, which lies adjacent to the S2 lobe cysteine and regulates agonist selectivity in GluR5 and GluR6 (Swanson et al., 1998), and S662 and S680, which in GluR6 are involved in phosphorylation by protein kinase A (PKA; Raymond et al., 1993; Wang et al., 1993; Basiry et al., 1999). The positions of these residues are marked with red asterisks in Fig. 2C.

The comparison of mutagenesis and molecular modeling in Fig. 2 and Table 2 provides support for the idea that all glutamate receptor subunits have a similar folding pattern, with ligand specificities probably accounted for by differences in amino acids at key positions as argued by Paas (1998). Analysis of mutants of the NR1 and NR2 subunits led to the proposal that the glycine coagonist docking site of NMDA receptors is exclusively on the NR1 subunit, whereas glutamate binds to the NR2 subunit (Kuryatov et al., 1994; Hirai et al., 1996; Laube et al., 1997; Anson et al., 1998; see Table 2).

The combination of structure determination and functional evaluation of mutants is thus producing internally consistent views of glutamate receptor subunit structures. Not surprisingly, before the GluR2 structure had been solved, numerous investigators had constructed molecular models by aligning the S1 and S2 sequences from glutamate receptors onto the structure of the amino acid-binding proteins, and then adjusting the new structure based on energy minimization in concert with functional information provided by mutants (Stern-Bach et al., 1994; Paas et al., 1996; Sutcliffe et al., 1996; Laube et al., 1997; Swanson et al., 1997a). The

general low-resolution picture provided by homology modeling is remarkably congruent across several glutamate- and glycine-binding sites of various glutamate receptor subunits. All models incorporated two hinged lobes that close upon an agonist molecule within a cleft, and this general scheme is confirmed by the GluR2 structure shown in Fig. 2. However, it is well known that identification of residues contacting ligands from electrophysiological evaluation of mutants is not straightforward, because the measured agonist EC_{50} is influenced not only by binding affinity but also by the ease with which the agonist-bound structure undergoes the conformational change leading to channel opening (e.g., Colquhoun and Sakmann, 1998). As caveats to the uncritical interpretation of mutagenesis experiments, one only needs to recall the once widely held but mistaken views that the acetylcholine-binding pocket of acetylcholinesterase consists of acidic and basic residues, and that the selectivity filter of potassium channels is derived from π-orbitals of aromatic residues. Both of these views were overturned when crystal structures became available (Harel et al., 1993; Doyle et al., 1998). Further understanding of the structure of the ligand-binding sites of glutamate receptors will require solving the structures of other subunits liganded to a variety of agonist and antagonists. This work is important to direct and interpret a myriad of mutagenesis studies and, in the long run, to facilitate the design of new drugs. The structure of the pore inferred from functional measurements is discussed below.

IV. RNA Modifications That Promote Molecular Diversity

As described elsewhere in this review, functional diversity in glutamate receptors is determined in large part by which genes are expressed in a given neuron. In addition, ionotropic glutamate receptor subunits are subject to post-transcriptional alterations—alternative splicing and RNA editing—both of which give rise to a high structural and functional diversity.

A. Alternative Splicing

All four AMPA receptor subunits occur in two alternatively spliced versions, flip and flop, that are encoded by exons 14 and 15 (in GluR2) positioned just before the M4 domain (Figs. 1–3) (Sommer et al., 1990; Monyer et al., 1991). Flip variants predominate before birth and continue to be expressed in adult rats, whereas flop variants are in low abundance before the eighth postnatal day and are up-regulated to about the same level as the flip forms in adult animals. The flip forms of most subunits desensitize more slowly and less profoundly than the flop forms (Table 4). Desensitization in the flip forms is more potently attenuated by cyclothiazide, whereas PEPA (4-[2(phenylsulfonylamino)ethylthio]-2,6-difluoro-phenoxyacetamide) preferentially reduces the desensitization of the flop forms (see below).
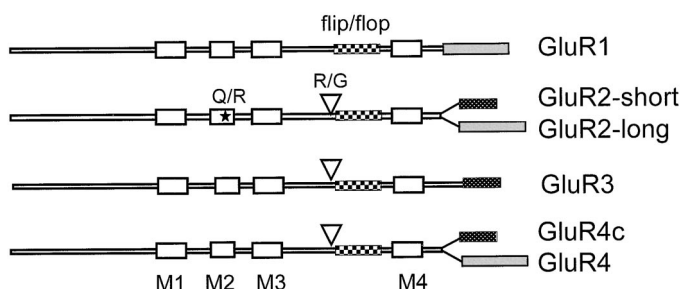
FIG. 3. Alternative splicing and editing of AMPA receptor subunits. The flip/flop and C-terminal splice variants of the AMPA receptor subunits are depicted schematically. The regions buried in the membrane are shown as boxes M1 to M4. The Q/R- and R/G-editing sites are indicated, as well as the defined phosphorylation sites in GluR1, Ser845 for PKA and Ser831 for PKC and CAMKII. Homologous C termini contain the same pattern.

C-terminal splice variants are found in GluR2, GluR4, and the kainate receptor subunits GluR5 to 7 (Figs. 3 and 4). A small percentage of GluR2 protein exhibits a long C terminus (Köhler et al., 1994). The cerebellum expresses GluR4c (Gallo et al., 1992), which has a C terminus that is shorter than that of GluR4 and is homologous to the tail of GluR2short. GluR5 cDNAs display four different C-tails and, additionally, an exon encoding 15 amino acids in the N terminus occurs in some transcripts (Sommer et al., 1992; Gregor et al., 1993). GluR6 and GluR7 each have two splice variants that differ in their C termini (Gregor et al., 1993; Schiffer et al., 1997a). When expressed as homomeric receptors in HEK 293 cells, GluR7a receptors gave rise to 5- to 10-fold larger currents than GluR7b (Schiffer et al., 1997a). Additional functional differences among the different splice variants have not been reported, but the different C termini may bind to different intracellular proteins and thus influence receptor targeting. For example, association of glutamate receptors with recently identified proteins containing PDZ domains is dependent on the C-terminal amino acids [e.g., GluR2 binding to glutamate receptor-interacting protein (GRIP), see below].

The NR1 subunit contains three alternatively spliced exons: exon 5 in the N terminus (also called the N1 cassette) and exons 21 and 22 in the C terminus (also called C1 and C2 cassettes). Exon 22 (C2) contains an alternate acceptor splice site that, when used, splices out part of exon 22 including the stop codon and engages a new reading frame that encodes an alternative cassette C2′ before a stop codon is reached. Several nomenclatures are used for the eight NR1 splice variants, some related to structure and others referring to the chronological appearance of the clones (see Hollmann et al., 1993; Zukin and Bennett, 1995). According to Hollmann's nomenclature, NR1-1 is the full-length clone containing both C-terminal exons, NR1-2 lacks exon 21, NR1-3 lacks exon 22, and NR1-4 lacks both (Fig. 5). The lower case letters a and b indicate the presence (a) or absence (b) of exon 5. These splice variants vary considerably in their properties and are differentially localized in the adult and developing animal (e.g., Laurie and Seeburg, 1994; Laurie et al., 1995; Nash et al., 1997; Paupard et al., 1997; Weiss et al., 1998). For example, recombinant NR1 receptors lacking exon 5 (N1 cassette) have a higher affinity for NMDA, are potentiated by Zn^{2+} when expressed without NR2 subunits in *Xenopus* oocytes but are more sensitive to block by Zn^{2+} and protons when expressed with NR2 subunits, and show stronger potentiation by polyamines through relief of proton inhibition (see below; Durand et al., 1993; Hollmann et al., 1993; Traynelis et al., 1995, 1998). Receptors containing N1 without C1 and C2 are more strongly enhanced by phorbol esters than those with C1 or C2 (Durand et al., 1993). Interestingly, after optic nerve crush, retinal ganglion cells down-regulated NR1 subunits and expressed preferentially receptors lacking exon 5 before cell death (Kreutz et al., 1998). However, the remaining NR1-b subunits seem to be crucial for survival because experimental reduction of NR1-b by treatment with antisense oligonucleotides increased retinal ganglion cell death after nerve crush.

The C1 cassette found in NR1-1 and NR1-3 is involved in receptor clustering, i.e., it binds to neurofilaments
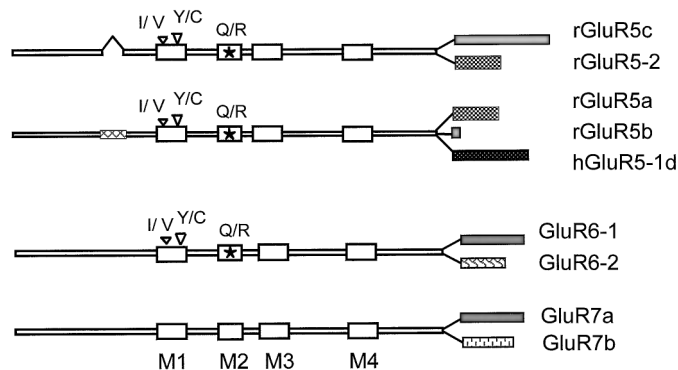

FIG. 4. Alternative splicing and editing of kainate receptor subunits. The diagram shows the basic structures of rat (prefix r) and human (prefix h) kainate receptor subunits, including the membrane-buried domains M1 to M4, the alternatively spliced cassettes, and editing sites in both M1 and M2 membrane domains.
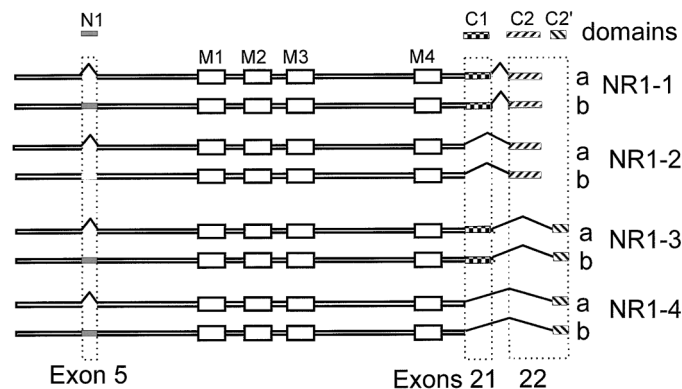

FIG. 5. Alternative splicing of NMDA receptor subunits. The different NR1 subunit splice variants arise from alternative splicing of the exons 5, 21, and 22, giving rise to the cassettes N1, C1, C2, and C2′.

and the intracellular protein yotioa (Ehlers et al., 1998; Lin et al., 1998). Furthermore, the C1 cassette contains protein kinase C (PKC) phosphorylation sites and binds to calmodulin. Clustering and interaction with these regulators can be inhibited by PKC phosphorylation in the C1 cassette (Ehlers et al., 1995). After kindling a transient reduction of C1-containing splice variants was found in rats (Kraus et al., 1996; Vezzani et al., 1995), but whether the expected functional receptor alterations contribute to the kindled state is unknown. NR1 variants with the C2″cassette (NR1-3 and -4) interact with postsynaptic density (PSD)-95 proteins (see below).

B. Editing of AMPA and Kainate Receptors

Some glutamate receptor RNAs are post-transcriptionally modified by RNA editing, which leads to single-amino acid exchanges (reviewed by Seeburg, 1996). In this process, selected adenosines are deaminated to inosines by dsRNA adenosine desaminases (Rueter et al., 1995; see below). Inosines base pair like guanosines, which changes the amino acid codon. To date, editing has not been demonstrated for any NMDA receptor RNA, but AMPA and kainate receptor RNAs are edited at multiple positions.

In the primary transcript of GluR2, GluR5, and GluR6, a glutamine codon in the M2 domain (C$\underline{A}$G) can be edited to an arginine (C$\underline{I}$G) at the Q/R site (Figs. 1, 3, and 4). The arginine in edited versions of GluR2 causes low calcium permeability (Hume et al., 1991), low single-channel conductance (Swanson et al., 1996), and an approximately linear current-voltage relation even in heteromeric receptors (Verdoorn et al., 1991; Hume et al., 1991; Egebjerg and Heinemann, 1993; Washburn et al., 1997). Editing at the Q/R site in GluR6 also controls anion permeability (Burnashev et al., 1996). Furthermore, some of the AMPA receptor subunits, GluR2-4, are edited at the R/G site, which is located just before the flip/flop exons (Fig. 3). The glycine codon (I$\underline{G}$A) replacing the genomically encoded arginine ($\underline{A}$GA) in GluR3 and GluR4 reduces and speeds up recovery from desensitization (Lomeli et al., 1994). Finally, the kainate receptor subunits GluR5 and GluR6 can be edited in M1 at the I/V and Y/C sites (Köhler et al., 1993). Nutt et al. (1994) identified two potential editing sites in human GluR7 that are not the result of adenosine deaminations, based on sequencing reverse transcription-polymerase chain reaction (RT-PCR) products of fetal and adult human brain, which revealed different codons in the amino-terminal region of GluR7: Ser310 (T$\underline{C}$C) or Ala (G$\underline{C}$C) and Arg352 (C$\underline{G}$G) or Gln (C$\underline{A}$G). Whether these changes are due to editing as originally proposed or to polymorphisms (Schiffer et al., 1997b) awaits resolution. Since in GluR6 the edited amino acids in M1 can influence the ion permeability in receptors that have a Q at the M2 Q/R site, it has been argued that M1 may influence the structure of the open channel (Köhler et al., 1993; Burnashev et al., 1995, 1996). Changing the homologous

amino acids in M1 of recombinant GluR4 had no effect on ion permeability, implying subtle differences in the channel structure between AMPA and kainate receptors. In single-cultured hippocampal neurons, editing of GluR5 and GluR6 occurred at different levels at all three sites, producing eight different species of mRNA (Ruano et al., 1995).

The enzymology of editing has received a lot of attention, with the ultimate aim of manipulating the editing process itself. Editing of glutamate receptors was initially found to depend on intronic sequences containing an editing complementary sequence, which base pairs with the exonic sequences (Higuchi et al., 1993; Egebjerg et al., 1994; Herb et al., 1996). The dsRNA structure is recognized by one of two editing enzymes, the first called DRADA or dsRAD (recently renamed ADAR1) and the second called RED1 or DRADA2 (now ADAR2) (Bass et al., 1997). Recombinant RED1 edits the GluR2 Q/R site (Melcher et al., 1996; Lai et al., 1997a; O'Connell et al., 1997), whereas the Q/R site of GluR6 can be edited by recombinant DRADA (Herb et al., 1996). Both recombinant enzymes can edit the R/G site. However, editing might not depend on the editing enzymes DRADA or RED1 alone, since some cells clearly express the mRNA for these enzymes but have no editing activity (Lai et al., 1997b). Moreover, the site selectivity and the efficiency of recombinant DRADA is changed by incubation with nuclear extracts (Dabiri et al., 1996), suggesting the contribution of additional proteins in the editing process. In addition to these deaminations that result in changes of the amino acid sequence, other adenosines are edited in introns and exons of GluR2 and GluR6, but do not change the coding sequence.

During development, editing occurs at different levels for all editing sites. The GluR2 Q/R site is the most vigorously edited site and this is even essential for survival. After E14 in rats >99% of GluR2 mRNA has arginine at the Q/R site. Removal of the editing complementary sequence in one **GRIA2** allele in mice, which reduced the efficiency of Q/R site editing by about 25%, resulted in epilepsy and early death, attesting to the importance of efficient GluR2 editing (Brusa et al., 1995). The physiological role, if any, of unedited GluR2(Q) is unclear, however, as mice engineered to contain a genomic arginine codon in the GluR2 Q/R site were phenotypically similar to normal mice (Kask et al., 1998). Also, in other species, GluR2 is nearly fully edited, with exceptions found in tissue from older humans (Nutt and Kamboj, 1994) and in fish, where the Q/R site arginine in GluR2 is genomically encoded (Kung et al., 1996). A lower extent of editing is found at the other editing sites, which gives rise to a high number of functional variants. During development, editing at the other sites increases gradually and, in the adult, only about 50% of GluR5 and 80% of GluR6 are edited at the Q/R site (reviewed by Seeburg, 1996). About 80 to 90% of GluR2-4 are edited at the R/G site, except for GluR4flip

(50%) (Lomeli et al., 1994). Kindling did not change editing of the Q/R site in GluR2, 5, or 6 (Kamphuis and Lopes da Silva, 1995), whereas transient ischemia in rats increased Q/R editing for GluR5 but reduced Q/R editing for GluR6 (Paschen et al., 1996). These results indicate that the editing processes are themselves tightly regulated.

V. Post-translational Modifications

Phosphorylation of ion channels is an important regulatory mechanism that may underlyy synaptic plasticity. The location of phosphorylation sites on glutamate receptor subunits and the functional consequences of phosphorylation have received much attention in the past 5 years. The responses of ionotropic glutamate receptors to agonists is usually potentiated after phosphorylation, but phosphorylation of NR1 can also disrupt channel clustering in transfected cells. The localization of the phosphorylation sites in glutamate receptor subunits was hotly debated for several years due partly to early uncertainties regarding receptor topology. Most of the controversies have recently been resolved and most experimentally veriified phosphorylation sites seem to be located intracellularly.

A. Phosphorylation of AMPA and Kainate Receptors

Like many other proteins, glutamate receptors are under tight control by various phosphokinases (reviewed by Roche et al., 1994; Soderling et al., 1994; Smart, 1997). Neuronal AMPA receptor activation can be potentiated by PKA (Knapp et al., 1990; Greengard et al., 1991; Wang et al., 1991; Blackstone et al., 1994), PKC (Wang et al., 1994a), calcium/calmodulin kinase II (CAMKII; McClade-McCulloh et al., 1993; Tan et al., 1994), and other unspecified kinases (e.g., Nakazawa et al., 1995). The potentiation by PKA of native AMPA receptors in cultured neurons appears to be due to an increase in channel open probability (Knapp et al., 1990) or open time (Greengard et al., 1991). Ser845 of GluR1 is a probable PKA target, because the C-terminus of the GluR1(S845A) mutant could not be phosphorylated after incubation with PKA (Roche et al., 1996). Moreover, the Western blot signal of hippocampal slices with antibodies directed againstphosphorylated Ser845 increased after forskolin treatment (Mammen et al., 1997). PKA activation appears to increase the open probability in recombinant GluR1 receptors but not in GluR1 (S845A) receptors (Banke and Traynelis, 1998). Changes in the phosphorylation state of Ser845 is specifically associated with synaptic plasticity. During chemically induced long-term depression, a decrease of phosphorylated Ser845, but not phosphorylated Ser831, was found (Lee et al., 1998). On the other hand, 15 min after a kindling stimulus phosphorylation of Ser845, but not Ser831, was specifically increased by about 25% (Wang et al., 1998). This suggests a role for the GluR1 PKA phosphorylation site in synaptic depression and enhancement. In Pur-

kinje cells, according to immunocytochemistry with antibodies directed against Ser696 of GluR2, phosphorylation is suggested to occur between the M3 and M4 segments, which is a proposed extracellular domain (Nakazawa et al., 1995). Exposure to AMPA increased immunostaining, but the kinases responsible have not been identified yet. However, several early studies of recombinant or native receptors did not find AMPA receptor phosphorylation by PKA in this region (Tan et al., 1994, Moss et al., 1993), leading to the suggestion that the association of PKA with A kinase-associated proteins (AKAPs) may in some cases be required for potentiation of AMPA receptors (Rosenmund et al., 1994).

GluR1 and GluR2/3 in the postsynaptic densities could be phosphorylated by endogenous kinases in the presence of calcium and calmodulin (Hayashi et al., 1997). Phosphorylation of AMPA receptors by CAMKII and possibly PKC was produced by electrical stimulation patterns that induced long-term potentiation (LTP). Phosphorylation by CAMKII correlated temporally with the increased AMPA receptor-mediated responses during LTP (Barria et al., 1997a). The idea that phosphorylation of AMPA receptors by CAMKII mightcontribute to synaptic plasticity is supported by the finding that mice engineered to lack the α subunit of CAMKII are devoid of LTP and short-term potentiation as well as long-term depression of synaptic transmission (reviewed by Soderling, 1996). Benke et al. (1998) used nonstationary noise analysis to find evidence for an increased single-channel conductance of synaptic AMPA receptors after LTP, but it is not known whether receptor phosphorylation is involved.

The CAMKII and most PKC phosphorylation sites were initially proposed to be located between M3 and M4, which would place them on the extracellular side of the membrane. However, later studies showed that Ser627, which was believed to be involved in the potentiation by CAMKII, is not phosphorylated (Yakel et al., 1995; Roche et al., 1996). Ser831 in the C terminus of GluR1 was recently identified as the CAMKII phosphorylation acceptor site using mutagenesis and antibodies directed againstphosphorylated peptides (Roche et al., 1996; Barria et al., 1997b; Mammen et al., 1997). This serine is also the target of PKC and is unique in GluR1, not being found in GluR2-4. In hippocampal slices, Ser831 and also the other PKA target Ser845, were found to be phosphorylated under basal conditions by immunoblotting (Mammen et al., 1997). Barria et al. (1997b) noted that Ser831 is not a consensus site for either PKC or CAMKII and is only poorly phosphorylated by CAMKII after LTP induction. The fact that Ser831 is not a good substrate might be a physiological checkpoint ensuring that only strong synaptic input would lead to phosphorylation and potentiation of postsynaptic currents.

PKA has also been shown to phosphorylate recombinant GluR6 homomeric receptors (Raymond et al.,

1993), and this phosphorylation of the GluR6 protein has been suggested to underlie an enhancement of whole-cell current responses (Raymond et al., 1993; Wang et al., 1993) similar to that observed with GluR1. These two studies have utilized site-directed mutagenesis to identify extracellularly localized serine residues (S684A and S666A) that are important for control of the PKA potentiation of GluR6, but it seems unlikely that these presumably extracellular serines themselves are phosphorylated (Basiry et al., 1999). Serines in the homologous position of GluR2 (S662 and S680) are located distant to the binding pocket in the crystal structure (Armstrong et al., 1998; red asterisks in Fig. 2C). The mechanism underlying the PKA-induced potentiation of GluR6, like that of GluR1, appears to be an increase in the open probability without any apparent change in response time course. Interestingly, calcineurin, a serine/threonine phosphatase that is colocalized with PKA, has the opposite effect, decreasing open probability (Traynelis and Wahl, 1997).

The effects of tyrosine kinases on non-NMDA glutamate receptors have received little study. Cotransfection of GluR1 and v-src resulted in phosphorylation of GluR1 (Moss et al., 1993), but this experiment could not distinguish between a direct or indirect effect of v-src. In synaptic membranes no phosphorylated tyrosines could be identified on GluR1 to GluR4, GluR6, GluR7, or KA2 (Lau and Huganir, 1995).

B. Serine/Threonine Phosphorylation of NMDA Receptors

NMDA receptors can be phosphorylated by PKA, PKC, and CAMKII, and the Ca^{2+}/calmodulin-dependent phosphatase calcineurin inhibits NMDA receptor function (Lieberman and Mody, 1994). In the brain, between 10 and 70% of NR1 and NR2 subunits seem to be phosphorylated at one or more sites by PKA or PKC. This variable proportion of phosphorylated subunits should substantially increase molecular and functional heterogeneity in the NMDA receptor family (Leonard and Hell, 1997).

PKC activation has been shown to enhance NMDA receptor function in different neuronal preparations. Activation of μ opioid receptors (Chen and Huang, 1992), the protease-activated receptor PAR1 (Gingrich et al., 1997), phosphoinositol-coupled metabotropic glutamate receptors (Aniksztejn et al., 1992), and muscarinic acetylcholine receptors (Markram and Segal, 1990; Dildy-Mayfield and Harris, 1994) all potentiated neuronal or recombinant NMDA receptors presumably via activation of PKC. Phosphorylation by PKC increases the opening probability and decreases the affinity for extracellular Mg^{2+} (Chen and Huang, 1992), but the mechanisms underlying these effects are unknown. Activation of PAR1 in hippocampal neurons by thrombin or an agonist peptide can also potentiate NMDA receptor responses in a Mg^{2+}- and voltage-dependent manner that

is reminiscent of PKC-induced relief of external Mg^{2+} blockade (Gingrich and Traynelis, 1998). Tingley et al. (1997) identified Ser890, Ser896, and Thr879 in the C1 cassette of the NR1 subunit as PKC targets by using antibodies directed against phosphorylated peptides. However, the C1 cassette does not seem to be responsible for PKC-induced potentiation, because recombinant homomeric NR1 receptors lacking the C1 cassette (NR1-2a) show even higher potentiation by PKC than receptors containing C1 (Durand et al., 1993). Some phosphorylation sites responsible for the Mg^{2+}-independent PKC-induced potentiation in NR1 subunits lacking C1 (Durand et al., 1993; Wagner and Leonard, 1996) might lie within NR1 but outside of C1, or might instead reside in the *Xenopus* XenU1 subunit (Soloviev and Barnard, 1997). However, this Mg^{2+}-independent potentiation also requires the carboxyl terminus of the NR2 subunit, suggesting it may be a property of the NMDA receptor channel region. Thus, receptors containing NR2C and NR2D are insensitive to phorbol ester-induced enhancement of function in *Xenopus* oocytes, whereas receptors containing NR2A and NR2B are potentiated (Mori et al., 1992). Alternatively, phosphorylation of sites in C1 may actually cause inhibition and the site(s) for potentiation may be on the NR2 subunit. Receptors without C1 would then be potentiated to a larger degree than receptors with C1. Additional work is needed to resolve these possibilities.

