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Chapter 13

Excitatory Amino Acids and Seizures

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I. INTRODUCTION

It has been known for four decades that focal application of the acidic amino acid glutamate to the surface of the cerebral cortex evokes local epileptiform activity.[1] The recognition of glutamate's pharmacological activity as a convulsant therefore preceded by more than a decade the emerging consensus that the amino acid is the major excitatory neurotransmitter in the mammalian central nervous system. However, it was not until the development in the early 1980s of subtype-selective excitatory amino acid (EAA) agonists and antagonists that the role of EAA receptor systems in the generation of epileptic activity was fully recognized. Of particular importance was the demonstration that activation of *N*-methyl-D-aspartate (NMDA)-type EAA receptors induces a pattern of burst firing in neurons reminiscent of the paroxysmal depolarizing shifts (PDS) recorded in the epileptic brain.[2] This observation, in conjunction with the demonstration by Croucher and co-workers[3] that NMDA receptor-selective excitatory amino acid antagonists had powerful anticonvulsant activity in a variety of animal seizure models, firmly established the concept that NMDA receptors play an important role in epileptic phenomena. These initial results stimulated a great deal of further work assessing the role of NMDA receptors in various *in vivo* and *in vitro* seizure models, and more recently in human seizure disorders. This explosion of research in turn engendered great enthusiasm for the development of NMDA antagonists as antiepileptic drugs. More recently, the availability of selective non-NMDA [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate] receptor antagonists has made it possible to demonstrate that non-NMDA receptors also participate in seizure generation and are potential targets for antiepileptic drugs.[4]

In this chapter, I shall review the evidence from both *in vitro* and *in vivo* studies implicating excitatory amino acids and their receptors in the development and expression of seizures. The status of EAA

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antagonists in anticonvulsant drug development recently has been reviewed[5,6] and will not be considered in detail here.

II. ROLE OF EXCITATORY AMINO ACID RECEPTORS IN SEIZURES

A. NMDA RECEPTORS

1. *In Vitro* Studies

In vitro evidence supporting a role of NMDA receptors in acute epileptic events takes two forms. In the first, excitatory amino acids when applied to regions of the brain *in vitro* have been shown to induce burst firing and seizure-like phenomena. In the second, NMDA antagonists were shown to diminish or block the epileptic activity in well-established *in vitro* epilepsy models.

The ability of NMDA to evoke burst firing has been demonstrated in a variety of brain regions, including the hippocampus where the effect has been shown to occur both in CA1 pyramidal neurons[2] and in granule cells.[7,8] In intracellular recordings from neurons in the CA1 region of the rat hippocampal slice, Peet et al.[2] have clearly shown that NMDA and quisqualate, an AMPA receptor agonist, produce distinct patterns of excitation. Quisqualate induces a rapid depolarization that evokes tonic action potential firing similar to that produced by current injection. In contrast, NMDA evokes bursts of fast action potentials superimposed on slower rhythmic depolarizing shifts of the membrane potential (30–50 mV in amplitude). Application of the Na^+ channel blocker tetrodotoxin (TTX) abolishes the fast action potentials without affecting the underlying rhythmic potentials. The TTX-insensitive potentials evoked by NMDA are believed to be generated as a consequence of the negative slope conductance region in the NMDA current-voltage (I-V) relationship.[9,10] The rectification results from the now well-known voltage-dependent Mg^{2+} block of the NMDA receptor channel.[11,12]

As reviewed by Dingledine et al.,[13] there are a variety of *in vitro* epilepsy models that exhibit burst firing and electrographic seizure-like discharges. NMDA receptor antagonists produce complete inhibition in some of the models and partial inhibition in others, and have no effect in the remainder. The extent to which NMDA receptors contribute to the epileptiform activity in each of these models can be assessed with selective NMDA receptor antagonists. The model most sensitive to NMDA antagonists is that produced by reducing extracellular Mg^{2+}. For example, under low Mg^{2+} conditions, Schaffer collateral-commissural pathway stimulation in the hippocampal slice produces an excitatory postsynaptic potential (EPSP) of prolonged duration with secondary waves of activity.[14,15] In addition, there are spontaneous population discharges and frank seizure-like discharges with patterns of activity reminiscent of electrographic tonic-clonic seizures.[16-19] Interestingly, in the hippocampus, the spontaneous epileptiform discharges recorded in Mg^{2+}-free medium appear to be generated by pacemaker neurons of the CA3 region that exhibit spontaneous synchronous PDSs. These neurons then synaptically excite CA1 neurons that are not capable of endogenous bursting inasmuch as the epileptiform discharges in CA1 cease when it is surgically isolated from CA3.[20] Unlike the CA1 and CA3 areas, in Mg^{2+}-free medium spontaneous epileptiform discharges are not observed in the dentate gyrus although synaptic activation does produce interictal burst-like events. Epileptiform activity is also produced in the neocortex[21] and basolateral amygdala[23] on exposure to low Mg^{2+}. Low Mg^{2+} epileptiform activity in the hippocampus is suppressed by the NMDA antagonist 2-amino-5-phosphonovalerate (APV), indicating that it is dependent to a large extent on intact NMDA receptor-mediated neurotransmission.[18-20] (However, low Mg^{2+} may also induce epileptiform events by other mechanisms such as increased transmitter release or charge screening effects; see References 24 and 25). Similarly, spontaneous epileptiform activity in neocortical and amygdala slices exposed to low Mg^{2+} are also highly sensitive to NMDA antagonists,[21,22,26] although it is only completely blocked in the presence of both NMDA and non-NMDA antagonists.[27]