The large potentiation by PKC in C1-lacking homomeric NR1 receptors might also be explained partially by the observation that without C1 there is no receptor clustering. Indeed, PKC activation and phosphorylation of NR1 at Ser890 within the C1 cassette can inhibit clustering of NR1 (Ehlers et al., 1995; Tingley et al., 1997). This might also explain why in some studies PKC reduced NMDA receptor-mediated currents (e.g., Markram and Segal, 1992). Of great interest is the observation that calcium influx through NMDA receptors appears to amplify the potentiation by PKC, as shown by Zheng and Sigworth (1997) who compared wild-type NMDA receptors with receptors carrying NR1 mutations that reduce calcium permeability.

Little is known about regulation of NMDA receptors by CAMKII or PKA. The substrate for CAMKII phosphorylation appears to be Ser1303 in NR2B and perhaps the homologous serine in NR2A. This site was found to be phosphorylated in hippocampal neurons, but its function is so far unknown (Omkumar et al., 1996). A PKA phosphorylation site resides in the C1 cassette of NR1 (Ser879; Tingley et al., 1997), but the physiological changes in NMDA receptor function observed after PKA activation seem to be indirect. In hippocampal neurons, PKA activation by β adrenergic receptors potentiated NMDA receptor activity apparently by inhibiting the phosphatase calcineurin (Raman et al., 1996). In contrast, the phosphorylation of NR1 by PKA or PKC can antagonize its interaction with spectrin in vitro and

might have direct functional consequences (Wechsler and Teichberg, 1998).

The activation of NMDA receptors can be inhibited by the serine and threonine phosphatases 1, 2A, or 2B (calcineurin). In acutely dissociated dentate gyrus granule cells, calcineurin can be activated by calcium entry through NMDA receptors and shorten the open time of NMDA receptors (Lieberman and Mody, 1994). Phosphatases 1 and 2A reduced the opening probability of NMDA receptors in cultured hippocampal neurons (Wang et al., 1994b). By second-to-second adjustment of the activity of kinases and phosphatases, the responsiveness of NMDA receptors to stimuli can thus be fine-tuned.

C. Tyrosine Phosphorylation of NMDA Receptors

Several studies showed that activation of tyrosine kinases increases NMDA receptor-mediated responses in neurons (reviewed by Wang and Salter, 1994; Köhr and Seeburg, 1996; Gurd, 1997; Lu et al., 1998; Zheng et al., 1998). Glutamate-activated currents in HEK 293 cells transfected with NR1/NR2A could be potentiated by including Src or Fyn kinases in the patch pipette (Köhr and Seeburg, 1996). Src and Fyn kinases were unable to potentiate a receptor consisting of NR1 plus a C-terminal truncation mutant of NR2A, however, suggesting that the tyrosine phosphorylation sites might lie within the C-terminal domain of NR2A (Köhr and Seeburg, 1996). Src appears to be an endogenous kinase that regulates NMDA receptors, because 1) an anti-Src antibody applied to the cytoplamic surface of spinal dorsal horn neurons reduced the open probability of NMDA receptors; 2) application to the cytoplasmic surface of a high-affinity peptide that activates Src [EPQ(pY)EEIPIA] increased channel activity in inside-out patches whereas the unphosphorylated peptide, which does not activate Src, was ineffective; and 3) anti-Src antibodies coimmunoprecipitated NR1 from synaptic membranes (Yu et al., 1997). These results taken together suggest that Src may be a regulatory component of the subsynaptic protein complex that contains NMDA receptors.

How does Src kinase potentiate NMDA receptor activation? Zheng et al. (1998) found that Src potentiation of NR1/NR2A receptors could be prevented by a trace concentration (10 μM) of EDTA or other divalent chelators, and also showed that intracellular application of Src altered the Zn^{2+} sensitivity of the receptor, thereby reducing tonic inhibition by ambient Zn^{2+} present as a contaminant in the solution. Three C-terminal tyrosines (Y1105, Y1267, and Y1387) that are found in NR2A were required for this effect of Src. Only Y1267 is unique for NR2A; both Y1105 and Y1387 are also found in NR2B and 2C. The effect of Src was traced to a reduction in Zn^{2+} potency for high-affinity block (IC_{50} = 90 nM) of NR1/NR2A receptors. With the likely assumption that ambient Zn^{2+} concentrations in the brain are in the range of several hundred nanomolar, Zheng et al. (1998)

proposed that one of the synaptic functions of Src may be to regulate the degree of tonic inhibition of NR2A-containing NMDA receptors by extracellular Zn^{2+}. Interestingly, NR1/NR2B receptors could also be potentiated by Src, but only in the presence of higher concentrations of Zn^{2+} that approximate the low potency (IC_{50} = 3–10 μM) block of these receptors by Zn^{2+}. The low potency for Zn^{2+} inhibition of NR2B, NR2C, and NR2D (see below) may explain the failure of Köhr and Seeburg (1996) to observe potentiation of NR1 coexpressed with NR2B, NR2C, or NR2D. In oocytes with a nominally Zn^{2+}-free bath solution, not only NR2A, but also NR2B and even NR2D-heteromeric and NR1-homomeric receptors were potentiated by Src and by an endogenous kinase that was activated by insulin (Chen and Leonhard, 1996). The potentiation by Src of NR1/NR2B and NR1/NR2D was much less than that of NR1/NR2A receptors, and the potentiation by insulin may be partially due to a PKC-dependent mechanism because insulin receptors are also linked to phospholipase C.

Which NMDA receptor subunits can be phosphorylated on tyrosine residues? Moon et al. (1994) reported that the major tyrosine-phosphorylated protein in the PSD is NR2B. Tyrosines in 2 to 4% of NR2A and NR2B subunits, but not the NR1 subunit, were found to be phosphorylated in synaptic plasma membranes (Lau and Huganir, 1995). This low proportion of tyrosine-phosphorylated subunits contrasts to the high degree of NR2 subunit phosphorylation by PKA and PKC (up to 70%). The lower level of tyrosine phosphorylation of NR2A may allow for large increases in response to environmental stimuli. Indeed, an endogenous tyrosine kinase was found to increase phosphorylation of NR2A but not NR2B or NR1 in synaptic membranes by about 7-fold (Lau and Huganir, 1995). The endogenous kinases responsible for tyrosine phosphorylation of NR2B in synaptic membranes have not been fully identified, although exogenous Fyn could phosphorylate NR2A and NR2B in the PSD fraction (Suzuki and Okumara-Noji, 1995) and Src and NMDA receptors coimmunoprecipitate (Yu et al., 1997). The phosphorylation of NR2B by Fyn kinase antagonized the interaction of NR2B with spectrin in vitro (Wechsler and Teichberg, 1998), so one function for Fyn kinase may be to target NMDA receptors to the subsynaptic membrane.

Tyrosine kinase-enhanced synaptic currents through NMDA receptor channels have been proposed to play a role in LTP induction. O'Dell et al. (1991) blocked LTP induction by tyrosine kinase inhibitors in the CA1 region, and fyn knockout mice are impaired in LTP and spatial learning (Grant et al., 1992). Src activation was reported to be necessary for induction of LTP in CA1 pyramidal cells (Lu et al., 1998). Electrical stimulation that produced LTP led to Src activation; conversely, blocking Src activation by perfusion with a Src-blocking peptide, Src(40-58), inhibited induction of LTP. On the other hand, direct Src activation by another peptide

[EPQ(pY)EEIPIA] was sufficient to enhance excitatory postsynaptic potentials, raising the question of whether Src activation alone might be sufficient for LTP. The Src-induced enhancement of synaptic excitatory postsynaptic currents (EPSCs) could be blocked by NMDA receptor antagonists (Lu et al., 1998), suggesting that this effect of Src was mediated by ongoing NMDA receptor activation. Src potentiates NMDA receptor-mediated currents and, in addition, potentiates AMPA-mediated responses dependent on NMDA receptor activation and Ca^{2+} influx. Tyrosine phosphorylation of NR2B is enhanced up to 2.5-fold beginning a few minutes after LTP induction and lasting up to at least 24 h (Rosenblum et al., 1996; Rostas et al., 1996). Moreover, tyrosine phosphorylation of NR1 and NR2B but not NR2A was increased after exposure to brain-derived neurotrophic factor (Lin et al., 1998). Brain-derived neurotrophic factor can modulate LTP and enhances EPSCs in hippocampal neurons by a postsynaptic, phosphorylation-dependent mechanism. Despite these studies, however, it is not yet clear whether LTP requires tyrosine phosphorylation of either NR2A or NR2B.

From studies with genetically engineered mice, it seems that either NR2A or NR2B is necessary for LTP depending on the synapse. The impairment of LTP by reduced NR2B and NR2A expression is synapse specific for CA3 cells that express both subunits (Ito et al., 1997). In adult heteromeric $NR2B^{-/+}$ mice, LTP was reduced at the fimbria-CA3 synapse but not at the commissural-associational input. On the other hand $NR2A^{-/-}$ mice showed specifically reduced LTP at the commissural-associational-CA3 synapse but not the fimbria-CA3 synapse. Whether this effect is due to the lack of phosphorylation or reduced association with intracellular binding proteins is not known. Mice lacking the C terminus of NR2A containing the phosphorylation sites had a similar phenotype as the NR2A knockout mice, with reduced LTP in the hippocampal CA1 region and impaired contextual learning (Sprengel et al., 1998); it is therefore likely that the C terminus and possibly its phosphorylation play some role. Interestingly, phosphorylation regulates clustering and association with NMDA receptor subunits with intracellular proteins (see below).

A number of other conditions are associated with tyrosine phosphorylation of NMDA receptor subunits. Transient global ischemia in the four-vessel occlusion rat model increased NR2A tyrosine phosphorylation by up to 29-fold for at least 24 h, with a smaller increase in the NR2B subunit (Tagaki et al., 1997). Moreover, tasting an unfamiliar substance increased tyrosine phosphorylation of NR2B in the insular cortex in a dose-dependent manner by about 60%, starting within minutes and lasting up to several hours (Rosenblum et al., 1997). In contrast to LTP, NR2B tyrosine phosphorylation after experiencing a novel taste was not blocked by AP5 and so apparently does not require NMDA receptor activation.

Conversely, endogenous tyrosine phosphatases may also regulate channel opening probability, because external application of a protein tyrosine phosphatase inhibitor in inside-out patches from rat spinal neurons increased open probability (Wang et al., 1996). Thus, tyrosine kinases and phosphatases also appear to play important roles in adjusting the activation properties of NMDA receptors.

D. Glycosylation, Proteolysis, and Covalently Bound Lipids

Glycosylation influences several properties of glutamate receptors, including channel activity and modulation by lectins. AMPA receptors contain 4 to 6 N-glycosylation sites, kainate receptors between 8 and 10, and NMDA receptor subunits 6 to 12 (Everts et al., 1997). Unglycosylated S1-S2 domains of GluR2 were crystallized with bound kainate (Armstrong et al., 1998), suggesting that agonist recognition does not absolutely require glycosylation. Inhibition of glycosylation by tunicamycin reduced the apparent molecular mass of GluR1 and GluR2 by about 4 kDa and prevented [³H]AMPA binding (Kawamoto et al., 1995a). Tunicamycin also inhibited the functional expression of kainate and AMPA-evoked currents in oocytes injected with rat brain total RNA (Musshoff et al., 1992). In contrast, in HEK 293 cells, tunicamycin reduced the maximal amplitude of kainate-induced currents mediated by recombinant GluR1flop only by about 50% (Hollmann et al., 1994). A subsequent study showed that flip and flop variants of GluR1 to GluR4 are differentially affected by tunicamycin treatment. The kainate- and glutamate-induced currents of the flop forms are decreased by tunicamycin treatment (except for GluR4flop), whereas currents through the flip variants are increased (with the exception of GluR3flip) (Everts et al., 1997). Tunicamycin occluded the potentiating effect of the lectin concanavalin A on all AMPA and kainate receptor combinations tested (Everts et al., 1997), in accord with the idea that the lectin-binding sites on AMPA receptors involve the attached sugars.

Glycosylation of the NMDA receptors is even more extensive, e.g., about 20 kDa of the 120-kDa NR1 and the 180-kDa NR2B protein, and about 10 kDa of the other NR2 subunits consists of sugar moieties (Kawamoto et al., 1995b; Laurie et al., 1997). Glycosylation is necessary for the binding of the NMDA receptor antagonist [³H]dichlorokynurenate (Kawamoto et al., 1995b) and channel function. Tunicamycin nearly abolished glutamate-evoked currents in NR2A- or NR2B-containing receptors, as well as in homomeric NR1, heteromeric NR1/NR2C, and NR1/NR2D receptors (Everts et al., 1997). Tunicamycin did not reduce the total number of binding sites for NR1/NR2A receptors expressed in HEK 293 cells but did interfere with receptor function as judged by [³H]MK801 binding and excitotoxicity assays (Chazot et al., 1995). These findings suggest that

glycosylation may not be necessary for subunit assembly per se but instead may instead be required for receptor function.

Recombinant GluR6 subunits could be palmitoylated in insect cells and HEK 293 cells (Pickering et al., 1995). Mutation of cysteines 827 and 840 prevented palmitoylation and reduced PKC-induced phosphorylation. The GluR1 C terminus as well as the NR1 and NR2 C termini might be targets for the calcium-dependent protease calpain (Bi et al., 1997, 1998). Calcium treatment of synaptic membranes or brain sections gave rise to a protein fragment recognized by antibodies directed against the GluR1 N terminus in Western blots. In contrast, the signal intensity obtained with antibodies directed against the GluR1 C terminus decreased. These effects were blocked by calpain inhibitors (Bi et al., 1997). Similar results were obtained with antibodies against the NR1 and NR2 C termini. Kainate treatment of cultured hippocampal slices seemed to induce calpain-mediated proteolysis of NR2 subunits, after which the antibodies also recognized 60-kDa and 52-kDa fragments, presumably from NR2 (Bi et al., 1998). Calpain-mediated proteolysis of GluR1 was observed in organotypic hippocampal slices after NMDA or glycine exposure, which induced global LTP (Gellerman et al., 1997; Musleh et al., 1997). The proteolysis product of GluRs seem to be rare or short-lived, since lower molecular mas species have not been described elsewhere to our knowledge. The serine protease thrombin also appears to cleave native and recombinant NR1 subunits, which may have relevance for pathological conditions in which significant amounts of thrombin enter the brain parenchyma (Butler and Traynelis, 1996).

VI. Receptor Activation and Desensitization

A. Agonists

Both NMDA and non-NMDA receptors are activated by the endogenous transmitter, L-glutamate, whereas the putative transmitter candidate, L-aspartate, appears to activate NMDA receptors exclusively (Patneau and Mayer, 1990); note, however, that aspartate can also activate receptors of unknown composition (Yuzaki et al., 1996). Glycine, which was first reported to potentiate NMDA receptor activation at submicromolar levels (Johnson and Ascher, 1987), was later shown to be an essential coagonist at NMDA receptors (Kleckner and Dingledine, 1988). Early reports that some NMDA receptors can be activated by glycine alone (Meguro et al., 1992; Kutsuwada et al., 1992) have not been confirmed or rebutted. One wonders whether coassembly of NR1 with the endogenous (to *Xenopus*) XenU1 subunit may help explain this result. As described above, the glycine-binding site appears to be located on the NR1 subunit, whereas the glutamate-binding pocket is on the NR2 subunit. The structural requirements of NMDA receptor agonists at both glutamate and glycine recognition sites

have been adequately covered in previous reviews (McBain and Mayer, 1994; Sucher et al., 1996).

The original classification of AMPA and kainate subtypes received support from work with recombinant receptors, which provided evidence that these agonists were selective for two different glutamate receptor subclasses. However, some members of each class of receptor can be activated by both agonists. For example, homomeric and heteromeric AMPA receptors can be activated by kainate (Boutler et al., 1990a), and certain heteromeric kainate receptors comprised of either GluR5, GluR6, or GluR7 plus either KA1 or KA2 can be activated by AMPA (Herb et al., 1992; Swanson et al., 1996; Schiffer et al., 1997a). More recent efforts have been directed at identifying selective agonists of AMPA and kainate receptors. AMPA receptor agonists are found in two major chemical classes, based on the structures of AMPA itself, or of willardiine (Gill, 1994; Fletcher and Lodge, 1996; Borges and Dingledine, 1998). Many analogs of AMPA have been synthesized that exhibit potent agonist properties, among them the carboxy derivative (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA) (Wahl et al., 1996) and a phenyl derivative, (*S*)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid ((*S*)APPA) (Ebert et al., 1994). In the willardiine series, 5-fluorowillardiine activates native AMPA receptors in hippocampus with a 46-fold higher potency than needed to activate native kainate receptors in dorsal root ganglia neurons (Wong et al., 1994). In addition, certain human neurotoxins—domoic acid from dinoflagellates, β-N-methylamino-L-alanine from cycad seeds—are also powerful AMPA receptor agonists, although domoate is also a potent kainate receptor agonist at GluR5 and GluR6 but not GluR7 receptors (Schiffer et al., 1997a).

No agonists have yet been identified that exhibit pronounced selectivity for particular AMPA receptor subunit combinations. Kainate receptors, however, are more heterogeneous in their responses to agonists. (2*S*-4*R*)-4-methylglutamate (SYM 2081) shows strong selectivity for kainate receptors (100–1000-fold compared to AMPA receptors) (Brauner-Osborne et al., 1997; Wilding and Huettner, 1997; Donevan et al., 1998). Some willardiine analogs (e.g., (*S*)-5-iodowillardiine) also show strong selectivity for kainate over AMPA receptors (Wong et al., 1994; Jane et al., 1997; Swanson et al., 1998), as does the trifluoro-kainate analog DZKA (Wils et al., 1997). DZKA has been used as a photoaffinity label of the high-affinity kainate-binding region of kainate receptors (Willis et al., 1997). Interestingly, both the *tert*-butyl AMPA derivative ATPA and 5-iodowillardiine specifically activate homomeric GluR5 receptors, the latter with no detectable affinity for homomeric GluR6 or GluR7 receptors (Clarke et al., 1997; Swanson et al., 1998). This difference in agonist sensitivity was traced to a single amino acid, N721, in GluR6 and GluR7, and S721 in GluR5 (Swanson et al., 1998). N721

is adjacent to one of the conserved disulfide-bonded cysteines in glutamate receptors and lies distant to the actual binding pocket (red asterisk in Fig. 2C). This asparagine may thus serve an allosteric role in positioning or shaping the binding pocket. This same residue was previously shown to be responsible for the small responses of homomeric GluR6 receptors to AMPA (Swanson et al., 1997a; Table 2). Although homomeric GluR6 receptors were insensitive to 5-iodowillardiine, coexpression of KA-2 and GluR6 subunits resulted in a kainate receptor that was weakly activated by this agonist (Swanson et al., 1998). The phosphono-isoxazole AMPA analog, ATPO, is another drug that has weak agonist activity at GluR5 but not GluR6 receptors (Wahl et al., 1998).

B. Competitive Antagonists: New Developments

The classical competitive antagonists of the glutamate site on NMDA receptors are phosphono derivatives of short-chain (five to seven carbons) amino acids such as AP5 and AP7, whereas halogenated quinoxalinediones and kynurenic acid derivatives were the first competitive glycine site antagonists to be identified (summarized in Priestley et al., 1995). More recently, certain phthalazinedione derivatives (Parsons et al., 1997) and benzazepinedione derivatives (Guzikowski et al., 1996) were found to be highly potent, selective, and systemically active glycine site antagonists. Although the glycine-binding site is located on the NR1 subunit, the affinity for glycine and other glycine agonists depends on which NR2 subunits are present, being about 10-fold lower for receptors containing the NR2A subunit than other NR2 subunits (Kutsuwada et al., 1992; Buller et al., 1994; Priestley et al., 1995). Honer et al. (1998) described a novel glycine site antagonist that photoaffinity labels the NR1 subunit but is more potent at NR2B-containing receptors than any other NR2 subunit. This compound, a dichloro-tetrahydroquinoline-2-carboxylic acid derivative (CGP 61594), may be useful for more precise identification of residues near the glycine-binding pocket and may also lead to drugs targeted to the NR1/NR2B subtype of NMDA receptors.

The first generation competitive blockers of non-NMDA receptors, the quinoxalines and quinoxalinediones, showed poor selectivity between AMPA and kainate receptors. Over the past 5 years much effort has being expended to develop more selective competitive blockers of AMPA and kainate receptors. Two of the earliest of these are tetrazole-substituted decahydroisoquinolines, LY293558 and LY294486, which block homomeric GluR5 kainate receptors at 1 to 10 μM but are inactive on GluR6 receptors (Bleakman et al., 1996a,b; Clarke et al., 1997). In binding assays, LY294486 showed 10- to 100-fold selectivity for GluR5 over the AMPA receptor subunits (Clarke et al., 1997). Wahl et al. (1998) showed that a phosphono analog of AMPA, ATPO, is a competitive inhibitor of recombinant AMPA receptors (Schild

K_b = 8 μM against GluR1 receptors), but had no effect on homomeric GluR6 or GluR6/KA2 receptors. As mentioned above, however, ATPO was a weak partial agonist at GluR5 and GluR5/KA2 receptors. Comparison of the profile of ATPO and its close structural analogs (e.g., ATPA) may point the way to the development of more potent and selective competitive AMPA receptor antagonists.

C. Noncompetitive Antagonists

Several classes of antagonist block NMDA receptors in a voltage-independent manner without causing significant reduction in agonist potency. Among these, ifenprodil and its analogs have received the most attention, and the mechanism of action of this phenylethanolamine has been eagerly sought. High-affinity block of the NMDA receptor by ifenprodil requires N-terminal residues on the NR2B subunit (Williams, 1993; Gallagher et al., 1996). Legendre and Westbrook (1991) first concluded from single-channel measurements that ifenprodil promotes transitions to a nonconducting state of the channel. More recent kinetic experiments of Kemp and colleagues (Kew et al., 1996, 1998; Fischer et al., 1997) have extended this conclusion by showing that ifenprodil stabilizes an agonist-bound state of the receptor that has low open probability. Mott et al. (1998) tied these observations together with the known allosteric block of NMDA receptors by protons (see below), and showed that ifenprodil increases the potency of ambient protons to block the NMDA receptor. By shifting the pKa for proton block of NMDA receptors to more alkaline values, ifenprodil binding causes a larger fraction of receptors to be protonated at physiological pH and, thus, inhibited. Mott et al. (1998) propose that the "low open probability" form of the receptor identified by Kew et al. (1996) is the protonated state.

Ifenprodil is neuroprotective in animal models of focal cerebral ischemia (Gotti et al., 1988). Unfortunately, ifenprodil and several of its analogs, including eliprodil and haloperidol (Lynch and Gallagher, 1996; Brimecombe et al., 1997), block certain serotonin receptors and calcium channels in addition to NMDA receptors, limiting their clinical usefulness. Several more selective derivatives of ifenprodil are being considered for clinical development, including CP101,606 (Mennitti et al., 1997), Ro 25-6981 (Fischer et al., 1997), and Ro 8-4304 (Kew et al., 1998). One indication that these compounds may be considered for is cerebral ischemia. An interesting feature of ischemic tissue is that the pH falls, sometimes to as low as 6.5 (Silver and Erecinska, 1992). Low pH increases the potency of some but not all phenylethanolamines, as determined by electrophysiological assays on recombinant receptors and neuroprotection assays of NMDA-induced toxicity in primary cortical cultures (Pahk and Williams, 1997; Mott et al., 1998). This novel mechanism of action of a potentially therapeutically useful class of compounds highlights the util-

ity of understanding, in detail, the mechanisms underlying allosteric modulation of NMDA receptor function. The mechanism of action of ifenprodil on the proton sensor suggests an approach to optimize the design of these compounds as neuroprotectants: one could search for phenylethanolamines that are inactive at physiological pH but have ifenprodil's sensitivity boost at ischemic pHs.