Epileptiform activity in hippocampal slices exposed to γ-aminobutyric acid A ($GABA_A$)-receptor antagonists is also dependent to some extent on NMDA receptor-mediated synaptic transmission. Thus, NMDA antagonists have been shown to partially, but not completely, reduce bursting in CA1 hippocampal pyramidal neurons exposed to picrotoxin or bicuculline,[28] neocortical neurons exposed to bicuculline[21,29,30] (however, see Reference 22), or amygdala neurons exposed to bicuculline.[31,32] Using selective NMDA and non-NMDA antagonists, Lee and Hablitz[33,34] were able to show that PDSs in the immature or adult rat neocortex could be completely eliminated by both NMDA and non-NMDA receptor blockade, but were only partially suppressed by antagonism of either receptor type alone. A great deal of experimental evidence supports the view that PDS events are mainly generated by synaptic receptor

currents although voltage-dependent conductances endogenous to the postsynaptic cell undoubtedly contribute to some extent.[35] It is clear that both NMDA and non-NMDA receptors participate in the generation of the potentials, but are not essential since the bursts continue in the presence of antagonists of either receptor class.

Traub et al.[35a,36] have developed a computational network model of burst firing in the disinhibited hippocampus that is useful for understanding the role played by NMDA and non-NMDA receptors in mediating the epileptiform burst firing occurring in the presence of $GABA_A$ antagonists. The various predictions of the model fit well with what is actually observed in electrophysiological recordings from brain slices exposed to $GABA_A$ antagonists. The model suggests that NMDA and non-NMDA receptors contribute to bursting in different ways. NMDA receptor-mediated synaptic currents do not initiate bursts, but serve to prolong their duration by inducing repetitive dendritic calcium spikes; these, in turn, generate sustained action potential firing in the soma. Blockade of NMDA receptors would be predicted to diminish the duration of bursts, but not to affect their initiation, as is generally observed experimentally. Non-NMDA receptors in the model serve to synchronize the network, and their blockade should prevent the occurrence of bursting. However, when non-NMDA receptors are blocked, NMDA receptors under certain circumstances can substitute for non-NMDA receptors in mediating synchronized firing so that bursting can still occur.

Another *in vitro* seizure model that is sensitive to NMDA antagonists is that produced by chronic exposure of hippocampal neuron cultures to kynurenate (a non-selective EAA antagonist) and elevated Mg^{2+}.[37] Upon withdrawal of the kynurenate and Mg^{2+}, such cultures exhibit PDSs and intense seizure-like sustained depolarizations. Although the cause of this epileptiform activity has not been well defined, it is reversed by reintroduction of kynurenate. The sustained depolarizations are also often suppressed by the specific NMDA antagonist APV, whereas the PDSs, as in the case of bicuculline-treated slices, are partially but not completely blocked by NMDA antagonists. Indeed, APV reduces the amplitude of the depolarizations and decreases their duration, as predicted by the model of Traub.[35a,36] Interestingly, similar seizure-like events occur in microcultures consisting of a single neuron, and these too are dependent on both NMDA and non-NMDA receptors.[38] Presumably, the seizures in these microcultures are mediated by autapic connections utilizing an EAA as transmitter. The various *in vitro* studies in conjunction with modeling of the hippocampal network indicate that NMDA receptors are particularly involved in prolonged seizure-like events. The importance of NMDA receptors in seizure-like phenomena is supported by studies demonstrating the *in vivo* anticonvulsant activity of NMDA receptor antagonists as discussed in the next section.

2. *In Vivo* Studies

The NMDA receptor is susceptible to blockade by drugs acting at several different sites on the receptor-channel complex. Antagonists may interact with the recognition sites for glutamate, glycine, and polyamines; or with the ionophore of the NDMA receptor-complex that can be physically occluded by channel-blocking compounds.[5] A common characteristic of these various NMDA antagonists is that they have broad-spectrum anticonvulsant activity in diverse animal seizure models. In fact, certain NMDA antagonists rank among the most potent anticonvulsant substances known. NMDA antagonists are particularly effective in the maximal electroshock (MES) test, a widely used screening model for anticonvulsant drugs.[39] In addition, NMDA antagonists are effective against seizures-induced by $GABA_A$ receptor antagonists and inverse agonists, but generally at higher doses than in the MES test.[40] Not surprisingly, seizures and lethality induced by systemic administration of NMDA are sensitive to NMDA antagonists, whereas the drugs are generally less effective in protecting against seizures caused by agonists acting at other EAA receptor subtypes. NMDA antagonists have also been reported to protect against seizures in the lithium-pilocarpine model of status epilepticus[41] (however, see Section VII). Finally, seizure protection is conferred by NMDA antagonists in genetically epilepsy-prone mice, rats, gerbils, chickens, and baboons.[40]

Overall, data accumulated during the past decade with selective NMDA antagonists support a role for NMDA receptors in virtually every seizure model. Unfortunately, clinical studies conducted to date have not been encouraging because the NMDA receptor antagonists tested have exhibited substantial toxicity. However, alternative strategies are being explored that seem to be more promising;[5,6] and indeed the recently approved broad-spectrum anticonvulsant felbamate may, at least in part, protect against seizures via its interaction with the NMDA receptor.[42]

B. NON-NMDA RECEPTORS

1. *In Vitro* Studies

As discussed in Section II.A.1, epileptiform activity, particularly the PDSs, occurring in many *in vitro* seizure models are dependent on activation of both NMDA and non-NMDA receptors. Treatment with a combination of NMDA and non-NMDA antagonists is required to substantially suppress or eliminate the epileptiform activity in these models. This clearly establishes the importance of non-NMDA receptors in mediating the epileptiform discharges in these models, but the situation is complicated by the fact that the discharges persist even in the presence of non-NMDA receptor blockers. In the case of the 4-aminopyridine (4-AP) model, the epileptiform activity is primarily dependent on non-NMDA receptors. 4-AP, a K^+ channel antagonist, is a powerful convulsant both *in vitro*[43-46] and *in vivo* (see below). The convulsant action of this drug may relate largely to its ability to promote neurotransmitter release. Both excitatory and inhibitory synaptic potentials are enhanced,[47] but the facilitation of excitation (enhancement of glutamate release) appears to be critical to the epileptiform activity. Unlike other *in vitro* seizure models, the discharges induced by 4-AP are mainly sensitive to non-NMDA antagonists[48] and have only a small NMDA component.[49] At present, the basis for the unique insensitivity of 4-AP-induced epileptiform activity to NMDA antagonists is obscure. One possible explanation is that, in contrast to the situation with $GABA_A$ antagonists, 4-AP preserves — and indeed facilitates — GABAergic inhibition so that the membrane potential level sensed by certain critical NMDA receptors is not allowed to become sufficiently depolarized to remove the Mg^{2+} block. Traub et al.[36] have speculated that in the absence of NMDA receptor current, non-NMDA receptors can by themselves generate sufficient dendritic depolarization to induce sustained bursting, and this is presumably the genesis of the bursting in 4-AP.

2. *In Vivo* Studies

The first evidence that selective non-NMDA antagonists could exert anticonvulsant effects *in vivo* was developed from studies with weak non-NMDA antagonists such as γ-D-glutamylaminomethyl sulfonate (GAMS) and 1-(*p*-bromobenzoyl)-piperazine-2,3-dicarboxylate (pBB-PzDA). When injected intracerebroventricularly, these compounds were able to protect against audiogenic seizures in DBA/2 mice[50] and were also able to block seizures induced by kainate, a non-NMDA antagonist, at doses that had little effect on NMDA-induced seizures.[4] The recent availability of systemically active, potent, and selective non-NMDA antagonists has made it possible to definitively confirm that non-NMDA antagonists have anticonvulsant activity. Indeed, like NMDA antagonists, such compounds have a broad spectrum of activity in diverse animal models.

To date, two classes of non-NMDA antagonist have been utilized in *in vivo* studies: quinoxalinediones, such as 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*F*]quinoxaline (NBQX), and 2,3-benzodiazepines, such as 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466). Among the quinoxalinediones (competitive non-NMDA antagonists), NBQX has been of particular utility since it is systemically active and has little effect on NMDA receptor responses.[51] NBQX is an effective anticonvulsant against reflex seizures[52,53] and is also protective in the MES test and against pentylenetetrazole (a $GABA_A$ receptor antagonist) seizures.[54] In addition, as has been demonstrated for less selective non-NMDA antagonists,[55] NBQX is modestly effective in protecting against the expression of kindled seizures.[56,57]

The noncompetitive non-NMDA antagonist GYKI 52466[58] has a similarly broad spectrum of anticonvulsant activity, as does NBQX.[52-54,56] However, it seems to be somewhat more effective against certain types of seizures such as those induced by 4AP,[54] which as noted above are believed to be dependent on massive synaptic glutamate release. In addition, GYKI 52466 was found to be more effective against seizures induced by systemic injection of AMPA or kainate,[54,59] although this is dependent on the experimental paradigm.[60] It has been postulated that the greater activity of GYKI 52466 in some models could be due to the fact that, as a noncompetitive antagonist, its blocking action would not be overcome by high levels of endogenous glutamate or exogenously administered glutamate agonists.[54] However, pharmacokinetic factors may also be important.[61]

Although non-NMDA receptor antagonists are effective anticonvulsants in animal seizure models, their practical utility may be limited by neurological toxicity.[54,56] Recently, however, it has been demonstrated that low, nontoxic doses of non-NMDA receptor antagonists can potentiate the anticonvulsant effects of conventional antiepileptic agents[62] and also NMDA antagonists.[57] Whether combined use of such compounds would result in improved efficacy with acceptable toxicity remains to be determined.