At intoxicating concentrations, ethanol is another noncompetitive NMDA receptor antagonist (Peoples and Weight, 1997 and references therein); trichloroethanol, the active metabolite of the sedative hypnotic chloral hydrate, also blocks NMDA receptors noncompetitively at anesthetic concentrations (Peoples and Weight, 1998). Block by ethanol appeared more potent with the NR2A or NR2B than the NR2C or NR2D subunits (Masood et al., 1994; Chu et al., 1995), but the degree of block was not affected by pH, Zn^{2+}, or the redox state of the receptor, suggesting that ethanol might act at a novel site on the NMDA receptor (Chu et al., 1995; Peoples and Weight, 1997).

Positively charged peptides can have multiple effects on NMDA receptors, best illustrated by the effects of the dynorphin peptides. In low extracellular glycine concentration (<100 nM), NMDA receptor currents are potentiated by dynorphin peptides that contain glycine residues (Zhang et al., 1997), perhaps as a result of proteolytic release of glycine from the parent peptide by the tissue. Dynorphin A(1-13) can also inhibit NMDA receptor activation in a voltage-independent manner that is noncompetitive with either NMDA or glycine (Chen et al., 1995a,b). DynA(1-13) potency is dependent on the NR2 subunit, being greatest for NR2A (Brauneis et al., 1996); potency also increases with increasing chain length, with DynA(1-32) being the most potent (Chen and Huang, 1998). Block by DynA(1-17) is unaffected by changes in pH or in the presence of Zn^{2+}, but is substantially weakened by dithiothreitol (Chen et al., 1995b). These results suggest that the dynorphin-binding site may involve the reduced form of the NMDA receptor, but no additional information is available regarding the mechanism of block. Highly basic toxins from the Conus marine snails are also potent NMDA receptors antagonists that appear to act at polyamine-sensitive sites in a noncompetitive fashion (Zhou et al., 1996). No detailed information is available regarding their mechanism.

No noncompetitive antagonists of the kainate receptors have yet been reported, but an important group of AMPA receptor antagonists are represented by the 2,3-benzodiazepines. These compounds, unlike the 1,4-benzodiazepines, have no affinity for the γ-aminobutyric acid (GABA)$_A$ receptor but block native and recombinant AMPA receptors in a noncompetitive manner (Donevan and Rogawski, 1993; Wilding and Huettner, 1995; Bleakman et al., 1996). The (−)-stereroisomer of GYKI 53655 (also known as LY300168) is the most potent of

these compounds, with an $IC_{50} < 1 \mu M$ on AMPA receptors expressed by rat cerebellar Purkinje neurons (Bleakman et al., 1996). GYKI 53655 has very low affinity for kainate receptors (Wilding and Huettner, 1995; Bleakman et al., 1996). AMPA receptors with a serine-to-glutamine mutation in the S/N site of the flip/flop exon were insensitive to cyclothiazide but normally sensitive to GYKI 53655 (Partin and Mayer, 1996); morover, the potency of these compounds was not affected by the flip/flop splice variant present (Johansen et al., 1995; Partin and Mayer, 1996). Both of these observations imply that the 2,3-benzodiazepine-blocking site is different from the site responsible for allosteric potentiation by cyclothiazide. As expected, GYKI 53665 noncompetitively protected cultured rat forebrain neurons against toxicity induced by exposure to AMPA (Kovacs and Szabo, 1997).

D. Uncompetitive Blockers

An uncompetitive blocker acts only on the activated receptor, not the receptor at rest. In addition to external Mg^{2+} ions and cytoplasmic polyamines (see below), a variety of other compounds are known to enter and block open glutamate receptor channels. A general feature of these blockers is that their binding site is made available once the channel is in the open state. Thus, the rate of the onset of block is use-dependent and is accelerated by increases in open channel probability. Once bound, however, the blocker can be trapped by channel closure. Recovery from the trapped blocked state is generally slow.

This trapping block mechanism has been exploited for NMDA receptors to estimate the open probability and the time required for the first opening of NMDA channels under experimental conditions typical of central synapses (Jahr, 1992; Dzubay and Jahr, 1996). It has been speculated that use-dependent blockers may be neuroprotective against acute and chronic neurological insults, such as stroke or epilepsy, by limiting the neurotoxic damage of excessive Ca^{2+} entry into cells via NMDA receptors (Lipton, 1993). Early hopes for open-channel blockers such as phencyclidine (MacDonald et al., 1991; Lerma et al., 1991) or MK-801 (Huettner and Bean, 1988; MacDonald et al., 1991; Jahr, 1992; Dzubay and Jahr, 1996) were disappointed by the appearance of neuropsychiatric and pathological side effects (Lipton, 1993). More clinically tolerable blockers such as the dissociative anesthetic, ketamine (MacDonald et al., 1991), dextromethorphan, and the related compound, dextrorphan (Netzer et al., 1993), the des-glycine metabolite of remacemide (Subramaniam et al., 1996), or amino-adamantane derivatives, such as memantine and amantadine (Bormann, 1989; Kornhuber et al., 1989; Chen and Lipton, 1997; Blanpied et al., 1997; Sobolevsky and Koshelev, 1998), may prove to be more useful combatants against neurotoxicity. The apparently favorable outlook for the use of memantine in the treatment

of neurological diseases may reflect its partial trapping in closed NMDA channels (Blanpied et al., 1997). Block produced by MK-801 or phencyclidine is difficult to reverse. In contrast, partial trapping and release of memantine from NMDA channels favor the occurrence of strong block only during sustained receptor stimulation, which might occur during brain trauma; more limited effects are expected on normal synaptic transmission (Blanpied et al., 1997). The molecular mechanisms that govern whether a blocker will be trapped, partially trapped, or escape before channel closure are not well understood. However, as suggested from a study of adamantane derivatives, only blockers that have a binding site sufficiently deep in the pore may be trapped following channel closure (Antonov and Johnson, 1996). Similarly, an understanding of the conformational transitions that NMDA receptors undergo may also account for the inability of glutamate or glycine to dissociate from their binding sites when open channels are blocked by 9-aminoacridine (Benveniste and Mayer, 1995).

For non-NMDA receptors, several polyamine amide toxins from arthropod venom that were originally identified using invertebrate preparations (Jackson and Usherwood, 1988; Jackson and Parks, 1989; Usherwood and Blagbrough, 1991) have been shown to also block open vertebrate non-NMDA receptors at nanomolar concentrations (reviewed in Bowie et al., 1999). The most frequently studied toxins or synthetic analogs have been argiotoxin (Herlitze et al., 1993; Brackley et al., 1993) and Joro spider toxin (Iino et al., 1996) from orb-web and Joro spiders respectively, or philanthotoxin (Bähring et al., 1997; Bähring and Mayer, 1998; Brackley et al., 1990, 1993) from the digger wasp *Philanthus triangulum*. Similar to cytoplasmic polyamine block, the affinity of externally applied toxins is dependent on editing at the Q/R site (Herlitze et al., 1993; Blaschke et al., 1993; Brackley et al., 1993; Washburn and Dingledine, 1996), which has also been shown to be true of adamantane derivatives (Magazanik et al., 1997). As a result, polyamine amide toxins have proved to be useful pharmacological tools in determining the subunit composition of native non-NMDA receptors in the central nervous system (CNS; Iino et al., 1996; Haverkampf et al., 1997; Tóth and McBain, 1998; Washburn et al., 1997).

E. Antagonists with Unknown Mechanism

Nitrous oxide (laughing gas) at anesthetic concentrations was recently shown to be an antagonist at NMDA receptors (Jevtovic-Todorovic et al., 1998). A half-maximal reduction of NMDA currents in voltage-clamped hippocampal neurons was produced by 40 vol% N_2O, comparable to the anesthetic concentration of 50 to 70%. Likewise, the IC_{50} for prevention of the death of arcuate neurons in adult rats injected s.c. with 100 mg/kg NMDA was 55 vol% N_2O. N_2O had no effect on AMPA receptor responses. The N_2O antagonism of NMDA receptors appeared to be mainly noncompetitive in nature,

but no additional mechanistic information is available. Interestingly, another volatile anesthetic, halothane, was shown to potentiate GluR6 responses in *Xenopus* oocytes (Dildy-Mayfield et al., 1996), an action that was traced to the M4 transmembrane domain and largely to a single amino acid (G819) in GluR6 (Minami et al., 1998).

Certain agonists at metabotropic glutamate receptors can also act as antagonists of NMDA receptors (Contractor et al., 1998). The phenylglycine derivative *d*-methyl-4-carboxyphenylglycine (MCPG), at a concentration often used to block metabotropic glutamate receptors (500 μM), reduced the current responses induced in hippocampal neurons perfused with high NMDA concentrations by 63% when the glycine concentration was very low, but had no effect when glycine was also saturating. In binding studies, however, MCPG was unable to displace radioligands that label either the glutamate- or glycine- binding site, leaving its mechanism of action unclear. A number of other drugs with actions on metabotropic glutamate receptors (e.g., (S)-3,5-dihydroxyphenylglycine (DHPG), *trans*-azetidine-2,4-dicarboxylate (*trans*-ADA)) had agonist activity at NMDA receptors, a finding that could be completely explained by the measured level of contamination of the commercial drug preparations with glycine and/or glutamate. This observation raises a cautionary note about interpretation of the effects of mGluR compounds on long-term potentiation (Bashir and Collingridge, 1994; Breakwell et al., 1998) and excitotoxicity (Nicoletti et al., 1996).

Endogenous sulfated steroids can modulate NMDA receptor activation (Table 3), and Weaver et al. (1997) took advantage of this observation to identify a synthetic steroid that blocks NMDA receptors in cultured hippocampal neurons with an apparently mixed competitive-noncompetitive mechanism. This steroid also appeared to have neuroprotective, anticonvulsant, and analgesic effects against chronic pain.

F. Glutamate Receptor Kinetics

The kinetic properties that underlie the time course of ligand-gated channel responses are important to understand since they provide clues as to the mechanisms governing the temporal aspects of fast synaptic transmission. A considerable amount of work on native receptor kinetics has allowed the elucidation of several features of glutamate receptor signaling that contribute to the time course of the synaptic current (reviewed by Jonas and Spruston, 1994; Edmonds et al., 1995; Jones and Westbrook, 1996; Trussell and Otis, 1996; Ozawa et al., 1998). Here, we will consider recent studies on the activation and desensitization of recombinant receptors, as well as the emergence of structural ideas about desensitization and deactivation. Both recombinant AMPA and kainate receptors are rapidly activated by high concentrations of glutamate with a high probability of opening (see Table 4). AMPA receptor activation apparently

TABLE 3
Endogenous extracellular voltage-independent modulation of NMDA receptor function

Modulator	Effect	EC_{50}		Maximal Effect[a]
Dynorphin	Inhibition	0.3 μM[b]	100%	Chen et al., 1995 a, b; Brauneis et al., 1996; Zhang et al., 1997; Chen and Huang, 1998
Osmotic pressure	Inhibition		75%	Paoletti and Ascher, 1994
Oxidizing agents	Inhibition		70%[c]	Aizenman et al., 1989, 1990, 1992; Tang and Aizenman, 1993 a,b
Protons	Inhibition	50–200 nM[d]	100%	reviewed by Traynelis, 1998
Sulfated steroids	Inhibition	150 μM[e]	100%	Park-Chung et al., 1994, 1997
Zinc	Inhibition	0.2–2 μM[g]	100–80%	Smart et al., 1994; Williams, 1996; Chen et al., 1997; Paoletti et al., 1997; Traynelis et al., 1998
Arachidonic acid	Potentiation	10 μM	2-fold	Miller et al., 1992; Petrou et al., 1993; Horimoto et al., 1996; Mishikawa et al., 1994; Tabuchi et al., 1997
PACAP[g]	Potentiation	<1 μM	3-fold	Liu and Madsen, 1997; Wu and Dunn, 1997
Polyamines, histamine	Potentiation	100 μM[h]	2-fold	Johnson, 1996; Williams, 1997a,b
Reducing agents	Potentiation		3-fold	Aizenman et al., 1989, 1990, 1992; Tang and Aizenman, 1993 a,b
Sulfated steroids	Potentiation	12 μM[i]	2.5-fold	Park-Chung et al., 1997

[a] Other exogenous modulators have been described: ethanol (Peoples and Weight, 1995, 1998; Masood et al., 1994; Peoples et al., 1997); ifenprodil (Legendre and Westbrook, 1991; Kew et al., 1996; Mott et al., 1998), and conanotokins (Zhou et al., 1996). Maximal inhibition (100% is full inhibition) or x-fold potentiation.
[b] Dynorphin A 1-32.
[c] The response of fully reduced receptors (e.g., dithiothreitol treated) is decreased by 70% by oxidizing agents.
[d] Proton inhibition depends on subunit composition.
[e] 3β-hydroxy-5β-pregnan-20-one sulfate.
[f] NR2A-containing receptors are much more sensitive to extracellular Zn^{2+} than receptors containing other subunits; NR2C and NR2D are much less sensitive to Zn^{2+}.
[g] Pituitary adenylyl cyclase-activating peptide.
[h] Spermine.
[i] Pregnanalone sulfate.

requires binding of two agonists (Clements et al., 1998), whereas two glutamate and two glycine molecules appear to be required for maximum activation of an NMDA receptor (Benveniste and Mayer, 1991; Clements and Westbrook, 1991). An interesting study by Liu et al. (1998) of cyclic nucleotide-gated channels with a constrained number of ligand-binding sites argues that four agonists may be needed even though the kinetic data suggest two functional sites. Although some kainate receptor (e.g., GluR6) dose-response curves have a Hill slope of unity, cooperativity at low agonist concentrations has been suggested (Heckmann et al., 1996; Schiffer et al., 1997a). Both kainate and AMPA receptors possess low-affinity binding sites for the endogenous transmitter glutamate, and thus are thought to deactivate quickly because of the brief mean boundtime of the agonist (Table 4). Rise times of these receptor responses recorded in excised patches approach the resolution of piezo-electric liquid filament exchange systems (0.2–0.4 ms), and agonist-binding rates may approach the diffusion limit.

Gating of AMPA and kainate receptors by glutamate is extremely fast in contrast to the slow gating of NMDA receptors. The EC_{50} values for the peak response to rapid glutamate application are similar for some AMPA and kainate receptors, although GluR7 receptors exhibit unusually low affinity (Table 4). Glutamate activates NMDA receptors with much higher potency (~1 μM), and the EC_{50} is controlled by subunit expression and splicing (see Table 4 and McBain and Mayer, 1994). The different affinity of NMDA and AMPA receptors for glutamate has an important functional consequence. Since AMPA and NMDA receptors are, in many cases, colocalized at central synapses, the rapid activation and brief open time of AMPA facilitates unblock of NMDA recep-

tors by Mg^{2+} and therefore participation of the more slowly activating NMDA receptors in synaptic currents.

All AMPA and kainate receptors desensitize rapidly and profoundly in the continued presence of glutamate, and understanding the biophysical and molecular nature of this process is central to understanding the function of these receptors in synaptic transmission as well as the effect of postsynaptic glutamate receptor function on information processing (Jones and Westbrook, 1996; Trussell and Otis, 1996). For AMPA and kainate receptors, the relative contribution of the deactivation and desensitization rates to the time course of the synaptic current is determined by the time course of glutamate in the synaptic cleft (Jonas and Spruston, 1994; Clements, 1996; Westbrook, 1996). If glutamate remains in the cleft for a very brief duration (i.e., approximately 1 ms or less), the deactivation kinetics of the postsynaptic glutamate receptors will dominate the time course of the synaptic current. However, receptor desensitization will limit the duration of excitatory postsynaptic currents when the synaptic glutamate concentration remains elevated for prolonged periods, e.g., as transmitter spills over to adjacent synapses during high frequency stimulation. AMPA receptors recover from desensitization with time constants that are approximately 10-fold faster than kainate receptors (Table 4), which is the one of the main kinetic distinctions between AMPA and kainate receptors activated by the endogenous neurotransmitter glutamate.

Many compounds have been identified that can interact with AMPA receptors (aniracetam, diazoxide, cyclothiazide, PEPA, thiocyanate) and kainate receptors (concanavalin A) to reduce desensitization (Vyklicky et al., 1991; Yamada and Rothman, 1992; Bowie and Smart, 1993; Yamada and Tang, 1993; Partin et al.1993,

TABLE 4
Kinetic parameters describing glutamate activation of AMPA, kainate, and NMDA receptors

	EC_{50}[a]	Popen[b]	τ-Deactivate[c]	Open Times[d]	τ-Desensitize	τ-Recovery	SS/Peak Ratio[e]
	μM		ms	ms	ms	ms	
GluR1flip	500	0.4–1.0	0.8–1.1		2.5–4.1	147	0.014
GluR1flop			0.8–1.1		3.2–3.7	147	0.007
GluR3flip					4.8	15–36[f]	0.024
GluR3flop					1.4		
GluR4flip	560		0.6	0.14, 3.3	3.6	6–14[f]	0.006, 0.040
GluR4flop			0.6		0.9	31–43[f]	0.003
GluR1flip/GluR2[g]					3.4–5.1	28–67[f]	0.009
GluR1flop/GluR2[g]					2.8–8.1		0.004
GluR3flip/GluR2[g]					2.9–4.9	15–26	0.015–0.022
GluR4flip/GluR2[g]				0.5, 1.3	3.7–6.1		
GluR4flop/GluR2[g]					0.8–1.1		
GluR5Q	630			0.3, 0.6	4.1–9, 68.6	50, 5000	0.01
GluR6Q	500	0.5–1.0	2.5	0.6, 2.3	4.3–4.9	1900–2400	0.04–0.009
GluR7a	5900				8.4–9[h]		0.04
GluR5Q/KA2				0.3	1.4	3000	
GluR6Q/KA2				0.4, 2.1	2.3		
GluR7a/KA1					7.6		0.03
GluR7a/KA2					5.3		0.03
NR1-1a/NR2A	1.8	0.36	33–70, 247–350[i]	0.06, 1.0, 3.6	649–750	300–618, 1200	0.28
NR1-1a/NR2B	0.9		71, 538	0.6, 2, 8		1014	
NR1-1a/NR2C	1.0		260–376[j]	0.6			
NR1-1a/NR2D	0.4	0.04	45, 4408	0.10, 0.9, 2.6	NA	NA	NA

NA, not applicable; NR1/NR2D receptors show no apparent desensitization in the continued presence of agonist. AMPA receptor data from Lomeli et al. (1994), Mosbacher et al. (1994), Partin et al. (1996), Swanson et al. (1997b), Wahl et al. (1997), and Banke and Traynelis, (1998). Kainate receptor data from Sommer et al. (1992), Heckmann et al. (1996), Swanson et al. (1996, 1997a), Schiffer et al. (1997a), and Traynelis and Wahl (1997). NMDA receptor data from Ikeda et al. (1992), Monyer et al. (1992), Stern et al. (1992), Ishii et al. (1993), Varney et al. (1996), Krupp et al. (1998), Vicini et al. (1998), Villarroel et al. (1998), and Wyllie et al. (1998).

[a] EC_{50} values were determined for the peak response to rapid application of glutamate.

[b] Popen was determined for AMPA and kainate receptors using non-stationary variance analysis and depends on the phosphorylation state, with phosphatases like calcineurin favoring low Popen and kinases such as PKA favoring high Popen (Traynelis and Wahl, 1997; Banke and Traynelis, 1998). NMDA receptor Popen was determined from individual activations of single channels (Wyllie et al., 1998).

[c] Deactivation time constants were measured in excised membrane patches in response to a 1-ms pulse of agonist.

[d] Individual open times are shown even for receptors for which single activations clearly occur in bursts (e.g., NMDA receptors).

[e] The ratio of the steady state to peak current was determined during prolonged application of glutamate. Editing of R to G at the R/G site increases the steady-state current for GluR4flop.

[f] See Lomeli et al. (1994) for control of the recovery from desensitization to 1-ms pulses by editing at the R/G site.

[g] See Mosbacher et al. (1994) for control of desensitization kinetics by GluR2 flip and flop.

[h] GluR7a and GluR7b possessed similar desensitization time courses.

[i] Similar decay times were found with NR1 splice variants.

[j] Deactivation was measured from the relaxation current following rapid removal of glutamate and glycine.

1996; Sekiguchi et al., 1997). Although the exact mechanisms of these drugs are still under investigation, the subunit and splice variant-specific manner of their actions (Partin et al., 1994; Johansen et al., 1995; Schiffer et al., 1997a) have helped to move the concept of receptor desensitization onto firmer molecular footing (see below).

In contrast to AMPA and kainate receptors, NMDA receptors activate slowly with a τ(rise) of 10 to 50 ms (Wyllie et al., 1998) and deactivate with a much slower time course (see Table 4). Slow deactivation could reflect entry into several closed states that precede receptor activation (reviewed by Benveniste and Mayer, 1991; Lester and Jahr, 1992; Edmonds et al., 1995). Kinetic analysis of receptor activation time course suggests that the binding of four agonist molecules (two glutamate and two glycine) is required for receptor activation (Clements and Westbrook, 1991; see discussion above). The probability that agonist-bound receptors will open has been estimated indirectly using slowly reversible open channel blockers and also from single-channel measurements to range between 0.04 and 0.3 (Dzubay and Jahr, 1996; Table 4). The deactivation time course of these receptors is much longer than the time course of

glutamate in most synaptic clefts (Lester et al., 1990; Clements, 1996), and thus will dictate the duration of the synaptic current. Because NMDA receptors have high affinity for glutamate, the peak synaptic glutamate concentrations are thought always to be high enough to fully activate these receptors. Although NMDA receptors desensitize in the continued presence of agonist, this desensitization is slow and complex, reflecting a variety of different processes that involve extracellular glycine, intracellular Ca^{2+} and certain intracellular proteins (see below). In summary, the kinetic properties of both NMDA and AMPA receptors seem well designed to serve their respective roles in synaptic transmission, where AMPA receptors supply a rapid depolarization in response to neurotransmitter release, which in turn can induce partial relief of the Mg^{2+} blockade of more slowly activating NMDA receptors.

G. Molecular Determinants of AMPA Receptor Deactivation and Desensitization

The rate of AMPA receptor desensitization is controlled by both subunit composition and the RNA splicing of the AMPA receptor flip/flop region (Mosbacher et al., 1994; see Table 4). The splice variants of GluR3flop

and GluR4flop desensitize 3 to 5 times faster than other homomeric receptors, and incorporation of GluR2flop also speeds up desensitization of flip variants. In addition, editing of the R/G site upstream of the flip/flop region in AMPA receptors (see above) can speed the recovery from desensitization about 2-fold, depending on the subunit composition (Lomeli et al., 1994). Partin et al. (1994) have also showed that flip/flop region determines steady-state desensitization for GluR1, which might reflect differences in their recovery rates from desensitization.

The allosteric transitions that lead to desensitization are favored by particular agonist structures; for example, AMPA but not kainate causes rapid desensitization of AMPA receptors. The structural determinants of a variety of compounds that relieve desensitization are also becoming elucidated. Both cyclothiazide and aniracetam have more pronounced effects on flip splice variants; cyclothiazide relieves desensitization almost completely on flip receptors, but only slows entry into desensitized states of flop splice variants (Partin et al., 1994; Johansen et al., 1995). The phenyoxyacetamide derivative PEPA also slows the onset of desensitization at flop splice variants (Sekiguchi et al., 1997). These unique desensitization properties of naturally occurring AMPA receptor isoforms as well as other data (Stern-Bach et al., 1994) have highlighted the region between M3 and M4 as a critical determinant of desensitization. Site-directed mutagenesis of residues in the flip/flop region has identified several residues that control the effects of cyclothiazide on AMPA receptor function. Ser750 and Asn750 appear pre-eminent among the structural determinants of GluR1flip and GluR1flop that have been investigated thus far. Conversion of Ser750 in GluR1flip to glutamine, which is the homologous residue found in the cyclothiazide-insensitive kainate receptors, abolishes cyclothiazide actions on AMPA receptors (Partin et al., 1995). A serine residue on either the GluR1 or GluR2 subunits of heteromeric receptors is sufficient to impart cyclothiazide sensitivity to the receptor (Partin et al., 1995). Similarly, introduction of a serine residue into the homologous site on GluR6 imparts some of the behavior of cyclothiazide to this modified receptor, suggesting the process of desensitization between kainate and AMPA receptor subunits is similar (Partin et al., 1995). The flip/flop domain is a helical region lying on a solvent-exposed surface of the subunit (Fig. 2, B and C). One structural model that has been proposed suggests that desensitization modulators bind directly at or near the flip/flop site, which critically interacts with other microdomains on the protein complex in a manner dependent on residue 750. Kinetic modeling of the effects of aniracetam suggest that this compound could exert its actions almost entirely through slowing of channel closing. By contrast, a more complex scheme was needed to account for the effects of cyclothiazide, with hypothesized stabilization of a nondesensitized state that could

occur through a 20-fold increase in the affinity of the agonist for cyclothiazide-bound receptor (Partin et al., 1996).

Mutagenesis has also suggested additional regions that may control various aspects of desensitization such as Leu646 in GluR1 (Mano and Teichberg, 1998) and Leu507 within the S1-binding domain of GluR3 as well as Leu497 in GluR1 (Stern-Bach et al., 1998; see Table 2). Aromatic substitutions at these latter S1 residues completely relieve desensitization independent of the flip/flop region, and suggest that this residue is critically important in conformations that govern entry into and exit from the desensitized state. Consistent with functional data, the homologous leucine residue within GluR2 points away from the flip/flop helix and is not part of the ligand-binding pocket (Armstrong et al., 1998).

H. Molecular Determinants of Kainate Receptor Deactivation and Desensitization

Although less effort has been devoted to unraveling the structural basis of desensitization for kainate receptors than for AMPA receptors, several recent reports have shed light on some structural requirements for desensitization of kainate receptors. For example, concanavalin A relieves desensitization of GluR5 and GluR6 receptors but not GluR7 (Schiffer et al., 1997a), suggesting that evaluation of glycosylation sites that differ between GluR7 and GluR5/6 might identify the binding site for this lectin. Alternatively, residues between M3 and M4 might control the effectiveness of bound concanavalin A. Furthermore, the kinetic properties of the responses to a variety of receptor agonists are distinct between AMPA and kainate receptor families, and even distinct for different subunits within a receptor family (Schiffer et al., 1997a; Swanson et al., 1998). For example, kainate induces a rapidly desensitizing response at GluR6 but a slowly desensitizing response at GluR5, and the subunit-specific attributes of the temporal response of various agonists are beginning to be used as tools to dissect outstructural features underlying kinetic properties (Swanson et al., 1997b, 1998). The deactivation rate for domoate is slower for GluR6 than for GluR5, and this difference seems largely a result of Asn721 in GluR6. Exchange of this residue in GluR6 for the corresponding residue in GluR5 (Ser721) reverses the relative domoate deactivation kinetics between the two receptors and also swaps the differential AMPA sensitivity (Swanson et al., 1997b; see Table 2). This same residue also appears to control the agonist selectivity of kainate receptor subunits (Swanson et al., 1998). Similarly, mutation of Ser689 to Ala speeds the deactivation of GluR5 to domoate; conversely, GluR6(A689S) appears to slow the desensitization to kainate. Residue 689 is homologous to S654 in GluR2 (Fig. 2D; Table 2), and thus may participate in agonist recognition.

I. Molecular Determinants of NMDA Receptor Deactivation and Desensitization

NMDA receptor desensitization reflects at least three distinct processes, and thus appears more complex than the conformation changes of the receptor protein that have been interpreted as desensitized states for AMPA and kainate receptors (reviewed by Mayer et al., 1995). First, in the continued presence of glutamate, NMDA receptor responses are diminished in a time-dependent fashion that reflects negative allosteric coupling between the glutamate and glycine-binding sites. In the presence of high glutamate concentration, this form of desensitization is manifest as a decrease in glycine affinity and can be overcome with high glycine concentrations (reviewed by Mayer et al., 1989; McBain and Mayer, 1994). Second, in the presence of high concentrations of glycine, NMDA receptor responses in dialyzed small cells or excised membrane patches rapidly desensitize by 50 to 80% (Sather et al., 1990; see Table 4). The relevance of this rapid glycine-insensitive desensitization is unknown since it only appears once the intracellular constituents are well dialyzed. Third, a Ca^{2+}-dependent form of desensitization (also referred to as Ca^{2+}-dependent inactivation) has been described that requires transmembrane movement of Ca^{2+} and has been proposed to occur at an intracellular site (Clark et al., 1990; Legendre and Westbrook, 1993; Rosenmund and Westbrook, 1993; Vyklicky, 1993). The calcium-dependent desensitization is sensitive to calcium buffers used in the patch pipette and can best be observed with low EGTA as the internal calcium buffer. Regulation of the NMDA receptor by Ca^{2+} can occur when Ca^{2+} enters the cell through routes other than the NMDA receptor. The time course of this form of NMDA receptor regulation is slow (on the order of seconds), and the process is thought to be modulated by second messenger systems and to occur during synaptic transmission (Rosenmund et al., 1993; Tong et al., 1995; Raman et al., 1996). Furthermore, time-dependent increases in Ca^{2+}-dependent desensitization might be controlled by dephosphorylation by calcineurin (Tong and Jahr, 1994). Ca^{2+}-dependent changes in NMDA receptor function have been suggested to involve filamentous actin and might occur through a rearrangement of intracellular linkages between the NMDA receptor protein and intracellular scaffolding proteins that are controlled by actin, Ca^{2+}, and ATP (reviewed by Rosenmund and Westbrook, 1993; McBain and Mayer, 1994).

NMDA receptor deactivation controls EPSC time course and is dependent on subunit composition (Table 4). Interestingly, deactivation is much slower for receptors containing the NR2D subunit, which is expressed early in development (Watanabe et al., 1992; Monyer et al., 1994). Such prolonged activation might be important for the formation, stabilization, or elimination of synapses during development. Although molecular studies have not yet been reported, evaluation of the amino acid residues that control the prolonged deactivation of NR2D compared to NR2A might shed further light on regions of the receptor that couple agonist binding to receptor activation. In this section, we will focus on recent advances in our understanding of the molecular determinants of these different states of NMDA receptors.

Like deactivation, all forms of NMDA receptor desensitization are subunit-dependent. For example, Ca^{2+}-dependent desensitization is prominent for NR2A and occurs to a lesser degree in NR2D-containing receptors, but is not significant in NR2B- or NR2C-containing receptors (Medina et al., 1995; Krupp et al., 1996). Because the glycine affinity is also influenced by the NR2 subunit (Kutsuwada et al., 1992; Ishii et al., 1993), the negative coupling between glutamate and glycine binding should also be sensitive to subunit composition. In general, NR2A seems to be unique among NR2 subunits in showing fast deactivation and the most prominent calcium-dependent, glycine-dependent, and glycine-independent desensitization. The functional significance of these unique properties of NR2A has not been fully explored.

Interestingly, NR1 C-terminal deletion mutants abolish Ca^{2+}-dependent desensitization, suggesting that this region of the receptor may be involved in this process. Ehlers et al. (1996, 1998) have identified two calmodulin-binding sites in the C-terminal region of NR1 and suggest that direct high-affinity binding of calmodulin to these binding sites may be involved in the calcium-dependent desensitization of NMDA receptors. Two groups have shown that mutations that disrupt the CBS1 calmodulin-binding site on the NR1 subunit interfere with Ca^{2+}-dependent desensitization (Zhang et al., 1998; Krupp et al., 1999). The C-terminal of NR1 also binds to α-actinin 2, an actin-binding protein, and the binding of α-actinin 2 is antagonized by calcium/calmodulin (Wyszynski et al., 1997; Allison et al., 1998). Overexpression of α-actinin 2 can reduce the Ca^{2+} calmodulin-dependent desensitization of recombinant receptors, suggesting that after Ca^{2+} entry into the cell, calmodulin may compete with α-actinin 2 binding to the NR1 C-terminal (Zhang et al., 1998; Krupp et al., 1999). Ca^{2+} may also directly reduce the affinity of α-actinin for the NMDA receptor C-terminal through its interaction with the EF hands of calcium-sensitive forms of α-actinin (Krupp et al., 1999). Krupp et al. (1999) go on to show that the C-terminal peptides can decrease Popen, suggesting that association of this part of the receptor with an intracellular surface recognition site on the protein following dissociation from α-actinin 2/actin might mediate Ca^{2+}-dependent desensitization (see also Zhang et al., 1998). Thus, α-actinin 2 may provide a link between the NMDA receptors and the actin filaments (see Rosenmund and Westbrook, 1993), and its displacement from

NR1 may play a role in calcium-dependent desensitization of NMDA receptors.

NR2C and NR2D receptors do not appear to show rapid glycine- and Ca^{2+}-independent desensitization (Krupp et al., 1996; Wyllie et al., 1998). Recently, two groups have exploited this observation and used chimeric NR2A and NR2C receptors to evaluate the structural determinants of this form of desensitization (Krupp et al., 1998; Villarroel et al., 1998). Both groups identified two N-terminal domains that differentially influence glycine-independent desensitization. A four-amino acid domain just upstream of M1 region and a 190-amino acid stretch with homology to leucine/isoleucine/valine-binding protein (LIVBP) that precedes the S1 region appear to collaborate to control the degree of desensitization. Both regions have been suggested to exert important effects on desensitization, since removal of either alone is insufficient to abolish desensitization. Two residues (Ala555 and Ser556) within the pre-M1 region have been identified as critical for desensitization of NR2A receptors, and data from the AMPA receptors also suggest that this portion of the glutamate receptor subunit can influence desensitization (Stern-Bach et al., 1998). Villarroel et al. (1998) showed that exchange of these two residues in the S1 region immediately upstream of M1 (Fig. 1) of NR2A to corresponding residues (proline and alanine) in NR2C eliminates a slower component of desensitization they observed ($\tau = 2$ s; but see Table 4), but leaves the fast component ($\tau = 0.3$ s) intact. On the other hand, substitution of a segment of the NR2C LIVBP region into NR2A abolished the fast component of desensitization but not the slow component. Introduction of alanine and serine into these two positions in NR2C enhanced the degree and rate of desensitization (Villarroel et al., 1998), perhaps suggesting the proline residue in NR2C places a conformational constraint on protein movement that prevents access to the desensitized state. Krupp and colleagues (1998) showed the contributions of these same two regions to the glycine-independent desensitization using a C-terminal deletion mutant of NR1 which abolishes the calcium-dependent desensitization, thereby simplifying their interpretation. In their experiments, glycine-dependent desensitization proceeded with a simple exponential time course. NR2A(A555P) and NR2A(S556A) had modest effects on the time course of desensitization, but more pronounced effects on the degree of desensitization. They found that substitution of the NR2C pre-M1 region and the LIVBP region into NR2A together abolish desensitization. These data reinforce the concept of a modular design of glutamate receptors and highlight regions that link agonist-binding domains to other portions of the receptor (including pore-forming elements) as critical determinants of the coupling between ligand binding and channel gating.

VII. Endogenous Allosteric Modulators

Relatively few forms of allosteric modulation by extracellular substances have been identified for AMPA and kainate receptors, suggesting their role as mediators of fast synaptic transmission may not be amenable to graduated fine tuning by the microenvironment. That is, the role of these receptors might be to deliver a depolarization to the postsynaptic neuron that is controlled in part by gene expression. Alternatively, their insensitivity to the extracellular environment may be a way of preserving full regulation exclusively for the second messenger-linked kinases and phosphatases that control synaptic plasticity. By contrast, at least a dozen forms of allosteric modulation of NMDA receptor function by endogenous substances have been reported, which can be taken as evidence of the importance of fine-tuning NMDA receptor function. In addition, many of the allosteric modulators provide tonic inhibition under physiological conditions (e.g., Mg^{2+}, H^+, Zn^{2+}), suggesting allosteric regulation can protect against the dangers of NMDA receptor overactivation. Table 3 summarizes the voltage-independent regulation of the NMDA receptor by a host of structurally unrelated compounds and ions. Of these, we will consider recent advances in regulation of NMDA receptors by Zn^{2+}, reducing and oxidizing agents, protons, and polyamines. The reader is referred to the primary articles for other modulators of NMDA receptors listed in Table 3 as well as other review articles (Hollmann and Heinemann, 1994; McBain and Mayer, 1994).

A. Extracellular Zinc

In addition to its role in biochemistry of various cells throughout the periphery, zinc is also concentrated by certain neurons into synaptic vesicles and can be released in a Ca^{2+}-dependent manner at certain synapses such as the mossy fiber–CA3 pyramidal cell synapse. Given this potential synaptic role of Zn^{2+} in the CNS, it has attracted a great deal of attention as a possible neuromodulator of ion channels as well as a neurotoxic agent (reviewed by Smart et al., 1994; Harrison and Gibbons, 1994; Choi and Koh, 1998; Cuajungco and Lees, 1998). It has been known for some time that group IIB transition metals such as Zn^{2+} and Cd^{2+} inhibit NMDA receptors by both a voltage-dependent and voltage-independent mechanism (reviewed by Peters et al., 1987; Westbrook and Mayer, 1987; Mayer et al., 1989; Christine and Choi, 1990; Legendre and Westbrook, 1990; McBain and Mayer, 1994; Smart et al., 1994; Trombley and Shepherd, 1996). Zn^{2+} also inhibits glutamate uptake (Spiridon et al., 1998) and potentiates AMPA receptors (Mayer et al., 1989; Rassendren et al., 1990; Dreixler and Leonard, 1997), suggesting release of Zn^{2+} might favor synaptic non-NMDA receptor activation.

Recent work has shown that recombinant NMDA receptors are inhibited in a similar fashion to native receptors (Williams, 1996; Chen et al., 1997; Paoletti et al., 1997; Traynelis et al., 1998). Voltage-dependent NMDA receptor channel block by Zn^{2+} is much weaker than for Mg^{2+} and appears to be qualitatively different, perhaps because of greater permeation of Zn^{2+} than Mg^{2+} through NMDA receptors (Mayer et al., 1989; Christine and Choi, 1990; Legendre and Westbrook, 1990; Paoletti et al., 1997). Interestingly, channel block by Zn^{2+} appears to involve some of the same pore-accessible residues as channel block by Mg^{2+} (Mori et al., 1992; Kawajiri and Dingledine, 1993; Sakurada et al., 1993; Paoletti et al., 1997). At the single-channel level, voltage-dependent Zn^{2+} block appears at low concentrations as a flickery block and at higher concentrations as a reduction in the single-channel amplitude because the individual blockages occur at frequencies beyond the recording resolution of current patch-clamp amplifiers (Christine and Choi, 1990; Legendre and Westbrook, 1990). Voltage-independent inhibition of native NMDA receptors by extracellular Zn^{2+} involves both a reduction in opening frequency and a decrease in open duration (Christine and Choi, 1990; Legendre and Westbrook, 1990).

Although heteromeric recombinant receptors containing NR1 + NR2 subunits are inhibited by Zn^{2+} in a fashion similar to neuronal receptors, NR1 subunits expressed in *Xenopus* oocytes appear to be potentiated by submicromolar concentrations of Zn^{2+} (Hollmann et al., 1993; Zheng et al., 1994), which may reflect properties of receptors comprised of NR1 coassembled with *Xenopus* glutamate receptor subunits (Soloviev et al., 1996). The voltage-independent Zn^{2+}-binding site appears to be strongly dependent on subunit composition, being influenced by the NR2 subunits as well as NR1 splice variants (Williams, 1996; Chen et al., 1997; Paoletti et al., 1997; Traynelis et al., 1998). Particularly interesting is the finding that the receptors comprised of the NR2A subunit are much more sensitive to Zn^{2+}, being inhibited in the nanomolar range by as much as 70 to 80% (Williams, 1996; Chen et al., 1997; Paoletti et al., 1997). Three important conclusions have been suggested from this finding. First, contaminant Zn^{2+} in experimental solutions as well as ambient Zn^{2+} present in the extracellular space could be high enough (hundreds of nanomolar) to tonically inhibit NR2A-containing NMDA receptors. This result could explain the lack of inhibition of certain neuronal receptors, which may have been fully inhibited by high ambient Zn^{2+} in control solutions (discussed in Paoletti et al., 1997). Second, a rapidly reversible form of "redox modulation" previously described for NR2A-containing receptors (Köhr et al., 1994) largely reflects chelation of contaminant Zn^{2+} from the extracellular medium by thiol-reducing reagents (Paoletti et al., 1997; Arden et al., 1998). Third, potentiation of NMDA receptors by the nonreceptor tyrosine kinase Src

appears to reflect reduction in Zn^{2+} sensitivity and subsequent relief of tonic Zn^{2+} inhibition for NR2A-containing receptors (Zheng et al., 1998). Although the concentration of Zn^{2+} in the brain and particularly the synaptic cleft remains a complex question (reviewed by Smart et al., 1994), it is clear that Zn^{2+} can have a multitude of effects on NMDA receptor function. Furthermore, Zn^{2+} permeation through unblocked NMDA receptors, AMPA receptors, and depolarization-activated Ca^{2+} channels could be detrimental to neuronal survival (Weiss et al., 1993; Koh et al., 1996; Sensi et al., 1997; Ascher, 1998; Yin et al., 1998).

What is the nature of the voltage-independent Zn^{2+}-binding site on the NMDA receptor? Given the impressive effect of the NR2 subunit on the IC_{50} value for Zn^{2+}, one might expect the Zn^{2+}-binding site to reside on this subunit. Although there is currently only indirect structural data describing the role of NR2 in presumably high-affinity Zn^{2+} binding (Köhr et al., 1994), site-directed mutagenesis of the NR1 subunit has provided some interesting clues to the structural nature of the Zn^{2+}-binding site. Amino acid substitutions at a variety of acidic residues (e.g., Glu342, Asp669) as well as presumed pore-forming residues (Asn616) and cysteine residues thought to be involved in redox modulation (Cys744, Cys798; Sullivan et al., 1994) all reduce the Zn^{2+} IC_{50} (Zheng et al., 1998; Traynelis et al., 1998). Although it is tempting to speculate that these substitutions might remove one of the electron donors to the Zn^{2+} coordination site, other interpretations are possible. Most notably, the shifts in the IC_{50} values for Zn^{2+} show a strong correlation with changes that these same mutations induce in the IC_{50} value for protons (Traynelis et al., 1998). If the proton sensor were a single ionizable residue that was closely associated with structural components of the gate, then one possibility is that this residue might also participate in Zn^{2+} coordination. Thus, any perturbation of the pKa at the proton sensor (e.g., through shielding by exon 5 or inductive effects of nearby charged residues) might also perturb Zn^{2+} binding. Although more work is needed to evaluate this possibility, these data nevertheless raise the idea that the NR1 subunit might control or participate in Zn^{2+} binding. The link between proton and Zn^{2+} regulation also suggests common structural determinants for these two important forms of extracellular regulation of NMDA receptor function (Wu and Christensen, 1996; Traynelis et al., 1998).

B. Reduction and Oxidation of Extracellular Cysteine Residues

Neuronal NMDA receptor function appears to be unusually sensitive to the oxidizing potential of the extracellular environment (reviewed by Aizenman et al., 1989; McBain and Mayer, 1994; Gozlan et al., 1994; Aizenman, 1994), and this redox modulation is controlled in recombinant receptors by two cysteine resi-

dues (Cys744 and Cys798) on the NR1 subunit (Sullivan et al., 1994), as well as which the NR2 subunit is present (Köhr et al., 1994; Sullivan et al., 1994; Omerovic et al., 1995; Brimecombe et al., 1997). The location of these cysteines in the GluR2 structure is shown in yellow in Fig. 2, B and C. When these cysteines are oxidized by experimental reagents such as 5,5′-dithiobis(2-nitrobenzoic acid), the receptor response is attenuated, and when they are reduced with compounds such as dithiothreitol the receptor response is enhanced. The reduced receptor is associated with a roughly 2-fold higher single-channel opening frequency and slightly lower EC_{50} value for NMDA, but no change in single-channel conductance (Tang and Aizenman, 1993a; Brimecombe et al., 1997). Voltage dependence of the channel was also unchanged when receptors were treated with reducing and oxidizing agents at physiological potentials (Tang and Aizenman, 1993b). Redox modulation appears to be functionally independent of modulation of the receptor by sulfated steroids (Park-Chung et al., 1997), ethanol (Peoples et al., 1997), and pH (Traynelis and Cull-Candy, 1991; Tang and Aizenman, 1993a). However, dithiothreitol treatment reduced the potency of dynorphin approximately 3-fold for the NMDA receptor (Chen et al., 1995b), and alkylation of the NMDA receptor presumably at thiols that participate in redox modulation has also been suggested to alter Mg^{2+} and Zn^{2+} inhibition (Tang and Aizenman, 1993a). It is noteworthy that the two NR1 cysteine residues that control redox modulation also control inhibition by Zn^{2+}, protons, and ifenprodil (Sullivan et al., 1994; Traynelis et al., 1998; Zheng et al., 1998; Mott et al., 1998).

A host of endogenous molecules have been described that are capable of oxidizing and reducing the NMDA receptor in a functionally relevant manner. These molecules include the oxidizing agents pyrroloquinoline quinone, lipoic acid, and reactive free radical oxygen species (Aizenman et al., 1990; Aizenman et al., 1992; Tang and Aizenman, 1993c; Aizenman, 1995; Scanlon et al., 1997) and reducing agents such as glutathione and dihydrolipoic acid (Gilbert et al., 1991; Manzoni et al., 1992; Tang and Aizenman, 1993b; Köhr et al., 1994; Varga et al., 1997). Nitric oxide (NO) donors also can inhibit the NMDA receptors, perhaps through the release of NO-derived compounds that support S-nitrosylation of the NMDA receptor (Lipton et al., 1993; Stamler et al., 1997). However, the exact mechanism of action of NO on NMDA receptors remains controversial (Hoyt et al., 1992; Fagni et al., 1995; Aizenman et al., 1998). Recently, additional compounds such as cyanide have been suggested to exert subunit-specific effects that appear to be linked to chemical modification through the redox site(s) of receptors containing NR2A (potentiation) or NR2B (depression; Arden et al., 1998). The triaminopyridine derivative, flupirtine, may also influence NMDA receptor activation via its redox site (Osborne et al., 1998).

Although a residual NMDA receptor response exists in the oxidized state, the difference between enhanced and oxidized responses is sufficient to suggest involvement in normal function as well as pathological situations (Levy et al., 1990; Puka-Sundvall et al., 1995; Sinor et al., 1997). This degree of regulation of the NMDA receptor by the extracellular redox state has been considered as a potential site for therapeutic intervention in ischemic cell death (Lipton, 1993; Lipton et al., 1993). Furthermore, some compounds that oxidize the NMDA receptor and thereby reduce NMDA receptor activity are anticonvulsant and neuroprotectant in experimental models (Jensen et al., 1994; Quesada et al., 1996, 1997). One important advantage of such modulation is the prospect of diminished side effects since oxidation does not fully inhibit the receptor. However, the feasibility of designing NMDA-specific redox modulators remains to be evaluated.

C. Extracellular Protons

The extracellular pH is highly dynamic in mammalian brain and influences the function of a multitude of biochemical processes and proteins, including glutamate receptor function. AMPA receptors are inhibited by protons at acidic pH values (near 6.0) that make this effect of more biochemical than physiological interest (Christensen and Hida, 1990; Traynelis and Cull-Candy, 1990, 1991; Traynelis et al., 1995). Somatic, postsynaptic (Gottfried and Chesler, 1994; Saybasili, 1998), and presynaptic (Chen et al., 1998) native NMDA receptors are inhibited by more physiologically relevant concentrations of extracellular protons. This inhibition occurs primarily through a voltage- and agonist-independent reduction in the single-channel opening frequency rather than through changes in the single-channel open time or single-channel conductance (reviewed by McBain and Mayer, 1994; Traynelis, 1998). The pH sensitivity of the NMDA receptor has received increasing attention for at least two reasons. First, the IC_{50} value for proton inhibition of exon 5- and NR2C-lacking receptors corresponds to pH 7.4, placing the receptor under tonic inhibition at physiological pH. Second, pH changes are extensively documented in the CNS during synaptic transmission, glutamate receptor activation, glutamate receptor uptake, and also during ischemia and seizures (Siesjo, 1985; Chesler, 1990; Chesler and Kaila, 1992; Amato et al., 1994). The acidification associated with these latter pathological situations should serve to inhibit NMDA receptors, which may provide negative feedback that minimizes their contribution to neurotoxicity (reviewed by Kaku et al., 1993; Munir et al., 1995; Vornov et al., 1996; Gray et al., 1997; O'Donnell and Bickler, 1994; Tombaugh and Sapolsky, 1993) and seizure maintenance (Balestrino and Somjen, 1988; Velisek et al., 1994). Such feedback inhibition might also delay the contribution of NMDA receptor activation to ischemic cell death to a point in time at which the pH

gradient has recovered before glutamate has been removed from the interstitial space. The pH sensitivity of glutamate uptake is consistent with this latter possibility (Billups and Attwell, 1996), which may enhance the opportunity for postinsult treatment of, for example, stroke with NMDA receptor antagonists (Tombaugh and Sapolsky, 1993).