C. METABOTROPIC GLUTAMATE RECEPTORS

1. *In Vivo* Studies

In addition to its actions as a fast excitatory transmitter that are mediated via ionotropic NMDA and non-NMDA receptor channels, glutamate also can affect neuronal excitability by actions on G protein-linked receptors coupled to ion channel systems either directly or via second messengers. Whereas the fast excitatory actions of glutamate are analogous to the nicotinic receptor-mediated actions of acetylcholine, the metabotropic actions of glutamate can be compared to the muscarinic actions of acetylcholine. Indeed, many of the cellular effects of metabotropic glutamate receptor activation are similar to those induced by activation of muscarinic cholinergic receptors. There is also a correspondence between the actions of muscarinic and metabotropic glutamate receptor activation *in vivo*. Thus, in rodents, treatment with high doses of cholinergic agonists is well known to induce persistent seizures and neuronal damage.[63] Similarly, intrahippocampal injection of the selective metabotropic glutamate receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) in adult rats produces limbic-like seizures similar to those observed in kindling,[64] whereas systemic administration in neonatal rats produces convulsions.[65] Neuronal damage is a long-term consequence of metabotropic receptor agonist treatment *in vivo*, as is the case for muscarinic receptor agonist treatment. An additional parallel between the effects of muscarinic and metabotropic glutamate agonists is that young animals show increased susceptibility to the neurotoxic effects of both types of agents.

There is increasing evidence that metabotropic receptor activation is required for the induction of certain forms of synaptic plasticity, such as long-term potentiation.[65-68] To the extent that kindling is related to these types of synaptic plasticity, metabotropic receptor activation would be expected to play a role in kindling development (see Section V.A). As yet, no firm support for this notion has been presented, although with the recent availability of selective metabotropic glutamate receptor antagonists such data may soon be forthcoming. In any case, it has been shown that metabotropic glutamate receptor-stimulated phosphoinositide hydrolysis is enhanced by kindling[69,70] (although no changes in the expression of the mRNA coding for metabotropic glutamate receptors has as yet been detected[71]). The role, if any, of these changes in the maintenance or expression of kindling has not been defined.

2. *In Vitro* Studies

Recordings in *in vitro* slice preparations have demonstrated that activation of metabotropic glutamate receptors can result in slow neuronal excitation.[72] The ionic basis of this excitation is complex and not yet completely characterized, but may involve the closure of voltage-dependent and Ca^{2+}-dependent K^+ channels.[73-75] In addition to this postsynaptic depolarizing action, metabotropic glutamate receptor activation may decrease glutamate release via presynaptic metabotropic autoreceptors on excitatory nerve terminals.[76,77] This latter action may be mediated by suppression of N-type voltage-dependent Ca^{2+} channels.[78,79] Finally, metabotropic glutamate receptor stimulation may induce membrane potential oscillations and burst firing reminiscent of epileptiform activity in some brain areas.[80,81] By virtue of the fact that glutamate is released during epileptic events, metabotropic glutamate receptors undoubtedly play a role in epileptiform activity. However, the precise role of these receptors and their potential as targets for antiepileptic drugs remain to be defined.

There have been only a limited number of studies examining the activity of metabotropic glutamate receptor agonists and antagonists on experimental epilepsy models *in vitro*. However, in the low Mg^{2+} model (see Section II.A.1), the metabotropic glutamate receptor agonist *trans*-ACPD (and, more specifically, its active isomer 1*S*,3*R*-ACPD) is able to reduce the frequency of spontaneous epileptiform events,[82,83] presumably as a result of the presynaptic action on glutamate release noted above. *Trans*-ACPD has additional effects on epileptiform burst events in this preparation that will require further investigation.[83] The emerging availability of selective agonists and antagonists[84] should allow the role of metabotropic glutamate receptors in the development and expression of epileptiform activity to be clarified. Due to the multiplicity of metabotropic glutamate receptors and the diversity of second messenger systems to which they are coupled,[85,86] the final story is likely to be complex.