Like voltage-independent Zn^{2+} inhibition, the inhibition of NMDA receptors by protons is also controlled by the NR2 subunit as well as alternative exon splicing in the NR1 subunit (see Traynelis, 1998). Inclusion of NR1 exon 5 reduces both proton and Zn^{2+} inhibition, and the same residues encoded by this exon appear to mediate both effects (see also, Zheng et al., 1994; Traynelis et al., 1995, 1998). These structural parallels extend to other portions of the molecule in that mutations that influence proton inhibition throughout the NR1 subunit similarly influence Zn^{2+} inhibition (Williams et al., 1995; Traynelis et al., 1998; discussed above). Interestingly, mutations that influence pH sensitivity are broad ranging in both NR1 and NR2 and include N-terminal acidic residues (Williams et al., 1995; Gallagher et al., 1997), cysteine residues that may participate in disulfide bond formation (Sullivan et al., 1994), residues in the extracellular M3-M4 loop (Kashiwagi et al., 1996), as well as residues that are thought to comprise the channel pore-forming region (Kashiwagi et al., 1997; Traynelis et al., 1998). This latter association between pore-forming residues and pH sensitivity might suggest that the proton sensor is tightly coupled to the movement of the gate.

What might the molecular composition of the proton sensor be? Whereas it could be lipid (discussed in Traynelis, 1998) or carbohydrate, it seems more likely to be a single amino acid residue that faces the extracellular solution. Cysteine and histidine residues (see Wu and Christensen, 1996) are obvious candidates given the similarity between their free solution side chain pKa values and the IC_{50} for proton inhibition at the NMDA. However, apart from the residues thought to be involved in disulfide bond formation, there are no other cysteine residue substitutions that alter the IC_{50} for proton inhibition (Sullivan et al., 1994). Furthermore, the pKa of a single residue within a protein can differ from its free solution value (see Traynelis, 1998 for examples), raising the possibility that residues with normally acidic or basic pKa values might comprise the proton sensor. For example, the pKa values of clustered intrapore acidic residues, which normally have free solution pKa near 4, are shifted above 7.5 for cyclic nucleotide-gated channels and Ca^{2+} channels (Root and MacKinnon, 1994; Chen et al., 1996; Chen and Tsien, 1997).

Identification of the residues or other molecular entities that constitute the proton sensor seems an important next step, since this information might provide structural clues to NMDA receptor function and regulation. Furthermore, such information might help frame structural models describing how exon 5 of the NR1

subunit (as well as polyamines and Mg^{2+}; see below) acts as a tethered modulator to relieve tonic proton inhibition at the surface of the receptor through shielding of the proton sensor (Paoletti et al., 1995; Traynelis et al., 1995; Johnson, 1996). This information would also help to illuminate the mechanism of ifenprodil's potentiation of proton inhibition (Mott et al., 1998) and could also be useful in the design of novel NMDA receptor antagonists.

D. Extracellular Polyamines

The interactions of endogenous polyamines and polyamine toxins with ion channels has received considerable attention in recent years, both because of the implications for neurophysiology and because of their potential as therapeutic agents. Endogenous polyamines such as spermidine and spermine have at least three effects on NMDA receptors. Extracellular polyamines can cause a voltage-dependent inhibition, a glycine-dependent potentiation, and a voltage- and glycine-independent potentiation of neuronal and recombinant NMDA receptor function (reviewed by Rock and Macdonald, 1995; Williams, 1995a, 1997a,b). The voltage-dependent block appears to involve the same intrapore residues as Mg^{2+} and Zn^{2+} block (Kashiwagi et al., 1997) and likely reflects fast-open channel block that is of lower affinity than Mg^{2+} with relatively weak voltage dependence (e.g., Rock and Macdonald, 1992; Araneda et al., 1992; Benveniste and Mayer, 1993). The voltage-dependent block has a similar subunit dependence as Mg^{2+} blockade, being less pronounced for NR2C-containing receptors when compared with receptors comprised of NR2A or NR2B subunits (Williams et al., 1994; Williams, 1995b). The structure and multivalent nature of the polyamines complicates interpretation of blocking data in terms of a binding site at a particular location within the electric field, although it has been suggested that more than a single charge enters the electric field if the channel behaves as a single ion pore; polyamines may also permeate the channel (Benveniste and Mayer, 1993; Igarashi and Williams, 1995).

Polyamines can stimulate NMDA receptor function to a greater degree at low glycine concentrations than at saturating glycine concentrations. This stimulation reflects approximately a 3-fold increase in glycine affinity (Benveniste and Mayer, 1993). Both glycine-independent and glycine-dependent forms of potentiation of NMDA receptor function are influenced by the NR2 subunit. However, whereas glycine-dependent potentiation occurs at NR2A- and NR2B-containing receptors, glycine-independent potentiation is observed exclusively at receptors that incorporate the NR2B subunit (Zhang et al., 1994; Williams et al., 1994; Williams, 1995b). Consistent with the subunit selectivity, NR1 subunit mutations that influence one process do not perturb the other (Williams et al., 1995; Kashiwagi et al., 1996). Glycine-dependent potentiation is not controlled by NR1 RNA

splicing, whereas glycine-independent potentiation is abolished when the N-terminal alternative exon 5 is incorporated into the mature transcript (Durand et al., 1993). Together, these results suggest that two separate binding sites might exist for glycine-dependent and -independent effects of spermine.

The glycine-independent form of potentiation has been suggested to arise from the relief of tonic proton inhibition at physiological pH. That is, polyamines (like alternative exon 5) shift the pKa of the proton sensor to acidic values, reducing the degree of tonic inhibition at physiological pH, which appears as a potentiation of function (Traynelis et al., 1995; Kumamoto, 1996). Whereas linkage of these two allosteric modulators (protons and spermine) explains their common dependence on splice variants, clearly other structural determinants must be invoked to explain the inability of polyamines to relieve proton inhibition of NR2A containing receptors. These structural determinants of NR2 polyamine potentiation appear to reside within the N terminus of this subunit, and can be attributed in part to several acidic residues that might be involved in polyamine binding (Gallagher et al., 1997).

The role of acidic residues within the NR1 subunit in polyamine-proton regulation has also become apparent through studies utilizing site-directed mutagenesis. Residues first predicted to be involved in polyamine potentiation through comparison of the NR1 sequence to the polyamine-binding protein PotD have also been shown to control proton sensitivity (Williams et al., 1995; Kashiwagi et al., 1996). Thus, mutagenesis through the NR1 and NR2 subunits supports the idea that glycine-independent polyamine potentiation reflects relief of tonic proton inhibition (Traynelis et al., 1995; Williams et al., 1995; Kashiwagi et al., 1996, 1997; Gallagher et al., 1997; Traynelis et al., 1998). Finally, although the physiological relevance of voltage- and glycine-independent polyamine potentiation of NMDA receptor function has remained unclear given the unknown concentrations of extracellular polyamines in vivo, recent data have identified two endogenous activators of the polyamine site, Mg^{2+} and histamine. Mg^{2+} acts with an IC_{50} value of 2 mM to partially reduce the pH sensitivity of NR2B-containing receptors under physiological conditions (Paoletti et al., 1995; Kumamoto, 1996). Histamine can act with an EC_{50} near 10 μM to potentiate neuronal and synaptic NMDA receptors (Vorobjev et al., 1993; Bekkers, 1993). This effect was originally suggested to involve polyamine potentiation on the basis of the nonadditive effects of spermine (Vorobjev et al., 1993). In addition, both the subunit and pH dependence of histamine potentiation support the idea that histamine potentiates NMDA receptors through action at the polyamine site (Williams, 1994; Yanovsky et al., 1995; Saybasili et al., 1995). Because histamine is released from widespread synaptic varicosities arising from the anterior hypothalamus, this form of

regulation may be relevant under physiological conditions. Finally, aminoglycoside antibiotics may also mimic the potentiating effects of polyamines, which might contribute to the ototoxicity observed with these compounds (Segal and Skolnick, 1998).

In summary, the last few years have seen the evaluation and considerable refinement of ideas about allosteric regulation in recombinant NMDA receptors. In addition to the continued discovery of new forms of regulation, one interesting trend to emerge from work on voltage-independent modulation of NMDA receptor function has been the convergence of regulatory systems. For example, site-directed mutagenesis has been used to suggest structural links between proton, zinc, polyamine, and redox modulation of the NMDA receptor. Although it would be oversimplistic to argue that these sites are identical, there is strong evidence to suggest that they may share either partially overlapping binding determinants or common downstream structural targets. Although convergence remains to be evaluated among the other modulatory systems, there is evidence that not all regulatory sites share structural and functional principles (Miller et al., 1992; Nishikawa et al., 1994; Chen et al., 1995b; Park-Chung et al., 1997; Peoples et al., 1997). The next few years are likely to be pivotal in identifying subgroups within Table 3 that share common features. This advance should facilitate the design of drugs that can modulate rather than fully abolish NMDA receptor function.

VIII. Molecular Determinants of Ion Permeation

From the wealth of biophysical data and recent structural information, the mechanisms and molecular determinants of ion permeation are best understood for voltage-gated Ca^{2+}, Na^+, and K^+ channels that discriminate among ions with a high degree of selectively. Traditionally, biophysical experiments suggest that selectivity is not governed simply by the physical constraints of the pore in a manner analogous to a sieving effect, but rather, ions may occupy several binding sites in the pore which are electrostatically coupled. Such an arrangement permits rapid ion transport through the channel while maintaining a high degree of selectivity. The recent elucidation of K^+ channel structure from *Streptomyces lividans* (Doyle et al., 1998) has provided structural information that creates a framework in which to interpret biophysical information in the future. In contrast, the mechanism of permeation and block of less selective channels, such as glutamate-activated receptors, remains unclear. Yet, understanding such mechanisms is important since permeation and block are controlled in a cell-specific manner through differential subunit expression and RNA editing. In view of this, we consider here the available biophysical data and recent mutagenesis studies of glutamate receptor pores that provide information on the pore diameter, single-chan-

nel conductance properties, and ion selectivity as well as block by endogenous ions such as Mg^{2+} or polyamines.

A. Pore Diameter

Traditionally, the minimum pore diameter of ion channels has been inferred from studies of permeating metal ions of differing hydrated radii or using organic ions with known space-filled volumes (Hille, 1992). Table 5 summarizes estimates of the minimum pore diameter for recombinant glutamate receptors determined by comparing the permeability sequence of a series of organic cations. Unlike most voltage-gated channels (see Table 5), the pores of glutamate receptors are physically larger, approximating the dimensions of nonselective endplate channels that may function in part as molecular sieves (Hille, 1992). Moreover, the dimensions estimated for the open channel of NMDA and non-NMDA receptors are large enough to account for permeation of Mg^{2+} ions at negative membrane potentials or polyamines at positive potentials (Bähring et al., 1997; Bowie et al., 1999).

Additional studies with impermeant organic cations also suggest that both NMDA and non-NMDA receptors have a short narrow region of constriction somewhere near or just past the middle of the membrane electric field, which is flanked by two wider vestibules that can accommodate cations as large as 0.73 nm (Fig. 6; Zarei and Dani, 1995; Villarroel et al., 1995). This hypothesized geometry is in agreement with the accessibility profile of cysteine-substituted residues (Kuner et al., 1996, 1997; discussed below) and differs somewhat from that of the axial fluctuations in pore diameter of K^+ channels (Doyle et al., 1998), even though glutamate and K^+ channels have been suggested to share some other features (Lee, 1992; Wood et al., 1995).

This geometry raises the possibility that NMDA channels may behave as single-occupancy pores with respect to the main permeant ion (Schneggenburger, 1996).

However, exceptions to this idea seem to exist for certain organic blockers since the NMDA receptor apparently can be occupied by both blocking and permeant ions simultaneously (Antonov et al., 1998). Consistent with the properties of single-ion pores, the unitary conductance reaches a sustained maximum in the absence of divalent ions of 90 pS with increasing concentrations of NH_4^+ as the charge carrier for hippocampal NMDA receptor (Zarei and Dani, 1994; Jahr and Stevens, 1993) and 60 to 160 pS with Na^+ as the charge carrier for recombinant NMDA receptors (Ruppersberg et al., 1994; Premkumar and Auerbach, 1996; Iino et al., 1997). This is in contrast to the multiphasic concentration-conductance curve that shows a minimum predicted for dual occupancy pores (see Hille, 1992). Whereas experiments with monovalent ions suggest that the biophysical basis of ion transport may differ between NMDA receptors (single-occupancy pores) and voltage-dependent Ca^{2+} channels (dual occupancy pores; discussed in Zarei and Dani, 1994; see Table 5), Ca^{2+} permeation appears to be more complex and may involve multiple binding sites (Premkumar and Auerbach, 1996; Wollmuth and Sakmann, 1998). Interestingly, mutation of residues within the glutamate receptor channel that control unitary conductance, Ca^{2+} permeability, and sensitivity to blockade by polyamines has little effect on the pore's minimum cross-sectional area (Table 5), suggesting that the electrostatics or coordination chemistry of permeant ions rather than purely steric considerations within the pore govern the functional effects of RNA editing on unitary conductance and ion selectivity.

B. Unitary Sublevel Conductances

One of the most intriguing features of the glutamate receptors apparent from the first single-channel studies in native channels (Nowak et al., 1984; Jahr and Stevens, 1987; Cull-Candy and Usowicz, 1987) is the striking variability in sublevel conductances of the open

TABLE 5
Comparison of pore dimensions of glutamate receptors determined by permeability to organic cations to measurements from other cation selective channels

Channel or Receptor	Pore Dimensions	Pore Area	References
	nm	*nm²*	
Neuronal AMPA[a]	0.6 diameter	0.28	Vyklicky et al., 1988
Neuronal NMDA[b]	0.45×0.57	0.26	Zarei and Dani, 1995
GluR1[c]	0.78 diameter	0.48	Burnashev et al., 1996
GluR1/GluR2	0.70 diameter	0.38	Burnashev et al., 1996
GluR6(Q)[c]	0.75 diameter	0.44	Burnashev et al., 1996
GluR6(R)[c]	0.76 diameter	0.45	Burnashev et al., 1996
GluR6(Q)/GluR6(R)	0.74 diameter	0.43	Burnashev et al., 1996
NR1-1a/NR2A	0.55 diameter	0.24	Villarroel et al., 1995; Wollmuth et al., 1996
5HT$_3$, serotonin[d]	0.76 diameter	0.45	Yang, 1990; Yakel et al., 1990
Muscle nicotinic	0.65×0.65	0.42	Dwyer et al., 1980
Na^+ Channel in frog nerve	0.31×0.51	0.16	Hille, 1992
K^+ Channel in frog nerve	0.17 diameter[e]	0.09	Hille, 1973; see Doyle et al., 1998
Ca^{2+} Channel in frog muscle	0.6 diameter[e]	0.28	McCleskey and Almers, 1985

[a] Cultured chick cortical and diencephalic neurons.
[b] Cultured rat hippocampal neurons.
[c] Homomeric receptors.
[d] N18 neuroblastoma cells.
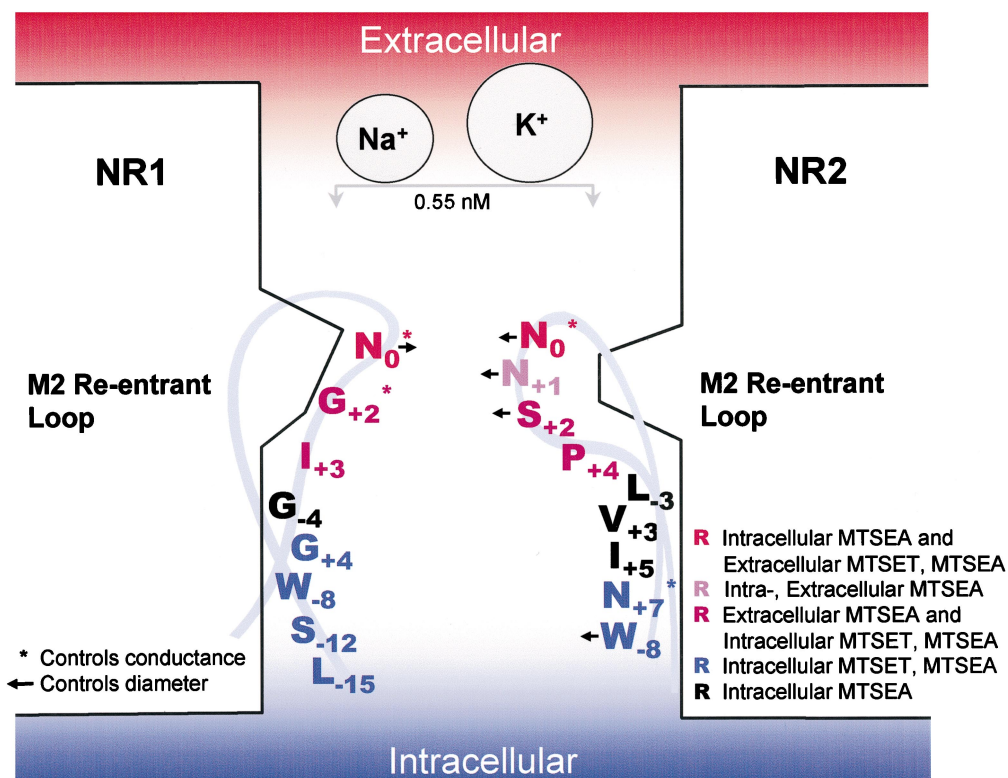[e] Diameter calculated from area.

FIG. 6. Cysteine-substituted residues in the NMDA receptor pore that are accessible to sulfhydryl reagents. The pore-forming region of the NMDA receptor is shown, with the extracellular solution and vestibule shaded red and the intracellular solution and vestibule shaded blue. Residues that are reactive with extra- or intracellular application of the thiol-modifying drugs methanethiosulfonate-ethyltrimethylammonium (MTSET; charged head group diameter 0.58 nm) and methanethiosulfonate-ethylammonium (MTSEA; charged head group diameter 0.36 nm) are shown as indicated by the color code. Residues that have been shown or suggested to control the narrow constriction of the pore are indicated by arrows. Residues at which mutations are known to alter single-channel conductances are marked with an asterisk. Figure summarizes unitary conductance data from Behe et al. (1995), Kupper et al. (1996), Premkumar and Auerbach (1997), Sharma and Stevens (1996a). Pore diameter data are from Kashiwagi et al. (1997) and Wollmuth et al. (1996, 1998a). Thiol-reactive cysteine mutageneisis data are from Kuner et al. (1996). The numbering scheme for M2 residues is that of Kuner et al. (1996), where N_0 in NR1 is Asn616 and N_0 in NR2 corresponds to Asn614 for NR2A using the initiating methionine as residue 1 rather than the cleavage site for the signal peptide, which has been assigned variable positions by different groups. Na^+ and K^+ ions shown using Pauling radii. The narrow constriction (0.55 nm; Table 1) is placed about 50% through the electric field (Zarei and Dani, 1995).

state both within a given cell type and across different preparations. Do the widely varying conductance levels observed result solely from multiple receptors or do they also reflect in part the properties of individual glutamate receptors? It now appears that most sublevels first observed in neurons can be accounted for in recombinant systems. The extreme variation in channel conductance (e.g., 0.2 versus 50 pS) for native non-NMDA receptors seems largely attributable to variable RNA editing of GluR5 or GluR6 (kainate receptors) and variable expression of fully edited GluR2 (AMPA receptors). The presence of arginine in the Q/R site lowers single-channel conductance substantially (Howe, 1996; Swanson et al., 1996, 1997; Traynelis and Wahl, 1997; Pemberton et al., 1998). A smaller contribution to variation in conductance levels is produced by subunit selection (Table 6). In addition, the varied conductance levels (and transitions between levels) of NMDA receptors observed in native neurons (Cull-Candy and Usowicz, 1987; Cull-Candy et al., 1988; Momiyama et al., 1996) closely match the multiple conductance levels of heteromeric combinations of NR1 and various NR2 subunits (Table 6). The multiple conductance levels of native non-NMDA recep-

tors (Wyllie et al., 1993) also show strong similarities with recombinant receptors (Table 6). Thus, the multi-sublevel conductances of glutamate receptors can be recapitulated in recombinant systems, providing perhaps the best evidence that sublevels arise from within the same receptor complex rather than from a heterogeneous mix of different channels expressed by neurons. Moreover, distinct receptors differing by only a single amino acid as a result of RNA editing appear likely to account for the wide variation in conductances observed across neuronal preparations.

Despite careful description of the conductance levels of neuronal and recombinant NMDA receptors, only marginal headway has been made toward understanding the basis for the different subconductance levels. A variety of possibilities have been raised as explanations for subconductance levels in general, including long-lived conformations with distinct pore properties, channels with multiple pores, channels exhibiting periods of rapid transition between open and closed states, alterations in electrostatic properties of the pore, protonation of residues that control ion transport, and physical changes in pore dimensions (Lauger, 1985; Fox, 1987;

TABLE 6
Single-channel conductance levels for various glutamate receptor subunit combinations

Receptor	pS	%	pS	%	pS	%	References
GluR2flip[a,b]					0.4		Swanson et al., 1997b
GluR4flip[b]	24	8	15	18	8	74	Swanson et al., 1997b
GluR4flip/GluR2			10	8	4	92	Swanson et al., 1997b
GluR5(R)[b-d]					0.2		Swanson et al., 1996
GluR5(R)/KA2[d]					1.0		Swanson et al., 1996
GluR5(Q)[b,d]	14	12	9	67	5	21	Swanson et al., 1996
GluR5(Q)/KA2[d]	17	5	9	18	5	77	Swanson et al., 1996
GluR6(R)[b,d,e]					0.35		Howe, 1996; Swanson et al., 1996; Traynelis and Wahl, 1997
GluR6(R)/KA2[d,e]					0.65		Howe, 1996; Swanson et al., 1996
GluR6(Q)[b,d]	25	4	15	15	8	81	Swanson et al., 1996
GluR6(Q)/KA2[d]	20	6	12	20	7	74	Swanson et al., 1996
NR1-1a/NR2A	51	77	38	23			Stern et al., 1992, 1994
NR1-1a/NR2B	51	83	39	17			Stern et al., 1992
NR1-1a/NR2C			36	75	19	25	Stern et al., 1992
NR1-1a/NR2D			35	61	17	39	Wyllie et al., 1996
NR1-1a/NR2A/N	75	ND	35	ND			Das et al., 1998

The three largest slope or chord single-channel conductance levels are shown for steady-state activation of recombinant glutamate receptors. Unless stated otherwise, glutamate (GluR1-6) or glutamate plus glycine (NR1/NR2A-D) were applied and responses were recorded in 0.85 to 1.0 mM external Ca^{2+}. The relative proportion of openings to each level is indicated as percent. ND, not determined.
[a] Similar results were obtained for both GluR2flip and GluR2flop.
[b] Homomeric receptors.
[c] Receptors were pretreated with concanavalin A.
[d] Receptors were activated by domoate.
[e] Receptors were activated by kainate; conductance was determined by variance analysis.
[f] Channel openings were recorded nM levels of extracellular Ca^{2+}; conductance levels may arise from separate channels.

Dani and Fox, 1991; Root and MacKinnon, 1994; Schneggenburger and Ascher, 1997). Although it remains unclear whether subconductance levels arise from physical changes in the pore or other more subtle changes in protein conformation, at least NMDA receptor subconductance levels do not appear to reflect rapid transitions between the open or closed state, multiple conductance paths (Premkumar et al., 1997), or protonation (Traynelis and Cull-Candy, 1991).