III. RELEASE OF EXCITATORY AMINO ACIDS DURING SEIZURES

Seizure-like discharges in *in vitro* systems are mediated by intense excitatory synaptic stimulation. The excitatory synaptic events result from glutamate (or aspartate) that is released from presynaptic nerve terminals. Although it seems reasonable that extracellular glutamate or aspartate levels would increase

during seizures, variable results have been obtained in studies of experimental seizures in animals. Moreover, in the cases where results have been positive, the magnitude of changes have generally been small. Early studies that did not use reuptake inhibitors often failed to see increases, presumably because the amino acids are avidly accumulated by uptake into neurons and glia.[87,88] However, some workers did observe small increases in glutamate release *in vivo* with focal[89] or generalized seizures,[90] particularly those induced by the potent acetylcholinesterase inhibitor soman.[91-93]

A recent study using a glutamate reuptake blocker demonstrated that pilocarpine, a muscarinic cholinergic agonist, is also able to produce small increases in extracellular aspartate and glutamate in the rat dorsal hippocampus.[94] This increase preceded the onset of seizure activity, and the levels actually returned to baseline during the seizure. It is noteworthy that the seizure models most likely to exhibit consistent elevations in cerebral glutamate release are those induced by cholinergic activation. Apparently the strong neuronal excitation produced by muscarinic cholinergic receptor stimulation is a more effective way of inducing glutamate release than is blockade of inhibition or exposure to excitatory amino acids. However, in one study of amygdala-kindled rats, glutamate levels in the ventricular perfusate taken immediately following a fully kindled seizure were twice the levels obtained preceding stimulation.[95]

Interestingly, several workers have observed increases in taurine release,[87,88,91,92,96] which are often greater than those of the excitatory amino acids. The significance of the changes is not known, but it may in some way be related to cell swelling.[91]

In contrast to the results of studies examining glutamate release, brain glutamate levels have been reported to decrease with sustained or repetitive seizures,[97-100] indicating that glutamate may become depleted with persistent seizure activity (status epilepticus).

Despite the variable results obtained in animal experiments, recent studies in human patients using intracerebral microdialysis have conclusively demonstrated an increase in EAA levels associated with seizures. The first such studies were conducted in anesthetized patients undergoing epilepsy surgery. Dialysis probes were inserted into epileptogenic hippocampal or cortical tissue prior to resection. A dramatic increase in extracellular aspartate and glutamate was observed during spontaneous and electrically evoked seizures; there were smaller increases in other amino acids including glycine, serine, and taurine.[101,102] More recently, During and Spencer[103] have reported microdialysis results in six awake patients being evaluated for epilepsy surgery. Dialysis probes were attached to depth electrodes inserted bilaterally into both hippocampi. Samples from the epileptic hippocampus indicated a sustained increase in the extracellular glutamate concentration that preceded the onset of complex partial seizures by 1.5 min and persisted for up to 16.5 min. Smaller increases were observed in the contralateral hippocampus beginning after the seizure onset. These results elegantly confirm that seizures are associated with the release of excitatory amino acids. Moreover, the observation that glutamate levels increase prior to the onset of electrographic seizures confirms the results obtained previously in the rat model[94] and suggests that glutamate may serve as a trigger for the transition to seizure.

IV. PLASMA GLUTAMATE ELEVATIONS IN GENETIC EPILEPSIES

There have been several reports of elevated plasma glutamate levels in patients with epilepsy and their first-degree relatives. The elevations were initially reported in kindreds with absence seizures,[104] but the observation has been extended to patients with primary generalized (absence and/or generalized tonic-clonic seizures) and partial seizures[105] (however, see Reference 106). The elevations were substantial, representing a 148–165% increase over levels in a control population for the primary generalized epilepsy patients and their unaffected first-degree relatives and a 139–177% increase for the partial epilepsy patients and relatives. It is unlikely that the plasma glutamate increases per se induce seizures because the glutamate would not be expected to cross the blood-brain barrier. Moreover, since the first-degree relatives had comparable elevations as the epileptic probands, there is not a simple relationship between the presence of the trait for increased plasma glutamate and seizures. In addition, the elevations cannot be attributed to seizures themselves or to the effects of antiepileptic medications because the relatives were seizure free. Thus, although the significance of the elevations is not obvious, it is conceivable that they could represent a marker for a defect in brain glutamate metabolism that predisposes to epilepsy. This defect is unlikely to be in glutamate dehydrogenase, the synthetic enzyme for glutamate, because leukocyte levels were no different from those of controls.[105]

V. ENDURING CHANGES IN EXCITATORY NEUROTRANSMISSION IN EPILEPSY

A. STUDIES IN THE KINDLING MODEL

The cellular basis of the increased neuronal excitability in epilepsy is not well understood. One attractive hypothesis is that there is an enhancement of excitatory neurotransmission, and indeed a great deal of effort has been directed toward evaluating this possibility. Virtually all of this attention has been focused on the kindling model of epilepsy in which the periodic delivery of an initially subconvulsive electrical stimulus to a discrete brain region (such as the amygdala or hippocampus) results in an enduring increase in excitability in the region stimulated, accompanied by generalized electrographic and behavioral seizures.[107]

Table 1 summarizes the many attempts to search for changes in excitatory neurotransmission and EAA receptors in kindling. This line of investigation was initiated by the work of Mody and collaborators,[108,109] who demonstrated that kindling induces an increase in the NMDA receptor component of excitatory synaptic potentials recorded in dentate gyrus granule cells. These investigators compared the intracellularly recorded excitatory synaptic responses of granule cells in hippocampal slices from control and kindled rats. Under normal conditions, the EPSP evoked in dentate granule cells by low frequency lateral perforant path stimulation failed to exhibit a component sensitive to the NMDA antagonist 2-amino-5-phosphonovalerate (APV). However, in slices from kindled animals, a slow Mg^{2+}- and APV-sensitive component of the EPSP was present. NMDA receptors are known to be present on dentate granule cells in high density,[110] but they apparently do not normally participate in low-frequency synaptic transmission. Kindling appears to result in an uncovering of these silent NMDA receptors. In other brain regions, such as the neocortex[111] and basolateral amygdala,[112] NMDA receptors do contribute to low-frequency synaptic transmission. However, at least in the basolateral amygdala, kindling induces the appearance of NMDA receptor-dependent epileptiform bursting[112] and an increase in the amplitude of the NMDA receptor-mediated component of the EPSP.[113] (The non-NMDA component was also enhanced as discussed below.)

In spite of the fact that there is now reasonably good evidence for an enhancement by kindling of NMDA-mediated neurotransmission in different brain regions, the basis for this increased responsiveness has been difficult to define. It has recently been reported that kindling induces an increase in the sensitivity of CA3 hippocampal pyramidal cells to NMDA,[114] implying a postsynaptic site for the plastic change. In line with this idea, some workers have been able to demonstrate effects of kindling on the biochemical consequences of NMDA receptor activation [e.g., NMDA inhibition of carbachol-stimulated phosphatidylinositol (PI) hydrolysis], while others have failed to find changes (e.g., NMDA-induced catecholamine efflux) (see Table 1). Several studies have shown an increased density of NMDA receptors in the hippocampus as assessed with radioligand binding techniques, whereas others have shown decreases or no change (Table 1). Recently, Köhr et al.[115] have reported changes in the biophysical properties of NMDA receptor single-channel currents recorded in cell-attached patches of granule cells acutely isolated from kindled animals. These changes — consisting of marked increases in the open times of the unitary currents as well as effects on their sensitivity to Mg^{2+} — could be due to differences in the NMDA receptor itself or to changes in its intracellular regulation (e.g., its phosphorylation state). The prolongation of NMDA receptor channel opening would be expected to increase the duration of the NMDA component of excitatory synaptic events, but how this would explain the previously reported recruitment of dormant NMDA receptors is not apparent.

In contrast to the view that the kindling effects on synaptic transmission are mediated postsynaptically, Rainnie et al.[113] have suggested that increased glutamate release accounts for the kindling-induced enhancement of the EPSP amplitude in the basolateral amygdala. Indeed, there is evidence from biochemical studies for enhanced stimulation-evoked glutamate release both in *in vivo* experiments[95] and in *in vitro* studies with cortical[116] and hippocampal[117,118] slices. Taken as a whole, the studies to date support the view that both presynaptic and postsynaptic factors may contribute to kindling-induced changes in NMDA receptor-mediated neurotransmission. However, as is apparent from Table 1, there is variability in the results obtained and a consistent picture has not yet emerged.

Kindling is a dynamic process that evolves through various stages. Some of the variability in the results summarized in Table 1 could therefore be due to differences in the kindling protocol and the relative times at which the measurements were made. Indeed, even within a specific study, there were often differences in the results obtained at different time points. Other differences could be due to

Table 1 Plasticity of Excitatory Amino Acid Receptor-Mediated Neurotransmission in Kindling