How might the pore properties be modified to account for subconductance levels? One interesting approach to this question relies on experiments in which all subunits of a homomeric receptor are bound by a competitive antagonist, and the dissociation of antagonist from one site (i.e., one subunit) at a time is studied in the presence of saturating agonist to evaluate the contribution of multiple agonist-binding steps to receptor function. Rosenmund et al. (1998; discussed above) have used this method to suggest that the different conductance states of homomeric GluR3 receptors might arise from channels with different numbers of bound ligands. If this interpretation proves correct (and antagonist binding does not alter subunit function), then these data suggest that consecutive ligand binding might ratchet open the pore to different dimensions or otherwise create a pore of uniform dimension but with incrementally changing properties. This might lead to different ionic permeabilities of the subconductances since the pore itself would be different. One indication that this might be true for glutamate receptors is the correlation between the unitary conductances and Ca^{2+} permeabilities among NMDA receptors containing different NR2 subunits (Burnashev et al., 1995; Stern et al., 1992; compare Tables 6 and 7). Another indication that subconductance

levels possess different ionic selectivities comes from work with mutant NMDA receptors containing NR1(N616Q), which exhibit two sublevels with different monovalent ionic selectivities (Schneggenburger and Ascher, 1997). Similar results also have been reported for mutant *Shaker* potassium channel sublevels (Zheng and Sigworth, 1997). Study of this same NR1 mutant subunit coexpressed with NR2A containing a similar mutation showed different sensitivity to divalent block between the two subconductance levels (Premkumar and Auerbach, 1996; Premkumar et al., 1997). Interestingly, similar to studies with channel-blocking compounds that suggested occupancy of the permeation path can alter channel gating (Antonov and Johnson, 1996; Bowie et al., 1998), Schneggenberger and Ascher (1997) show that permeant ions can also influence gating under certain conditions.

Do receptors activated by the rapid synaptic release of glutamate open to the same conductance levels as those produced by steady-state application of other receptor agonists (e.g., domoate, AMPA, kainate, quisqualate)? One useful approach to this question is nonstationary variance analysis (Traynelis and Jaramillo, 1998), which can be used to evaluate the weighted mean conductance of the channels that constitute the response to synaptically released glutamate. Using this method, a variety of investigators have measured a weighted mean conductance of 6 to 29 pS for native non-NMDA channels that open in response to high concentrations of rapidly applied glutamate, conditions that mimic synaptic transmission (Hestrin, 1992; Jonas et al., 1994; Raman and Trussell, 1995; Spruston et al., 1995; Koh et al., 1995b). Nonstationary variance analysis of synaptic currents mediated by non-MDA receptors has provided sim-

ilar conductance estimates (Traynelis et al., 1993; Silver et al., 1996), and also the surprising result that synaptic conductance might increase in some forms of plasticity (Benke et al., 1998). Although these conductance values are not directly comparable to those obtained from excised patches exposed to non-natural agonists, they are at least similar in range (see Table 6).

C. Ionic Selectivity

Glutamate receptors are permeable to cations and, with the exception of homomeric GluR6 and GluR2 (Table 7), largely exclude anions from the pore. Sodium and potassium are thought to be nearly equally permeable, and thus extensive comparisons between the two ions have not been made. However, because Ca^{2+} has the ability to couple electrical to biochemical signaling and alter intracellular ion concentrations (Brocard et al., 1993), its permeability through various glutamate receptors has received considerable attention. Consistent with previous results in neuronal glutamate receptors (McBain and Mayer, 1994), recombinant NMDA receptors appear to be more permeable to Ca^{2+} than non-NMDA glutamate receptor subtypes (Table 7) and other cation-selective receptors (Rogers and Dani, 1995). Ca^{2+} permeation is more complex in NMDA than AMPA receptors, and the increased Ca^{2+} permeability of NMDA receptors may reflect the presence of multiple intrapore Ca^{2+}-binding sites, which could cooperate to enhance Ca^{2+} flux (Premkumar and Auerbach, 1996; Wollmuth and Sakmann, 1998). NMDA receptors also possess lower unitary conductances in high Ca^{2+} (Jahr and Stevens, 1987; Ascher and Nowak, 1988; Gibb and Colquhoun, 1992; Iino et al., 1997; Wyllie et al., 1996), as expected if Ca^{2+} hesitates longer in the channel and thus permeates more poorly than monovalent ions. Interestingly, AMPA receptors lacking the GluR2 subunit (for which the Q/R site codon is almost 100% edited to one encoding arginine; see above) are also Ca^{2+} permeable (see Table 7), as are kainate receptors with glutamine in the Q/R site of the M2 region. Since the elu-

cidation of the molecular determinants of Ca^{2+} permeability in AMPA receptors (Hollmann et al., 1991; Hume et al., 1991; Burnashev et al., 1992a), there have been a wide range of reports demonstrating Ca^{2+}-permeable AMPA receptors in identified neurons and glia (Muller et al., 1992; McBain and Dingledine, 1993; Jonas et al., 1994; Geiger et al., 1995; Otis et al., 1995; Koh et al., 1995b; Zhang et al., 1995; Steinhauser and Gallo, 1996 and references therein; Washburn et al., 1997). The extensive regulation of Ca^{2+} permeability by RNA editing and cell-specific GluR2 expression underscores the importance of this property for neuronal and glial function. Moreover, the unedited receptors expressed by interneurons are typically rapidly gated compared with receptors in principal neurons (Geiger et al., 1995), which may contribute to the burst-firing patterns of many interneuron populations.

Mutagenesis of residues that impact permeation properties has led to partial understanding of the structural basis for ionic selectivity. The genomic codon for Gln607 in the GluR2 primary transcript is almost completely edited in the mRNA to an arginine codon (see above), and inclusion of even a single copy of this subunit in the receptor complex appears to render the resulting receptors less Ca^{2+} permeable (Geiger et al., 1995; Washburn et al., 1997). Likewise, editing of the codon at Gln621 of GluR6 reduces Ca^{2+} permeability (Table 7); editing in both receptor classes also reduces the single-channel conductance (Table 6). Interestingly, the presence of the positively charged arginine within the GluR6 pore causes a substantial increase in Cl^- permeability (Table 7). After these studies of non-NMDA receptors, homologous residues in the NR1 (Asn616) subunit and the homologous and adjacent asparagine residues in the NR2 subunit (Asn614 and 615 in NR2A) of the NMDA receptor were found to influence Ca^{2+} permeability (Burnashev et al., 1992b; Kawajiri and Dingledine, 1993; Sakurada et al., 1993; Wollmuth et al., 1996; Schneggenburger, 1998) as well as single-channel conductance (Behe et al., 1995; Premkumar and Auerbach,

TABLE 7
Ionic permeabilities relative to monovalent ions for glutamate receptors of known subunit composition

Receptor	$P_{Ca}/P_{Na,Cs,K}$[a]	P_{Cl}/P_{Na}[a]	$P_{f(Ca)}$[b]	
GluR1	2.34		0.032	Burnashev et al., 1995
GluR2		0.14		Burnashev et al., 1996
GluR1/GluR2	0.05	~0	0.005	Burnashev et al., 1995, 1996
GluR4			0.039	Burnashev et al., 1995
GluR6(R)[c]	0.12[d]	0.74	0.002[e]	Burnashev et al., 1995, 1996
GluR6(Q)	0.21–1.2	~0	0.016[e]	Egebjerg and Heinemann, 1993; Burnashev et al., 1995, 1996
GluR6(R)/GluR6(Q)		~0	0.006[f]	Burnashev et al., 1995, 1996
NR1-1a/NR2A	3.10–11	0.1–0.19		Burnashev et al., 1995; Wollmuth et al., 1996; Sharma and Stevens, 1996b; Schneggenburger, 1996, 1998
NR1-1a/NR2B	17		0.175	Iino et al., 1997; Schneggenburger, 1996
NR1-1a/NR2C	2.23		0.082	Burnashev et al., 1995

Na^+ and Cs^+ are considered equally permeable.
[a] Lewis equation derived from the Goldman-Hodgkin-Katz equation.
[b] Proportion of whole-cell current carried by Ca^{2+} determined from simultaneous measurement of whole-cell current and Ca^{2+} fluorescence.
[c] Results are shown only for studies which either directly measure Ca^{2+} flux or take into consideration the Cl^- permeability.
[d] Not significantly different from zero.
[e] Editing of transmembrane region 1 had no effect fractional Ca^{2+} current through GluR6(R), but slightly decreases fractional Ca^{2+} current through GluR6(Q).
[f] Expressed at a ratio of 1:1.

1996). In addition, mutations at acidic residues near the C-terminal end of M2 (see also discussion of GluR3 Asp616 in Dingledine et al., 1992; Chazot et al., 1993; NR1 Glu621, Schneggenburger, 1998) as well as a leucine and tryptophan residue (e.g., GluR1 Leu592, NR1 Trp611, NR2B Trp 607; Ferrer-Montiel et al., 1996, 1998; Williams et al., 1998) in or near M2 in NR1 also control Ca^{2+} permeability. In AMPA receptors, replacement of arginine by the smaller (but still positively charged) lysine in the Q/R site apparently increased Ba^{2+} permeability, as expected if this residue influences permeation (Dingledine et al., 1992).

Thus, in all three classes of glutamate receptor there are strong data arguing that structural perturbations at the Q/R/N site and several nearby residues within the M2 region of the receptor alter ion selectivity. M1 may also contribute to selectivity, although effects are not striking. The simplest interpretation of these data is that these critical residues all reside in the pore of the receptor. In such a case, either the side chains or main chains may interact with passing ions or help define the environment and conformation of the pore.

IX. Molecular Determinants of Channel Block

A. External Mg^{2+} Block of NMDA Receptors

One unique feature of the NMDA receptor compared to other ligand-gated ion channels is the dual dependence of function on agonist binding and membrane potential. This property renders the Ca^{2+} flux through NMDA receptors a coincidence detector for depolarization and synaptic release of glutamate. Interestingly, the NMDA receptor's voltage dependence follows directly from channel block by submillimolar concentrations of extracellular Mg^{2+} rather than from the voltage dependence of conformational changes (Nowak et al., 1984; Mayer et al., 1984; Jahr and Stevens, 1990a,b). Binding of extracellular Mg^{2+} within the pore is strongly voltage-dependent, and this property dominates the physiological role of NMDA receptors. At resting membrane potentials, most (but not all; see below) subtypes of NMDA receptor undergo rapid channel block by extracellular Mg^{2+}, which reduces the NMDA receptor component of synaptic currents considerably. However, when neurons are depolarized, for example, by intense activation of colocalized postsynaptic AMPA receptors, the voltage-dependent block by Mg^{2+} is partially relieved, allowing ion influx through activated NMDA receptors. The resulting Ca^{2+} influx can trigger a variety of intracellular signaling cascades, which can ultimately change neuronal function through activation of various kinases and phosphatases.

Based on the measured voltage dependence of block, Mg^{2+} has been proposed to bind to a site deep within the pore, probably near or past the middle of the electric field (Ascher and Nowak, 1988; Johnson and Ascher, 1990; Ruppersberg et al., 1994; Zarei and Dani, 1994;

Premkumar and Auerbach, 1996; Wollmuth et al., 1998a). For receptors containing NR2A or NR2B, Mg^{2+} would bind with a voltage-dependent K_d near 10 μM at -80 mV; Mg^{2+} potency at 0 mV is much lower, with a K_d of 2 to 7 mM (Wollmuth et al., 1998a). NR2C receptors are ~10-fold less sensitive (Monyer et al., 1992, 1994; Ishii et al. 1993), and the structural determinants of this decreased sensitivity appear to reside within M1, M4, and the intervening M2-M3 linker (Kuner and Schoepfer, 1996). At resting potentials, block rapidly reaches equilibrium within the duration of an NMDA receptor burst such that individual blockages and unblockages are readily apparent in single-channel records. At very hyperpolarized potentials (or in the absence of other ions; Stout et al., 1996), Mg^{2+} can be driven through the channel, suggesting that it is a permeant blocker (e.g., Mayer and Westbrook, 1987; Ascher and Nowak, 1988; Wollmuth et al., 1998a). The permeability of various divalent cations inversely correlates with their energies of dehydration (Mayer and Westbrook, 1987; Ascher and Nowak, 1988), suggesting that ions with tightly bound water have difficulty passing through the channel. The idea that permeation requires dehydration fits well with the size of hydrated blocking ions such as Mg^{2+} (0.64 nm) and the size of the NMDA receptor pore (<0.6 nm in diameter; see Table 5). Although highly permeable Ca^{2+} and poorly permeable Mg^{2+} appear to bind to separate sites on the basis of their voltage dependence of block, the extent of channel block of wild-type and mutant receptors by Mg^{2+} can be altered by extracellular Ca^{2+} (Mayer and Westbrook, 1987; Sharma and Stevens, 1996b; discussed in McBain and Mayer, 1994). Similarly, the voltage dependence can also be influenced by different intracellular ions (Ruppersberg et al., 1994).

The structural determinants of external Mg^{2+} block are strikingly similar to those governing Ca^{2+} permeability through NMDA receptors; a similar situation is also apparent in non-NMDA receptors with comparison of polyamine blockers and Ca^{2+} permeability (see below). Whereas individual substitutions may have differing effects on Ca^{2+} permeability versus Mg^{2+} block, the same residues typically influence both (Fig. 7). Residues critical for extracellular Mg^{2+} block fall into two categories: a polar Q/R/N site and a nearby hydrophobic site. Substitutions at the Q/R/N site on NR1 or at similar positions on NR2 can relieve Mg^{2+} blockade (Burnashev et al., 1992b; Mori et al., 1992; Sakurada et al., 1993; Kawajiri and Dingledine, 1993; Sharma and Stevens, 1996a; Wollmuth et al., 1998a). For example, substitution of arginine for asparagine at residue 616 of NR1 reduced Mg^{2+} blockade more than substitution of glycine, serine, glutamine, or aspartate. In contrast, the same substitutions at NR2A Asn615 all strongly reduced Mg^{2+} block (Wollmuth et al., 1998a). These results have been interpreted to suggest that the asparagine residue on NR2A may be more critical for block, perhaps by participating in Mg^{2+} chelation. Mg^{2+} block
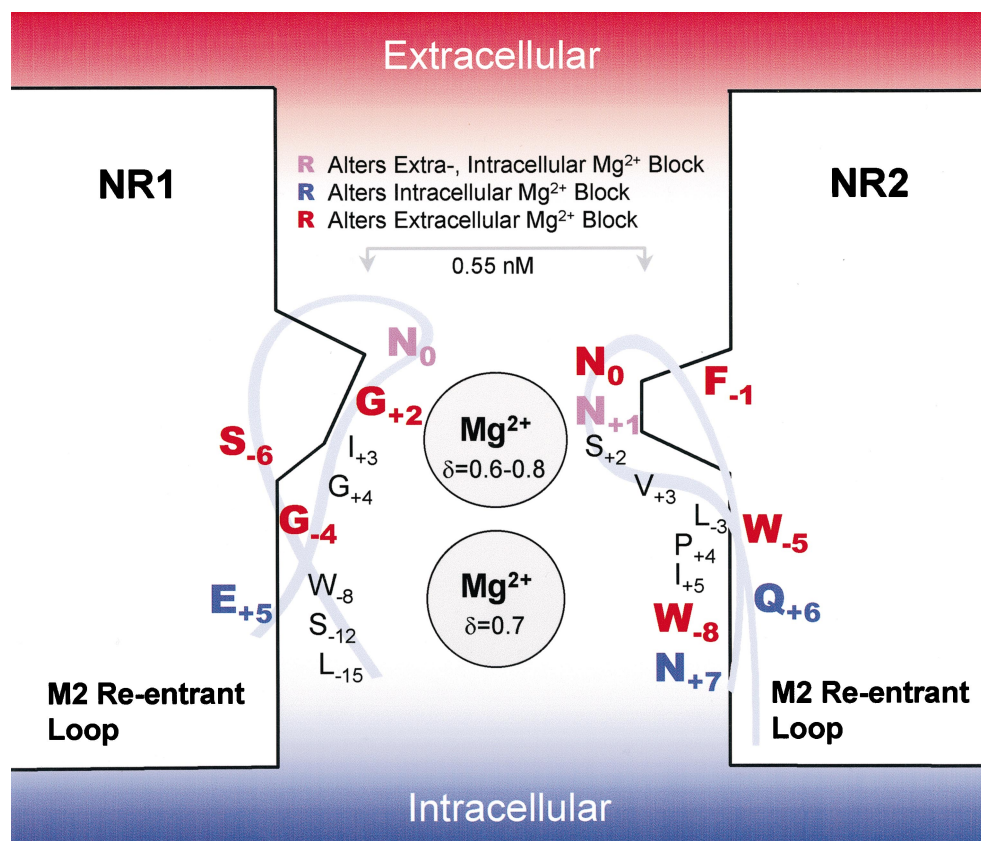
FIG. 7. Residues involved in block of the NMDA receptor by Mg^{2+}. The pore-forming region of the NMDA receptor is shown, with the extracellular solution and vestibule shaded red and the intracellular solution and vestibule shaded blue. All residues are shown for NR2A although experiments supporting their accessibility and control of voltage-dependent Mg^{2+} block were performed in receptors containing NR1 plus NR2A, NR2B, or NR2C subunits. Thus, we ignore NR2 subunit variations in pore conformation and Mg^{2+} blockade, as well as the possibility that different subsets of the same subunit in the receptor complex may contribute complementary yet distinct subsets of these residues to the pore (i.e., N_0 and N_{+1} might be on one NR2 subunit and S_{+2} and P_{+4} might be contributed by a second NR2 subunit). Residues that are presumably accessible to the pore are shown within the pore (Kuner et al., 1996; Fig. 6), even though they may contribute their main chain carbonyl groups rather than side chains to the pore environment. Bold indicates residues at which mutations have been shown at physiological potentials to reduce the voltage dependence of Mg^{2+} block, reduce the affinity of Mg^{2+} block, or increase Mg^{2+} permeability. Block by intra- and extracellular Mg^{2+} was not examined in all residues shown. Residues are color-coded to indicate whether they control extracellular Mg^{2+} blockade (red), intracellular Mg^{2+} blockade (blue), or both (purple). This figure summarizes data from Kuner et al. (1996), Kupper et al. (1996, 1998), Williams et al. (1998), and Wollmuth et al. (1998ab). δ is the distance through the electric field from outside to inside and is from Wollmuth et al. (1998a) and Li-Smerin and Johnson (1996); Mg^{2+} shown using Pauling radius.

does not seem to be determined by pore size, although the voltage dependence of block may be influenced by increased permeability of Mg^{2+} in mutant receptors with larger pore diameters (Wollmuth et al., 1998a).

Williams et al. (1998) have recently shown that certain tryptophan residues in the M2 region of NR2 also control permeability and block by Mg^{2+}. Exchange of tryptophan residues on NR2B (Trp607) and NR2A (Trp606) for nonaromatic residues (Ala, Asn, or Leu) reduced Mg^{2+} blockade, whereas similar mutations on NR1 had no effects. When Trp607 in NR2B was mutated to large hydrophobic residues (Y or F), there was no effect on Mg^{2+} IC_{50} at -70. These latter substitutions suggest that a large hydrophobic residue at this position is critical for Mg^{2+} block. These authors speculate that π bonding of delocalized electrons to the metal might be important determinants of Mg^{2+} binding, and that this residue might constitute a barrier predicted from modeling to exist between the intra-and extracellular Mg^{2+}-blocking sites (Li-Smerin and Johnson, 1996; Kashiwagi

et al., 1997). However, structural data do not support an interaction between the required consensus sequence for K^+ channel selectivity and permeating ions, suggesting that the molecular nature of the effects of mutations at Trp606/607 in the NR2 subunits requires more investigation.

The chemical nature of the intrapore Mg^{2+}-binding site is impossible to ascertain from any of the standard approaches presently used to study channel block. Nevertheless, numerous authors have proposed that the Q/R/N site and certain tryptophan residues in the NMDA receptor (NR1 Asn616, NR2A Asn 615, NR2B Trp607) physically bind to Mg^{2+} (Mori et al., 1992; Kawajiri and Dingledine, 1993; Sakurada et al., 1993; Sharma and Stevens, 1996a; Williams et al., 1998; Wollmuth et al., 1998a); the available evidence supporting NR2A-asparagine interaction with Mg^{2+} is perhaps the most compelling (Wollmuth et al., 1998a). What this means for asparagines is that a lone pair of electrons from the oxygen within the amide group would satisfy

one of the hybrid orbitals that dictate Mg^{2+} coordination chemistry. Extensive π bonding in aromatic residues such as tryptophan can also coordinate metals (Kumpf and Dougherty, 1993). If there is a ring of similar amide or aromatic groups contributed from NR1 and NR2 subunits at roughly the same position in the pore, they should be able to satisfy some of the coordination sites of Mg^{2+}. Alternatively, main chain carbonyls may particpate in coordination of Mg^{2+} at its binding site.

One might not expect full coordination of the permeating ion by the protein given the correlation between dehydration energies and lack of permeability for various divalent ions (Mayer and Westbrook, 1987; Ascher and Nowak, 1988). The correlation implies that Mg^{2+} is poorly permeable because it does not easily shed its hydration shell and remains as a hydrated complex that is too big to permeate. However, because the dehydration energy is a function of the charge-to-surface area ratio of an ion, it is also a reflection of the small diameter of Mg^{2+}. Thus, it is possible that fully dehydrated Mg^{2+} might be exactly the right diameter to form a coordination complex with geometrically fixed intrapore asparagine and/or tryptophan residues or other electron donors. In this case, the diameter of other divalent ions would dictate their ability to coordinate with this site in the pore. If coordination with such a site (and thus channel block) was dependent on small diameter, permeability would appear to be correlated with low energy of dehydration. Consistent with this notion is the finding that mutations that increase the pore diameter do not always alter Mg^{2+} blockade (Wollmuth et al., 1998a). In any model, it is important to remember that the molecular nature of the pore is uncharted and probably does not fit conventional notions of a water-filled space with a dielectric constant near $80\epsilon_o$. New techniques and structural data will be required to evaluate the nature of the complex interactions of Mg^{2+} with pore-forming residues.

B. Internal Mg^{2+} Block of NMDA Receptors

Intracellular Mg^{2+} can also exert a voltage-dependent block of NMDA receptor channels (Johnson and Ascher, 1990). Assuming that the voltage dependence of block reflects only the position of the blocker in the membrane electric field, the blocking site for internal Mg^{2+} lies $\sim35\%$ of the electric field from the intracellular side (Johnson and Ascher, 1990; Wollmuth et al., 1998b). The dissociation constant for Mg^{2+} binding is 8 mM at 0 mV, similar to that calculated for extracellular block (Ascher and Nowak, 1988). However, the absence of resolvable blocked states with internal Mg^{2+} (i.e., flickers) reflects a weaker voltage dependence of blocker affinity that is observed experimentally as an apparent reduction in single-channel current amplitudes (Johnson and Ascher, 1990). The proposed electrical distances of the internal and external Mg^{2+} sites present a problem in that access to each site entails either complete or near crossing of

the other. Resolution of this latter problem requires new models of channel block (Ruppersberg et al., 1994) or revision of our interpretation of δ as a measure of the relative position of the blocker in the electric field (Woodhull, 1973). One possible explanation to account for this dichotomy is that Mg^{2+} occupancy may be coupled to movement of the permeant ion from its site (i.e., the narrow constriction), which would steepen the apparent voltage dependence of external block (Zarei and Dani, 1994, 1995; Antonov et al., 1998). Other possibilities include the presence of a reduced dielectric constant within a portion of the pore that might increase the effectiveness with which a charge senses an electric field. Ion-ion interactions between permeating and blocking ions within the pore and permeation by Mg^{2+} upon unbinding can also complicate the interpretation of the measured values for δ (Wollmuth et al., 1998a; Kupper et al., 1998).

Mutations at the NR1 residue Gly618 disturb external Mg^{2+} blockade without affecting block by internal Mg^{2+}. Conversely, mutations made downstream of the N615 site, at NR1 Glu621, NR2A Gln620, or Asn621, appear to influence internal block by Mg^{2+} but have little effect on block by external Mg^{2+} (Kupper et al., 1996, 1998; Wollmuth et al., 1998a,b). These structural data confirm, in part, biophysical results suggesting distinct blocking sites on the basis of different Mg^{2+}-bound dwell times, with unbinding rates differing by more than 100-fold (dissociation from the internally accessible site is faster; Johnson and Ascher, 1990; Li-Smerin and Johnson, 1996). Although mutagenesis data from recombinant receptors suggests that the internal and external Mg^{2+}-blocking sites are physically distinct, mutagenesis has also hinted at complex interactions between the structural determinants of these two binding sites. For example, studies of internal Mg^{2+} block of receptors with substitutions at NR1 Asn616 and NR2 Asn615 suggest that the NR1 subunit plays a larger role in internal Mg^{2+} block than the NR2 subunit. However, single-channel studies show that this difference is due, in part, to effects of internal Mg^{2+} on open probability of these mutant channels, and suggest that NR1 N616S and NR2 N615S mutations reduce internal Mg^{2+} block to a similar extent (Kupper et al., 1998).