Receptor Type/ Brain Region	Preparation	Method	Effect	Ref.
NMDA				
Dentate gyrus	Slice	Intracellular (current clamp) recording	Uncovering of NMDA component of EPSP (24 h to 6 weeks)	108, 109
Basolateral amygdala (contralateral)	Slice	Intracellular (current clamp) recording	Enhancement of NMDA component of EPSP (2–12 weeks)	113
Hippocampus	Slice	Grease-gap recording of NMDA-induced depolarization	Enhanced sensitivity in CA3 but not CA1 (1–2 months)	114
Hippocampus	Slices	Carbachol-stimulated PI hydrolysis	Long-lasting increase in inhibitory effect of NMDA (24 h and 28–35 d), but not kainate or phorbol-12,13-diacetate	164
Amygdala, hippocampus	Slices	NMDA-induced [^{3}H]NE release, [^{3}H]TCP binding	No change at 3–6 weeks	165
Striatum, amygdala	Slices	NMDA-induced DA efflux	No change at 5–7 d	166
Amygdala, hippocampus	Homogenates	[^{3}H]TCP binding	Decreased density in hippocampus but not amygdala at 72 h	167
Hippocampus	Homogenates	[^{3}H]CPP, [^{3}H]glycine, [^{3}H]TCP binding	Increased density of all sites at 28–32 d but not 1 d	168
Hippocampus, striatum	Coronal sections	[^{3}H]glutamate (NMDA-sensitive), [^{3}H]MK-801 autoradiography	No change at 48 h, except for inconsistent reductions in glutamate binding	169
Hippocampus, neocortex, amygdala	Coronal sections	[^{3}H]glutamate (NMDA-sensitive) autoradiography	Decreased density in cortex and hippocampus at 28 d, but not at 1 d	170
Hippocampus, cortex, amygdala, striatum, nucleus accumbens, substantia nigra	Horizontal sections	[^{3}H]glutamate (NMDA-sensitive) autoradiography	Increased density in hippocampus and cortex at 5 d (ipsilateral)	171
Amygdala, hippocampus, striatum, cortex, cerebellum	Washed membranes	[^{3}H]MK-801, [^{3}H]glycine, [^{3}H]spermidine binding	Decreased MK-801 (but not other ligand) binding in amygdala at 7 d (but not 28 d) with amygdala kindling; no effect of hippocampal kindling	172
Neocortex, hippocampus, striatum, cerebellum	Homogenates	[^{3}H]TCP binding (NMDA-receptor specific)	No change at 3 d	173
Hippocampus, dentate gyrus, amygdala, pyriform cortex	Coronal sections	NMDAR1 mRNA by *in situ* hybridization	No change at 28 d	71
Hippocampus	Sections	Nr1, NR2A, NR2A and NR2B by *in situ* hybridization	Decreased expression of NR2B mRNA in dentate cells during partial rapid kindling; decreased NR1 and biphasic changes in NR2A and NR2B in full rapid kindling at 0 to 4 h, but not at later times; no changes in pyramidal cells	179
Hippocampus	Transverse sections	NMDAR1, NR2A, NR2B, NR2C, NR2D by *in situ* hybridization	No effect at 24 h and 28 d. (Increase in NMDA-displaceable [^{3}H]glutamate and [^{3}H]CPP but not [^{3}H]CGS-19755 binding at 24 h and 28 d)	180

Table 1 (continued) Plasticity of Excitatory Amino Acid Receptor-Mediated Neurotransmission in Kindling

Receptor Type/ Brain Region	Preparation	Method	Effect	Ref.
Dentate gyrus	Acutely isolated granule cells	Whole cell and single-channel recording	Longer duration open times, lower affinity for Mg^{2+}, but no effect on single-channel conductance	115
Dentate gyrus	Acutely isolated granule cells	Single-channel recording	Increased agonist sensitivity at 1 d	115a
AMPA/KAINATE				
Hippocampus	Slice	Grease-gap recording of AMPA-induced depolarization	No change	114
Basolateral amygdala (contralateral)	Slice	Intracellular (current clamp) recording	Enhancement of non-NMDA component of EPSP	113
Hippocampus	Coronal sections	[^{3}H]kainate autoradiography	Decrease at 1 d (greater in CA3 than in dentate)	174
Hippocampus	Coronal sections	[^{3}H]kainate autoradiography (high affinity)	Increase in dentate and CA3, but not CA1, with amygdala kindling; increase in dentate only with entorhinal kindling (associated with mossy fiber sprouting)	175
Neocortex, hippocampus, amygdala	Coronal sections	[^{3}H]kainate and [^{3}H]glutamate autoradiography	Bilaterally decreased densities of kainate receptor binding in dorsal hippocampus and dentate (but not other areas) and AMPA-sensitive glutamate binding in hippocampus and neocortex at 1 d, but not at 28 d	176
Hippocampus, striatum	Coronal sections	[^{3}H]AMPA, [^{3}H]kainate, [^{3}H]glutamate (NMDA insensitive) autoradiography	No change at 48 h, except for inconsistent reductions in AMPA binding	169
Hippocampus, cortex, amygdala, striatum, nucleus accumbens, substantia nigra	Horizontal sections	[^{3}H]glutamate binding (AMPA/kainate sensitive)	Increased density hippocampus and cortex at 5 d (ipsilateral)	171
Amygdala, hippocampus, striatum, cortex, cerebellum	Washed membranes	[^{3}H]kainate, [^{3}H]AMPA binding	No change	172
Hippocampus	Coronal sections	GluR1 and GluR2 mRNA by *in situ* hybridization	Bilateral enhanced expression of flip (but not flop) variants at 24 h but not 1 month (dentate, but not CA1 and CA3)	177
Hippocampus, amygdala and pyriform cortex	Coronal sections	GluR2 and KA1 mRNA by *in situ* hybridization	Bilaterally enhanced expression of KA1 (but not GluR2) in CA3 (but not other regions) at 28 d	71
Hippocampus	Coronal sections	GluR1 mRNA by *in situ* hybridization	Decreased expression of GluR1 at 24 h but not at 2 h	178
Limbic forebrain, piriform cortex/ amygdala, hippocampus, entorhinal cortex	Dissected brain regions	Quantitative Western blotting with subunit specific antibodies	Decrease in GluR2 (but not GluR1 or GluR4) in limbic forebrain and piriform cortex/amygdala at 24 h, but not at 1 week or 1 month	119a

Note: All studies were performed in the rat.