C. Internal Polyamine Block of AMPA and Kainate Receptors

Similar to Mg^{2+} block of NMDA channels, calcium-permeable kainate and AMPA receptors are tonically blocked at resting membrane potentials by cytoplasmic polyamine ions (Bowie et al., 1998; Rozov et al., 1998). The initial observations demonstrating that freely diffusible polyamines produce strong voltage-dependent block finally provided a molecular understanding of the complex rectification of native and recombinant calcium-permeable kainate and AMPA receptors, and additionally explained the loss of rectification observed in ex-

cised membrane patches (Bowie and Mayer, 1995; Donevan and Rogawski, 1995; Isa et al., 1995; Kamboj et al., 1995; Koh et al., 1995a; Bowie et al., 1998; Rozov et al., 1998). Bound and unbound polyamines are found in millimolar amounts in virtually all eukaryotic and prokaryotic cells (Watanabe et al., 1991). Most naturally occurring polyamines, such as spermine, spermidine, and putrescine, form complexes with nucleic acids, proteins, and phospholipids, which have implicated them in cell growth and differentiation (Pegg, 1986). More recently, however, the presence of freely diffusible polyamines in the cytoplasm (5–100 μM for spermine and spermidine) (Watanabe et al., 1991) has been shown to exert a profound effect on the gating properties of a number of ion channel families (Nichols and Lopatin, 1997; Williams, 1997a,b; Bowie et al., 1999). In view of the relatively high binding affinity of polyamines for ion channels, this property may, in fact, represent one of their most specific roles in mammalian cells.

Internal polyamine block is not exclusive to non-NMDA receptors, being first identified to account for the "intrinsic" channel gating of inwardly rectifying K^+ channels (Lopatin et al., 1994; Ficker et al., 1994; Fakler et al., 1995) and more recently for neuronal nicotinic acetylcholine receptors (Haghighi and Cooper, 1998). In contrast to K^+ channels and neuronal nicotinic acetylcholine receptors, Mg^{2+} appears to make no significant contribution to rectification of non-NMDA receptors (Bowie and Mayer, 1995). Similar to polyamine block of K^+ channels, the order of potency of naturally occurring polyamines is spermine > spermidine $\gg$ putrescine with dissociation constants calculated at 0 mV of 5 μM, 25 μM, and 1.2 mM, respectively, for homomeric GluR6(Q) channels (Bowie and Mayer, 1995). Recent kinetic studies suggest that the rate of binding by polyamines to the open channel is diffusion-limited and relatively voltage-insensitive, whereas the voltage dependence of equilibrium block is governed largely by the blocker's exit rate from the open channel (Bowie et al., 1998). Furthermore, structure-activity studies suggest that the blocker's residency time in the open channel is not determined exclusively by electrostatic attraction, as might be expected, but is jointly determined by hydrophobic interactions (Cui et al., 1998). Two amino acid residues have, at present, been identified as important structural elements for polyamine binding to the open channel. The first, the Q/R site of the M2 domain, is believed to contribute to the selectivity filter since it determines other permeation properties as described above. The second is a negatively charged aspartate, four amino acid residues downstream of the Q/R site (Dingledine et al., 1992). A recent model proposed by Washburn et al. (1997) suggests that glutamine residues at the Q/R site form a ring of carbonyl oxygens that contribute to or represent the binding site for external Ca^{2+} ions, whereas polyamines interact with the downstream aspartate as well as the Q/R site. This model explains earlier findings that replacement of aspartate with a neutral asparagine of similar size reduced polyamine block without affecting Ca^{2+} permeability (Dingledine et al., 1992).

A major difference between K^+ channels and non-NMDA receptors is the relief of polyamine block observed at extreme positive membrane potentials in the latter case. An early explanation for relief of block was that, at high membrane electric fields, cytoplasmic polyamines permeate non-NMDA receptors, which is observed experimentally as the appearance of outward current flow at very depolarized membrane potentials (Bowie and Mayer, 1995; Koh et al., 1995). This explanation is consistent with estimates of pore dimensions, which are generally accepted to be smaller for K^+ channels (0.3 nm in diameter) (Hille, 1992) than non-NMDA receptors (0.70–0.78 nm diameter) (Burnashev et al., 1996). Experimental evidence directly supporting polyamine permeation was finally obtained by comparing block with polyamines of differing cross-sectional diameter and under experimental conditions where external polyamines were the sole charge carriers (Bähring et al., 1997).

Biochemical estimates of free cytosolic concentrations of polyamines (Watanabe et al., 1991) and knowledge of their affinity as channel blockers (Bowie and Mayer, 1995) suggest that a combination of spermine and spermidine may contribute to channel block. The voltage dependence of putrescine was too weak to account for the block observed in intact HEK293 cells. The ratio of the free concentration of spermine and spermidine in biochemical studies is 1:3 (Watanabe et al., 1991). Using this information with estimates of each blocker's affinity to constrain their fits, Bowie and Mayer (1995) proposed that, from the analysis of the voltage dependence of whole-cell responses, a combination of approximately 50 μM spermine and 150 μM spermidine could account for rectification in intact cells. As yet, it is not known whether the free polyamine concentration is under dynamic regulation although there are extrusion and uptake mechanisms that may be important to consider in this context (Khan et al., 1994).

The recent proposal that polyamines may access both closed and open channels has revealed a novel activity-dependent regulation of calcium-permeable AMPA and kainate receptors that was not considered previously (Bowie et al., 1998; Rozov et al., 1998). Bowie et al. (1998) have proposed that in the closed conformation, kainate receptors possess a water-filled cytoplasm-facing cavity that is accessible to polyamines, an arrangement consistent with the accessibility profile of cysteine-substituted residues on NMDA (Kuner et al., 1996) and AMPA (Kuner et al., 1997) receptors and the structure of a K^+ channel pore (Doyle et al., 1998). Unlike the open state, occupancy of this site has been shown to be insensitive to membrane potential, which may suggest that water molecules in the pore shield

polyamines from the voltage drop across the membrane (Bowie et al., 1998) or that key determinants of polyamine binding lie just beyond the electric field in the closed conformation. Whether the acidic residue four amino acids downstream of the Q/R site in all AMPA and kainate receptor subunits contributes to this polyamine-binding site is unknown. Once in the open state, polyamines are able to "sense" the membrane electric field and re-equilibrate with their binding site in a voltage-dependent manner. The process of re-equilibration is experimentally observed as a voltage-dependent slowing of response rise times by polyamines (Bowie et al., 1998; Rozov et al., 1998). This explanation is favored by the observation that when polyamine affinity is reduced in high permeant ion concentrations, response rise times are faster, reflecting a more rapid re-equilibration of block rate (Bowie et al., 1998).

The relatively slow block of closed AMPA receptors by polyamines accounts for the facilitation of peak responses observed following a train of brief agonist pulses, which may represent a mechanism of short-term plasticity for calcium-permeable non-NMDA receptors (Rozov et al., 1998). A kinetic model of polyamine block also predicts these observations, but additionally suggests that polyamines accelerate channel closure either through an allosteric mechanism or by emptying the pore of permeant ions (Bowie et al., 1998). As yet, it is not known whether activity-dependent modulation of polyamine block fulfills an important role in the CNS. In the hippocampus, for example, basket cells of the dentate gyrus express Ca^{2+}-permeable AMPA receptors that exhibit rapid gating characteristics (Geiger et al. 1995, 1997) believed to be pivotal in defining the functional roles of these interneurons in network oscillatory activity (Jefferys et al., 1996) and feed-forward and feedback inhibition (Buzsáki and Chrobak, 1995). Although Ca^{2+} permeability and gating properties are undoubtedly important factors in sculpting neuronal behavior, these recent findings suggest that cytoplasmic polyamines may be central to plasticity mechanisms previously thought to be absent in hippocampal and cortical interneurons (McBain, 1998; Bowie et al., 1999).

X. Molecular Composition of the Pore

A. Outer Vestibules

As described above, experiments designed to probe the cross-sectional diameter of the permeation pathway concluded that NMDA receptors contain a narrow constriction between broad outer and inner vestibules. Although the molecular nature of the vestibules is unknown, recent reports suggest that the outer vestibule contains a divalent ion-binding site that occupies a shallow position within the electric field (Jahr and Stevens, 1993; Paoletti et al., 1995; Premkumar and Auerbach, 1996; Sharma and Stevens, 1996b). Similarly, studies of polyamine block suggest that polyamines may occupy an inner vestibule before gating (Bowie et al., 1998). Thus, both vestibules may serve unique physiological functions that may control glutamate receptor function.

B. Narrow Constriction and Selectivity Filter

Do residues implicated by mutagenesis studies in permeation and block actually present side chains to a presumably water-filled pore? Residues within the M2 region of both NR1 and NR2 of the NMDA receptor appear to be accessible to covalent labeling by reactive sulfur-containing ions (Kuner et al., 1996, 1997). Particularly interesting is the finding that residue NR1 Asn616 (the Q/R/N site) and equivalent positions in NR2 subunits are sensitive to channel-blocking thiol-reactive compounds applied to both the extra- and intracellular sides of the channel in the presence of glutamate and glycine. This NR1 residue has been suggested to be at the hairpin turn of the re-entrant M2 loop that lines the channel lumen on the basis of the observation that larger thiol-reactive compounds can modify residues on either side of this loop when applied internally, whereas NR1 Asn616 (and equivalent positions in NR2) are the only residues reactive toward these compounds when applied from the extracellular surface. This suggestion is supported by mutagenesis data suggesting that NR1 Asn616 and Asn615 help to form the narrowest portion of the pore (Wollmuth et al., 1996, 1998a). Moreover, other positions such as a hydrophilic stretch of amino acids on the downstream side of Asn616 are reactive to cysteine-modifying reagents, suggesting that these residues might comprise the selectivity filter and narrow pore constriction (Kuner et al., 1996; Fig. 6). Wollmuth et al. (1998a) have argued that NR2 Asn residues form the selectivity filter that discriminates external Mg^{2+} from other ions, whereas NR1 Asn616 largely acts to limit channel diameter (Wollmuth et al., 1996, 1998a; Kuner et al., 1996; Williams et al., 1998). As expected, pore-enlarging mutations in either subunit render even more residues reactive with pore-blocking cysteine-modifying reagents (Kuner et al., 1996), but unexpectedly do not alter external Mg^{2+} blockade or permeability. Thus, a central point that has emerged in recent years is the asymmetrical contribution of NR1 and NR2A residues to 1) pore diameter, 2) external Mg^{2+} block, and 3) Ca^{2+} permeability. Such asymmetry may be mirrored by AMPA and perhaps kainate receptors in which varying proportions of different subunits can be incorporated. Thus, functional results from studies of mutant and differentially edited receptors as well as biochemical data in the form of covalent-labeling experiments that suggest M2 residues reside in the pore. Moreover, the many parallels between the effects of the Q/R/N site and nearby residues in NMDA receptors and polyamine block and Ca^{2+} permeability of non-NMDA receptors suggest that the both classes of receptors might share similar pores.

XI. Association of Glutamate Receptors with Intracellular Proteins

A variety of intracellular proteins that bind to glutamate receptors have just recently been described. They appear to be structurally and functionally quite important not only for receptor targeting or clustering, but also for modulation of receptor activity and activation of signaling pathways. Moreover, receptor targeting and clustering is regulated during development (Rao et al., 1998), depends on synaptic activity, and might even play a role in LTP. For example, tetanic stimulation of hippocampal slice cultures induced long-lasting AMPA receptor clustering observed by introduction of recombinant GluR1 tagged with green fluorescent protein (Shi et al., 1998).

The yeast two-hybrid system was instrumental in the initial identification of several glutamate receptor-associated proteins, including proteins containing PDZ domains [e.g., proteins of the PSD-95 family, GRIP, AMPA receptor-binding protein (ABP)]. Some PDZ domains, which are 90-amino acid repeats that are known to be involved in protein-protein interactions, associate with the C termini of certain AMPA and NMDA receptor subunits. Coimmunoprecipitation confirmed the association of glutamate receptors with PDZ domain-containing proteins and signaling molecules (Src, calmodulin, G proteins). Some of these proteins compete for binding to the receptor (often dependent on the calcium concentration) and in some cases binding can be regulated by phosphorylation.

A. AMPA Receptors

The first ABP cloned was GRIP. GRIP contains seven PDZ domains and interacts through its fourth and fifth PDZ domains with the C-terminal motif SVKI* (*denotes a stop codon) of GluR2, GluR3 and possibly GluR4c (Dong et al., 1997). At least two other synaptic proteins interact with the C-tails of these subunits, Pick1 (Xia et al., 1998) and ABP (Srivastava et al., 1998). Like GRIP, ABP binds to the very C terminus of GluR2/3, and both proteins can form homo- and heteromultimers through PDZ-PDZ domain interactions. However, GRIP and ABP do not interact with PSD-95, another PDZ domain protein that binds to NMDA receptors (Srivastava et al., 1998). Thus, the PDZ domain-containing proteins that bind AMPA and NMDA receptors might form distinct complexes. The binding of the C-tail of GluR2/3/4c to Pick1 may be involved in the clustering of AMPA receptors. In fibroblasts cotransfected with GluR2 and Pick1, Pick1 induced clustering (Xia et al., 1998). That the GluR2 C-tail is important for receptor clustering is confirmed by the observation that injection of GluR2 C-tail peptides, which compete with the binding to Pick1 or other proteins, disrupted AMPA receptor clustering in cultured hippocampal neurons (Dong et al., 1997).

GluR2 and GluR4c, but not GluR1, GluR3 and GluR4, specifically interact with an N-ethylmaleimide-sensitive fusion protein (NSF) but not other chaperone-like proteins (Osten et al., 1998; Nishimune et al., 1998; Song et al., 1998). The homohexameric NSF is an ATPase involved in various membrane fusion events, such as inter-Golgi protein transport and exocytosis of synaptic vesicles. The synaptic NSF attachment proteins (SNAPs) are an essential component for the latter. The NSF binding site is distinct from the GRIP/ABP-binding site and resides in the cytoplasmic C-tail of GluR2 and GluR4c at about 20 to 10 amino acids from the very C-terminal amino acid. The interaction appears to occur with three residues (Lys844, Gln853, and Asn851 in rat GluR2) that are only found in GluR2 and GluR4c (Nishimune et al., 1998). GluR2, NSF, and SNAP were coimmunoprecipitated and colocalized in dendrites and axonal shafts using immunohistochemistry (Osten et al., 1998; Song et al., 1998). One NSF hexamer seems to associate with one AMPA receptor. The binding of GluR2-NSF to SNAP was ATP-dependent (Osten et al., 1998). Intracellular perfusion with synthetic peptides that compete for the NSF-binding site on AMPA receptors decreased the amplitudes of miniature EPSCs in cultured hippocampal neurons (Song et al., 1998) and AMPA-mediated EPSCs in CA1 neurons in slices within minutes (Nishimune et al., 1998). Similar results were obtained with anti-NSF antibodies in CA1 neurons, which suggeststhat postsynaptic AMPA receptor function can be regulated rapidly by NSF. From these results it is proposed that rapid (minutes to less than 1 h) AMPA receptor turnover occurs at the synaptic membrane and that NSF acts as a chaperone for (re)insertion of new or recycled AMPA receptors into the plasma membrane. In addition, F-actin plays a role in localizing AMPA receptor clusters to synapses and in some neurons also in the clustering of AMPA receptors. In cultured hippocampal GABAergic neurons, disruption of F-actin by latrunculin A disturbed the synaptic localization but not the clustering of GluR1-containing receptors (Allison et al., 1998). In contrast, in pyramidal neurons, both synaptic localization and clustering were partially reduced by latrunculin A. However, it is not known how AMPA receptors associate with actin. Finally, the A kinase-anchoring protein AKAP-79 functions as a signaling scaffold for PKA, PKC, and calcineurin at postsynaptic densities in neurons. Calcineurin is inactive when bound to AKAP, and anchoring of PKA to AKAPs seems to be required for the modulation of AMPA receptors (Rosenmund et al., 1994).

A new concept is the recently reported regulation of AMPA receptors by G proteins (Wang et al., 1997). In cultured cortical neurons and in membrane preparations, AMPA inhibited both the ADP ribosylation of $G\alpha_{i1}$ induced by pertussis toxin and forskolin-induced cAMP elevations. Both of these effects were blocked by CNQX. This demonstrates that G_i proteins were activated by

AMPA, a compound that is thought to be inactive on all metabotropic glutamate receptors. $G\alpha_{i1}$ could be coimmunoprecipitated with GluR1 by an anti-GluR1 antibody in AMPA-treated cultures but not in control cultures, suggesting an involvement of ionotropic AMPA receptors in the metabotropic signaling cascades. The mechanism of interaction between AMPA receptors and G proteins is still unclear, although an adaptor protein might be involved. In addition, AMPA application to cultured cortical neurons activated mitogen-activated protein (MAP) kinase, which could be inhibited by pertussin toxin or by lack of extracellular calcium (Wang and Durkin, 1995). The calcium entry through Ca^{2+}-permeable AMPA receptors may be responsible for the MAP kinase activation. Furthermore, AMPA induced the association of the G protein β subunit with the Ras complex, Raf kinase, and MEK1. This could result if AMPA receptor activation induced the release of $G\beta\gamma$ subunits from $G\alpha$, which in turn activates the Ras protein complex and subsequently MAP kinase. It is becoming clear from all of these findings that AMPA receptors are embedded within a complex protein network, which points to a more complicated role than simple ion transport.

B. NMDA Receptors: Signaling Molecules and Proteins Lacking PDZ Domains

A variety of signaling proteins, actin binding, and filamentous proteins can also bind to NR1, NR2A, or NR2B subunits. Src was found to be associated with NMDA receptors by coimmunoprecipitation (Yu et al., 1997; see above), tyrosine-phosphorylated NR2A and NR2B bind to the src homology domain region 2 (SH2) of phospholipase $C\gamma$ in vitro (Gurd and Bissoon, 1997), and autophosphorylated CAMKII has a high affinity for the NR2B but not NR2A C-tail (Strack and Colbran, 1998). The association between these signaling molecules suggests functional coupling. Indeed, Src can phosphorylate NMDA receptors and enhance their activity (see above). Whether phospholipase C is functionally coupled to NMDA receptors is to our knowledge unknown. However, the phosphorylation of AMPA receptors by autophosphorylated CAMKII targeted to the PSD by its interaction with the C-terminal 49 amino acids of NR2B is thought to increase synaptic strength during LTP (see above).

α-Actinin is an actin-bundling protein that binds not only to NR1 but also to the NR2B C terminus and may play a role in localization of the NMDA receptor (Wyszynski et al., 1997). Depolymerization of F-actin by latrunculin A completely dispersed α-actinin and reduced the number of synaptic NR1 clusters by 60% in cultured hippocampal neurons (Allison et al., 1998). The NMDA receptor clustered together with PSD-95 appeared to relocate to the cell bodies. In whole-cell recordings actin depolymerization induced by calcium, but not destabilization of microtubuli by colchicine, reduced

NMDA receptor activity (Rosenmund and Westbrook, 1993). Furthermore, the reduced calcium-dependent depolymerization of actin in hippocampal neurons of gelsolin knockout mice was associated with reduced current rundown of NMDA receptors (Furukawa et al., 1997). However, the finding that NMDA receptors in patches from cultured mouse neurons are mechanosensitive (Paoletti and Ascher, 1994) probably has a different explanation than an association between NMDA receptors and cytoskeletal proteins, because lysophospholipids that are thought to alter lipid packaging in membranes mimicked the effect (Casado and Ascher, 1997).

Moreover, in biochemical experiments the NR1, NR2A, and NR2B subunits interacted with the actin-binding protein spectrin (Wechsler and Teichberg, 1998). Spectrin seems to bind to different sites of the NR2B C terminus than actinin or PSD-95. The association between NR2B and spectrin can be weakened by calcium and phosphorylation by the Fyn kinase. In contrast, the spectrin interaction with NR1 can be antagonized by calcium/calmodulin or by phosphorylation of NR1 by PKA or PKC. The functional consequences of the spectrin assocation are not yet known.

Depending on the calcium concentration, calmodulin can bind to two different sites in the NR1 C terminus. Calcium/calmodulin seems to bind with high affinity close to the serines in the C1 cassette, and PKC activation can weaken this interaction (Hisatsune et al., 1997). The lower affinity binding site also binds α-actinin 2 and is found in all NR1 splice variants in the C0 cassette that precedes C1. Calcium-dependent binding of calmodulin to this site seems to mediate calcium-dependent inactivation of NMDA receptors (Zhang et al., 1998), possibly via calcium/calmodulin-dependent release from the cytoskeleton (e.g., from α-actinin). At the single-channel level calcium-dependent calmodulin binding to NR1 reduced the open probability of NMDA receptors by up to 4- to 5-fold (Ehlers et al., 1996; Hisatsune et al., 1997). The C1 cassette of NR1 not only binds calmodulin but also the protein yotiao, which is expressed in muscle, pancreas, and brain (Lin et al., 1998). The filamentous protein can be coimmunoprecipitated with NR1 in brain. Yotiao is found in the PSD fraction, is somato-dendritically localized, and colocalizes with NR1. In addition, the NR1 C1 cassette interacts with the ends of the 68-kDa neurofilament subunit NF-L, which is colocalized in dendrites and cofractionates with NR1 (Ehlers et al., 1998). Furthermore, in quail fibroblasts, this cassette was found to be crucial for the formation of receptor-rich domains (Ehlers et al., 1995). Interestingly, the clustering was disrupted by PKC activation, which was dependent on Ser890 in the C1 exon (Tingley et al., 1997). In summary, NMDA receptor activation might regulate its own activity, its association with intracellular proteins, and its clustering.

C. NMDA Receptors: PDZ Domain-Containing Proteins

In addition to the actin system, other molecules containing PDZ domains form complex networks with NMDA receptors and possibly the tubulin system, which seems to play an important role for the localization, clustering, and function of NMDA receptors. A variety of proteins from the PSD-95 family named after their first discovered member, PSD-95, can bind to the C termini of the NMDA receptor subunits. Using the yeast two-hybrid system and the NR2A C-tail as bait, Kornau et al. (1995) identified PSD-95 as an NR2A-interacting protein (reviewed by Kennedy, 1997). The PSD-95 family proteins are also called channel-associated proteins of synapses (chapsyns) and consist of at least four proteins: SAP90/PSD-95, SAP97, PSD-93/chapsyn-110, and SAP102 (Kennedy, 1997). Each chapsyn contains three PDZ domains, one src homology domain 3 (SH3) and one guanylate kinase (GK) domain. The SH3 and GK domains as well as the PSD domain can mediate protein-protein interaction. The GK domain has no kinase activity, but can bind GMP. Among the three PDZ domains of PSD-95, the second has the highest affinity for the E(T/S)XV* motif in the C termini of all four NR2 subunits as well as the NR1-3 and NR1-4 splice forms (which bear the C2′ terminus). PSD-95 is an abundant cytoskeletal protein, found in the postsynaptic fraction of synaptosomes, but it also occurs presynaptically. The C termini of NR2A, B, and C can also interact with PSD-93/chapsyn-110, SAP97, and SAP102 (Müller et al., 1995, 1996; Kim et al., 1996; Niethammer et al., 1996). All of these proteins have also been found associated with shaker-type K channel subunits and the plasma membrane Ca^{2+} ATPase isoform 4b, which have similar C-terminal motifs (Kim et al., 1998). In heterologous cells, chapsyn-110 can heteromultimerize with PSD-95 to promote clustering of NMDA receptors and Kv1.4 potassium channels, which are otherwise diffusely distributed (Kim et al., 1996). SAP97 is found predominantly in axons and in glutamatergic terminals (Müller et al., 1995). The postsynaptic protein SAP102 was coimmunoprecipitated with NR1 subunits by anti-NR1 antibodies, and binds also to the very C termini of recombinant NR2A and 2B and the cortical cytoskeleton (Müller et al., 1996; Lau et al., 1996). Another protein containing a PDZ domain but with an "inverse" structure is the synaptic scaffolding molecule (S-SCAM; Hirao et al., 1998). S-SCAM contains five C- but not N-terminal PDZ domains which interact with NMDA receptors and neuroligins, and in addition the N- but not C-terminal GK-like domain binds to SAP90/PSD-95-associated proteins (see below).

The PDZ domain contain proteins and others such as actinin and spectrin may provide a scaffold for the localization of signaling proteins to position them close together. For example, anti-PSD-95 antibodies coimmunoprecipitated NR1, NR2B, and α-actinin (Wyszynski et

al., 1997). Moreover, neuronal nitric acid synthase can bind to PSD-95 via a PDZ-PDZ interaction and could, thus, be localized close to NMDA receptors (Brenman et al., 1996). Recently, a brain-specific synaptic Ras-GTPase-activating protein, synGAP, was cloned that binds to PSD-95 (Kim et al., 1998; Chen et al., 1998). SynGAP coimmunoprecipated and colocalized with NMDA receptor subunits and PSD-95 and is enriched at excitatory synapses. In vitro, synGAP stimulated the GTPase activity of Ras, suggesting that it might be a negative regulator of Ras at certain synapses (Kim et al., 1998). However, the Ras-GTPase-activating activity of synGAP can be inhibited by phosphorylation by CAMKII, which would allow the activation of the MAP kinase pathway by Ras after NMDA receptor activation (Chen et al., 1998).

Different members of the PSD-95 family and S-SCAM can bind to several proteins that may function as scaffold proteins, e.g., neuroligins (Irie et al., 1997), cysteine-rich interactor of PDZ three (CRIPT; Niethammer et al., 1998), microtubule-associated protein 1A (MAP1A; Brenman et al., 1998), the different isoforms of guanylate kinase-associated proteins (GKAPs; Kim et al., 1997; Kawashima et al., 1997), and the SAP90/PSD-95-associated proteins (Takeuchi et al., 1997). Neuroligins are membrane-spanning cell adhesion molecules that interact with β-neurexins and form intercellular junctions. Neuroligins bind to the third PDZ domain of PSD-95 family proteins, which then could recruit NMDA receptors that have high affinity for the second PDZ-domain. The third PDZ domain of PSD-95 can also associate with CRIPT, which could link to the tubulin system, since CRIPT coimmunoprecipitated with PSD-95 and tubulin (Niethammer et al., 1998). In COS-7 cells, CRIPT reorganized the microtubuli to thick fibers and recruited PSD-95 to the microtubuli. Another link to the tubulin system may occur via the GK domain of PSD-93/chapsyn-110, which can interact with MAP1A (Brenman et al., 1998). MAP1A regulates microtubule dynamics.

The GKAPs were originally isolated using the yeast two-hybrid system with the GK domain of PSD-95 as bait. They can interact with the GK domain of SAP97, SAP102, and chapsyn-110 and are enriched in the PSDs (Kim et al., 1997; Kawashima et al., 1997). Furthermore, in cultured hippocampal neurons, the postsynaptic GKAP seems to be specifically expressed at glutamatergic but not GABAergic synapses (Naisbitt et al., 1997). The functions of GKAP are not clear, but the evidence points to an association with cytoskeletal proteins. During the development of synapses in cultured hippocampal neurons, PSD-95 and GKAPs are clustered at presumptive postsynaptic sites opposite presynaptic terminals before NMDA and AMPA receptors appear at these sites (Rao et al., 1998). Thus, it seems that the scaffold is provided before the postsynaptic receptors cluster.

Taken together, these findings point to an informationally rich cluster of signaling proteins that target glutamate receptors and probably their associated kinases and phosphatases to subsynaptic membranes. NMDA receptors are clustered by chapsyns and neurofilaments and are localized within the cell by actin filaments. Moreover, NMDA receptors not only directly bind to signaling molecules (calmodulin, CAMKII) but in addition are indirectly complexed in combination with other signaling molecules (K^+ channels and possibly Ca^{2+} pumps, and the Na^+ channel α subunit) via interaction with actin-binding and PDZ domain-containing proteins.

D. Kainate Receptors

The functional regulation of kainate receptors by association with intracellular proteins is just being uncovered. Members of the PSD-95 family (PSD-95/SAP90, SAP97, and SAP102) colocalize and associate with some kainate receptor subunits (Garcia et al., 1998). The interaction between kainate receptor subunits and SAP90 occurs via SAP90's PDZ1 domain and the GluR6 C terminus (ETMA*) and via SAP90's SH and GK domains and KA2. SAP90 clusters GluR6 and KA2 subunits when heterologously expressed. Moreover, when SAP90 was coexpressed with GluR6/KA2 or GluR6 homomers, receptor desensitization was reduced, attesting to a functional role of PDZ domain-containing proteins (Garcia et al., 1998).

XII. Genetic Regulation of Receptor Expression

The level of expression of each glutamate receptor subunit is determined at any particular time by the balance of the rates of gene transcription, mRNA translation, mRNA degradation, and protein degradation; other processes such as receptor assembly and synaptic targeting mechanisms provide additional controls over the appearance of functional receptors where they are needed. Glutamate receptor expression is not static but varies in a cell-specific manner throughout development and in response to trauma and other environmental factors. Examples are the developmental switch from NR2B to NR2C subunits in cerebellar granule cells about 2 weeks after birth (Watanabe et al., 1992), the altered expression of different AMPA receptor subunits after ischemia or seizures (Pollard et al., 1993; Pellegrini-Giampietro et al., 1994; Prince et al., 1995; Ying et al., 1997; Gorter et al., 1997), and the induction of GluR1 expression during chronic exposure to morphine or other drugs of abuse (Fitzgerald et al., 1996). The injection into the ventral tegmental area of a GluR1-expressing herpes simplex vector sensitizes rats to certain behavioral effects of morphine (Carlezon et al., 1997), pointing to a functional role for GluR1 up-regulation by opioids. The relative abundance of GluR2 among other AMPA receptor subunits differentially affects Ca^{2+} permeability and rectification, as one consequence of the variable

rather than fixed number of GluR2 subunits in an AMPA receptor (Washburn et al., 1997). All of these findings indicate that the mechanisms controlling the expression of glutamate receptor subunits are important regulatory determinants of receptor function. Below we provide a brief discussion of progress made in the past few years on the transcriptional and translational control of subunit expression. A more detailed review, including a discussion of insights from genetically modified animals, appears elsewhere (Myers et al., 1999).

A. Transcriptional Control

To date the 5'-flanking regions containing the proximal promoter have been cloned and preliminarily characterized for the following genes: NR1 (Bai and Kusiak, 1995; Bai et al., 1998), NR2B (Sasner and Buonnano, 1996; Klein et al., 1998), NR2C (Suchanek et al., 1995, 1997), GluR1 (Borges and Dingledine., 1998), GluR2 (Köhler et al., 1994; Myers et al., 1998), and KA2 (Huang and Gallo, 1997). The glutamate receptor genes characterized to date have several features in common. Promoters in all of these genes appear to be GC-rich, to lack TATA/CAAT boxes, and to have multiple transcriptional start sites. Regulatory elements have been identified both up- and downstream of the principle transcriptional start site in several genes (Suchanek et al., 1997; Huang and Gallo, 1997; Myers et al., 1998), and one expects this to be true for most of these genes. The Sp1 regulatory element as well as neuron-restrictive silencer elements appear to be present in most, if not all, of these promoters.

We are ultimately interested in understanding the mechanisms by which gene expression responds to environmental signals within an individual neuron. However, most laboratories have focused initially on understanding the basis of neuron-specific expression. For example, GluR2 is expressed nearly exclusively in neurons in the brain, and this pattern is recapitulated in cultured cortical neurons transfected with a luciferase reporter plasmid driven by a segment of the GluR2 promoter sequence as short as 250 bp (Myers et al., 1998). A combination of gel shift and mutation analysis, and cotransfection with plasmids encoding putative transcription factors, identified three functional regulatory elements in the GluR2 gene, Sp1, NRF-1, and the neuron-restrictive silencer RE1/NRSE; a 25- to 32-bp sequence was also identified that did not itself contain any regulatory elements but instead served as an important spacer or bridge sequence between the Sp1 and NRF-1 elements. The RE1/NRSE sequence that acts in some other genes as a powerful silencer in non-neuronal cells (Kraner et al., 1992) served only as a fine-tuning modulator of expression in the GluR2 promoter. Moreover, careful mutation analysis of the entire sequence showed that no single regulatory element was required for neuron-specific expression of the GluR2 gene in rat cortical and glial cultures (Myers et al., 1998). Thus, the basis

for neuron-specific GluR2 expression remains to be found (see Myers et al., 1998 for speculation on possible mechanisms).

Similar to the situation with the *GluR2* gene, the RE1/NRSE silencer makes only a minor contribution to cell-type specific expression of the *NR1* gene (Bai et al., 1998). Elements that recognize two single-stranded DNA-binding proteins were also identified in the *NR1* gene (Bai et al., 1998), but their role in neuron-specific expression is unknown. Sasner and Buonnano (1996) described an 800-bp region of the NR2B promoter that was sufficient to limit reporter expression to neurons in transgenic mice, and deletion analysis was later used to identify a 150-bp region surrounding the major transcriptional start site that confers neuron-specific expression of NR2B (M. Sasner and A. Buonnano, submitted). Again, however, the mechanism of neuron selectivity has not yet been established. Likewise, neuron-selective expression is conferred by promoter sequences for *KA2* (Huang and Gallo, 1997) and *NR2C* genes (Suchanek et al., 1997), but the regulatory elements responsible and the mechanisms conferring cell-type specificity on these promoters are yet to be identified.

B. Translational Control

The 5′ UTR of many of the glutamate receptor mRNAs is unusually long, for example, at least 282 bases for NR2A (Meguro et al., 1992; full-length mRNA may be longer), up to 481 bases for GluR2 (Myers et al., 1998), and up to 772 bases for NR2C (Suchanek et al., 1995) depending on the transcriptional start site. The exceptionally long 5′ UTR of NR2B (up to 1199 base pairs) is broken up by three introns (Klein et al., 1998). These long 5′ UTRs often exhibit stetches of high GC content and sometimes contain multiple out-of-frame AUG codons that could act as decoys for scanning ribosomes. There are up to five upstream AUGs in the GluR2 mRNA (Myers et al., 1998), and 12 in NR2B (Klein et al., 1998). In contrast, only 10% of eukaryotic 5′ leader sequences contain any AUG codons (Kozak, 1991; note that the 772 base 5′UTR of NR2C has no AUG codons). Translational suppression has been inferred for NR1 mRNA natively expressed in PC12 cells because no trace of NR1 protein can be detected in these cells despite a moderately high mRNA level (Sucher et al., 1993). However, translational supression mediated by the 5′UTR has so far been demonstrated only for the *NR2A* and *GluR2* genes.

Wood et al. (1996) used a combination of in vitro translation in rabbit reticulocyte lysate and the *Xenopus* oocyte expression system to study the translatability of NR2A mRNAs possessing different 5′ UTRs. They found that removal of most of the 282 bases of 5′ UTR increased electrophysiological responses mediated by NR1/NR2A receptors by over 100-fold. Removal of each individual upstream AUG only minimally increased translation, whereas mutations within a 15-base se-

quence of the 5′ UTR that disrupted a proposed stem-loop structure relieved translation inhibition considerably. A similar situation may hold with GluR2 translation. Myers et al. (1998) found that the 5′UTR of GluR2 mediated a 30- to 60-fold translational suppression in *Xenopus* oocytes and an in vitro rabbit reticulocyte translation system. The upstream AUG's played a minor role, but a broad region near the 5′ end of the mRNA that harbored a repeat sequence appeared to mediate much of the translational inhibition. Interestingly, transcriptional start sites straddled this control region, such that some but not all GluR2 mRNAs would be expected to be translationally suppressed. This finding highlights the need for cautious interpretation of methods (in situ hybridization, single-cell RT-PCR) designed to infer subunit distribution based on mRNA localization.

From these initial studies, it is clear that regulation of at least the NR2A and GluR2 subunits can occur at the translational as well as transcriptional level. It will be important to understand the conditions under which such translational control is actually realized in neurons, because constitutive suppression would make little biological sense.

XIII. Therapeutic Potential: Clinical Trials

It is well known that glutamate and aspartate can be neurotoxins, especially when energy supply is compromised (Greene and Greenamyre, 1996; Choi, 1998). This has given rise to the proposal that neurodegeneration associated with a variety of acute and chronic disorders (ischemic stroke, epilepsy, Parkinson's Disease, AIDS dementia, among others) may be caused in part by overactivation of glutamate receptors. Indeed, there is evidence from animal studies for marked neuroprotective effects of NMDA and AMPA receptor antagonists in models of ischemic stroke, epilepsy, and Parkinsonism. There is also growing support for the proposal that chronic pain can be relieved by antagonists of NMDA and perhaps kainate receptors (see below). Finally, there are suggestions that slowing AMPA receptor desensitization may have a cognitive enhancing effect. Rather than summarizing a large number of animal studies, we intend to review primarily evidence from clinical trials testing the use of drugs targeted to glutamate receptors in humans. The NMDA receptor antagonists are further along in clinical studies of stroke, Parkinson's disease, and pain than are the AMPA or kainate receptor antagonists.

Epilepsy has been considered a potential therapeutic target for glutamate receptor antagonists. Indeed, the common anticonvulsant valproate, in therapeutic concentrations, has been shown recently to bind to AMPA receptors in the human hippocampus (Künig et al., 1998). Thus valproate may act as an anticonvulsant partly by blocking AMPA receptors. However, epilepsy as a target, although attractive from a mechanistic point

of view, has waned in recent years with the introduction in the mid-1990s of five new anticonvulsants, and the natural reluctance of physicians to expose their patients to the possibility of an unexpected seizure with untested drugs. To our knowledge, there are no ongoing clinical trials of glutamate receptor antagonists for any of the epilepsies.

A. Ischemic Stroke

Stroke was the first clinical indication considered for glutamate receptor antagonists. Aside from the considerable challenges involved in crafting a drug that has appropriate pharmacokinetics and bioavailability, there are at least three additional hurdles to be overcome to develop a useful glutamate receptor antagonist for ischemic stroke. First, Grotta (1995) and Lees (1997) point out the extreme heterogeneity of human stroke patients, and the long delay until treatment compared with animal studies; they question whether the relatively homogenous animal models can provide information with good predictive value. They also point out the usefulness of being able to predict which stroke victims would show the strongest responses to glutamate receptor antagonists. Second, drug concentrations high enough to be neuroprotective typically block glutamate receptors in healthy brain tissue, which makes it very difficult to achieve a satisfactory side effect profile. Cardiovascular side effects (hypo- and hypertension) have been the most prominent and dose-limiting in many small-scale human studies. To solve this problem, one must either identify particular receptor subtypes that contribute to neuron damage but play little role in healthy brain or develop a drug that blocks receptors only in the ischemic region (i.e., context-dependent block). The recent demonstration that the potency of some ifenprodil analogs is increased at the acidic pHs typical of ischemic tissue (Pahk and Williams, 1997; Whittemore et al., 1997; Mott et al., 1998) points the way to optimizing the ifenprodil structure to maximize effects at acidic pH values that occur during ischemia. Third, the earlier the drug is administered the more protection one sees in animal models; therefore, one would like to identify a drug that can be taken prophylactically for those at risk for stroke. Along these lines, it may be possible to develop an ifenprodil analog with a large enough potency boost at low pH that at therapeutic concentrations the drug is inactive at normal pH (Mott et al., 1998). Low-affinity uncompetitive blockers may offer an alternative approach, because strong block would result during periods of intense activation, but recovery from block might be fast enough to have minimal impact on normal synaptic transmission.

To date, this constellation of features is not found in any individual drug, but a number of NMDA receptor antagonists have been subjected to early clinical trials for stroke. These include the uncompetitive channel blocker aptiganel (Cerestat, CNS1102), the competitive

glutamate site blocker selfotel (CGS 19755), the competitive glycine site antagonist ACEA 1021, and the ifenprodil analog eliprodil. The selfotel trial was terminated early due to lack of efficacy at tolerable doses (Davis et al., 1997), as was the eliprodil trial (reported in Lees, 1997). In the case of eliprodil (an ifenprodil analog), the drug is known to block N-, P-, and Q-type Ca^{2+} channels at doses that also block NMDA receptors (Bath et al., 1996; Biton et al., 1997); this secondary effect could be dose-limiting. Thus, the results to date have not been especially encouraging, but the marked therapeutic effects of NMDA receptor antagonists in animal models of focal ischemia gives rise to continued optimism. Phase III trials with magnesium sulfate and aptiganel are currently in progress.

Early stroke trials with the AMPA receptor antagonist NBQX were discontinued due to the insolubility of the drug, which precipitated in kidneys causing necrosis. Second-generation AMPA receptor antagonists that are much more water soluble are under development in several companies. For example, the novel water-soluble AMPA receptor antagonist, YM872, is neuroprotective in several in vitro assays (Small et al., 1998). Likewise, introduction of a methylphosphonate group into a quinoxalinedione produces a water-soluble AMPA receptor antagonist (ZK200775) which, after systemic administration in rats, appears to exhibit an unusually long therapeutic window (>4 h) for neuroprotection following occlusion of the middle cerebral artery (Turski et al., 1998).

B. Neuropathic Pain

Chronic pain, such as that due to injury of peripheral or central nerves, has often proved very difficult to treat, even with opioids. An early study concluded that chronic pain in patients associated with spinal cord injury could be markedly reduced by very low-dose ketamine (Eide et al., 1995). Subsequently several case reports indicated that systemic administration of amantadine or ketamine could substantially reduce the intensity of trauma-induced neuropathic pain, in some cases eliminating the pain altogether (e.g., Eisenberg and Pud, 1998). Small-scale double-blind, randomized clinical trials corroborated that amantadine could significantly reduce neuropathic pain in cancer patients (Pud et al., 1998), and ketamine could reduce pain in patients with peripheral nerve injury (Felsby et al., 1996), peripheral vascular disease (Persson et al., 1998), or kidney donors (Stubhaug et al., 1997). "Wind-up pain" produced by repeated pinpricking was also dramatically reduced. These findings suggest that central sensitization caused by nociceptive inputs can be prevented by these drugs. Local infiltration of ketamine (5 ml of 5.3 mM) produced transient relief from pain associated with first- or second-degree burns, but this was ascribed to a local anesthetic action (Pedersen et al., 1998). Wiesenfeld-Hallin (1998) summarized clinical studies that indicate ketamine can

also reduce the need for opiates in the treatment of severe pain. Most of these findings are very encouraging but suffer from the problem of demonstrating that the therapeutic effects of ketamine and amantadine were indeed mediated by block of NMDA receptors. In this regard, Mathisen et al. (1995) showed that chronic neuropathic orofacial pain could be relieved transiently by racemic ketamine and its two stereoisomers, with effective serum concentrations of the three drugs related to their affinity for the NMDA receptor. It will be important to examine more specific NMDA receptor antagonists to evaluate adverse effects of these drugs more carefully and to confirm these findings with larger scale clinical trials.

One potential problem with administering NMDA antagonists chronically to treat chronic pain is the impairment in memory and attention that may result (e.g., Malhotra et al., 1996). NMDA receptor antagonists such as ketamine can also produce a psychotic state in humans reminiscent of schizophrenic symptoms (Krystal et al., 1994). These considerations have encouraged investigators to explore other molecular targets. The prominence in dorsal root ganglion cells of both functional kainate receptors (Huettner, 1990) and the GluR5 subunit (Partin et al., 1993; Bahn et al., 1994) suggests the possibility of targeting kainate receptors for chronic pain. Several biotechnical and pharmaceutical companies are currently testing kainate receptor antagonists in animal models of chronic pain. For example, Simmons et al. (1998) showed that a selective GluR5 antagonist, LY382884, an isoquinoline derivative that has very low affinity for all AMPA receptors as well as for GluR6, exhibited analgesic actions in formalin-injected rats. Intraperitonial doses of LY382884 that were analgesic had no detectable ataxic effects. These results are encouraging, and one anticipates a rush of similar reports in the upcoming year.

C. Parkinson's Disease

There is ample rationale from animal studies to suspect that glutamate receptor antagonists might be beneficial in Parkinson's disease (Blandini and Greenamyre, 1998). The anti-Parkinsonian drug, amantadine, is now known to be an NMDA receptor channel blocker (Blanpied et al., 1997). Amantadine is seldom used alone due to limited efficacy. However, a small-scale clinical trial demonstrated the value of amantadine as add-on therapy with L-dopa. Amantadine reduced the severity of dyskinesias by 60% in these patients without reducing the antiparkinsonian effect of L-dopa itself (Verhagen Metman et al., 1998). It is not clear, however, whether the therapeutic mechanism involves NMDA receptor blockade or some other action of the drug. Other small-scale clinical trials with ifenprodil or dextromethorphan, both NMDA receptor blockers, were mildly supportive (Metman et al., 1998) or inconclusive (Montastruc et al., 1997).

D. Cognitive Enhancement

Both NMDA and AMPA receptors play critical roles in learning and some forms of associative memory in animals (e.g., Tsien et al., 1996). Could drugs that facilitate glutamate receptor transmission enhance certain forms of cognition in humans? One such compound under current study is the benzoylpiperidine CX516, which produces a mild relief from desensitization in AMPA receptors (Arai et al., 1996). In one double-blind, placebo-controlled study, CX516 produced a dose-dependent improvement in the ability to recall nonsense syllables for 30 elderly individuals (Lynch et al., 1997). Similarly, CX516 was reported to improve memory scores in four other placebo-controlled, double-blind tests in a small sample of young individuals (Ingvar et al., 1997). In a multicenter, placebo-controlled, double-blind randomized study of 91 Alzheimer's patients, a 10-week regimen of D-cycloserine, a partial agonist at the glycine recognition site of NMDA receptors, appeared to improve recall in an implicit memory test (Schwartz et al., 1996). How prolongation or enhancement of excitatory synaptic currents throughout the brain could lead to a selective enhancement of memory rather than an increase in "synaptic noise" that might instead be disruptive or perhaps even neurotoxic is unclear, at least to us. Nonetheless, the initial reports are reasonably encouraging; larger scale clinical trials and studies that demonstrate the mechanism of action of these drugs for improving memory would be very interesting.

XIV. Outlook

There have been three truly landmark events in the history of glutamate receptor research: the identification of the first selective NMDA receptor antagonists by Watkins and colleagues in the early 1980s, the cloning of the first cDNA encoding an AMPA receptor subunit in 1989 by Heinemann's group, and in late 1998 the identification of the first structure for a ligand-binding site by Gouaux's group. The first two discoveries triggered an avalanche of progress in the ensuing years; we can expect the same from the structural advance.

Much progress has been made in the past 5 years in the identification of the subunits and structural elements important for 1) transmembrane topology, 2) three of the four elemental functions of these receptors (i.e., ligand recognition, desensitization, and ion permeation), 3) receptor modulation by drugs, and 4) the functional consequences of cytoplasmic modifications. The fourth elementary function of ligand-gated ion channels, gating, has so far proved recalcitrant to study, although recent data suggest that new insights may be close at hand.

NMDA and AMPA receptors have received the most intense study, probably because their cDNAs were the first to be cloned. During the same time period the first wave of transgenic, knockout and knockin animals have

been generated, mostly for the NMDA receptors; results from these mice confirm the wide range of functional, behavioral, and developmental roles for glutamate receptors. The early clinical trials for treating stroke with NMDA or AMPA receptor antagonists have been a disappointment, in part due to the inability to administer a therapeutic dose of drug without adverse effects appearing. However, second-generation antagonists for AMPA and NMDA receptors are being introduced, leading to guarded optimism. Selective antagonists for kainate receptors are just now being developed. Clinical trials for relief of chronic pain by kainate and NMDA receptor antagonists are being planned, and currently it appears that pain may be the most likely initial indication to be successful clinically. For all of these reasons, the biology of glutamate receptors continues to attract significant attention and resources in biomedical research.

To date, all pharmacological approaches that manipulate glutamatergic synaptic transmission have involved the use of receptor antagonists or modulators of desensitization. Given that different subunit combinations confer specific functional features to glutamate receptors, an alternative strategy would involve the judicious genetic manipulation of subunit expression with the objective of more selectively influencing the properties of synaptic receptors. Indeed, given sufficient knowledge of the functional consequences of changing subunit stoichiometry, this genetic approach, while currently still unfeasible, may eventually offer an alternative and perhaps more systematic means to alter receptor function than the search for new antagonists.

As a final comment, the insertion of genetics into the glutamate receptor field is furnishing new technical approaches for manipulating receptor activation, and is providing the expected return on the investment by the research community of time, careers, and money. One expects efforts in this field to pay off in the next 5 years or so with the introduction of a new generation of pharmaceuticals directed to some of the most difficult clinical problems in neurology—chronic pain and stroke. Continued effort and collaboration among neurologists, physiologists, anatomists, and molecular biologists, in addition to a good deal of capital, will be necessary to realize this goal.

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