variability in the fundamental mechanisms underlying kindling in different brain regions. On the other hand, there is reason to question the importance of any changes in NMDA receptor number or functional activity as a fundamental mediator of the enhanced excitability of kindling. As will be discussed in Section VI.A, NMDA receptor antagonists are highly effective in preventing the development of kindling, but have far less activity against fully kindled seizures. Therefore, while NMDA receptors are clearly involved in the kindling process, their role in the expression of seizures in fully kindled animals is less certain. If plasticity of NMDA receptors is not the key to kindling, is it possible that changes in non-NMDA receptors account for the enhanced excitability? With the exception of a report demonstrating an enhancement of non-NMDA receptor-mediated EPSP's in the basolateral amygdala,[113] there is little physiological support for this concept. Moreover, as is the case for NMDA receptors, studies of non-NMDA receptor binding have revealed variable effects (Table 1). However, two recent reports have demonstrated selective enhancements in the expression of specific non-NMDA receptor subunit mRNAs in the dentate gyrus and other regions of kindled rats. Thus, the possibility that there are plastic changes in non-NMDA receptors is worthy of further investigation. However, the recent demonstration by Meldrum et al.[119] that non-NMDA receptor antagonists are not more effective anticonvulsants in kindled rats than in other epilepsy models does not provide strong encouragement that non-NMDA receptor upregulation specifically mediates the increased excitability associated with kindling. Interestingly, a recent study using quantitative Western blotting has demonstrated a transient, regionally specific decrease in the expression of GluR2 subunit protein in amygdaloid kindled rats.[119a] AMPA receptors lacking the GluR2 subunit have inwardly rectifying current-voltage relationships and high Ca^{2+} permeability.[119b,119c] It is conceivable, therefore, that the enhanced expression of Ca^{2+}-permeable AMPA receptors could play a role in the development of kindling. Moreover, it has also been demonstrated that persistent seizure activity can result in reduced expression of GluR2 subunit mRNA in hippocampal regions most vulnerable to neurodegeneration.[119d] This reduction occurs with a time course that parallels the neuronal cell loss, supporting a role for Ca^{2+}-permeable AMPA receptors in the seizure-induced pathology.

Although most studies of excitatory amino acid receptors in animal models of epilepsy have used the kindling paradigm, Geddes et al.[120] examined the densities of NMDA and non-NMDA receptors in the frontal cortex and hippocampus of two baboon species; one had photosensitive epilepsy, and the other did not. There were no differences noted in the densities of either receptor type.

B. STUDIES OF HUMAN TISSUE

What is the relevance of the animal experiments with the kindling model to human epilepsy? There have been three studies of excitatory amino acid (EAA) receptor binding in tissue obtained at surgery from patients undergoing temporal lobe resection for medically intractable complex partial epilepsy. In the first of these there was a loss of [^{3}H]kainate and NMDA-sensitive [^{3}H]glutamate binding in areas of the hippocampus with mesial sclerosis.[121] However, there was an increase in binding of the two ligands in the parahippocampal gyrus. Another study of epileptic hippocampi also found decreases in the density of NMDA receptors (in area CA3) as assessed by [^{3}H]thienylphencyclidine (TCP) and NMDA-sensitive [^{3}H]glutamate binding.[122] However, there was a 91–108% increase in [^{3}H]AMPA binding in the dentate gyrus; other regions of the hippocampus showed no changes. The third study[123] also showed reductions in [^{3}H]TCP binding in various regions of the hippocampus, but there were increases in the binding of [^{3}H]glutamate (in the presence of quisqualate to label NMDA receptors) and [^{3}H]glycine (in the presence of strychnine to label the glycine site on NMDA receptors) in CA1 and the dentate gyrus. This study also found an increase in [^{3}H]glutamate binding to non-NMDA receptors in CA1 but not in the dentate. It is difficult to know the meaning of these various changes in receptor density. It has been proposed that the increases in non-NMDA receptors could be associated with the aberrant sprouting of mossy fibers that has been observed in patients with complex partial epilepsy,[124,125] and could contribute to the increased hippocampal excitability in this condition. On the other hand, the decreases in NMDA receptors (particularly as assessed with channel-blocking ligands such as [^{3}H]TCP) may reflect selective loss of NMDA-receptor bearing neurons, which would be expected to be especially sensitive to glutamate-mediated excitotoxicity.

Because of the lack of available tissue, there is little information on excitatory amino acid receptors in human seizure disorders other than medically intractable complex partial epilepsy. However, in a study of five hippocampi obtained from children age 8 months to 15 years dying of various severe epilepsy syndromes, Represa et al.[126] have reported an increase in high-affinity [^{3}H]kainate binding sites in the

CA3 region and the supragranular layer of the fascia dentata that was believed to be due to mossy fiber sprouting.[127]

VI. ROLE OF EXCITATORY AMINO ACID RECEPTORS IN EPILEPTOGENESIS

A. NMDA RECEPTORS

Although the role of excitatory amino acid receptors in mediating the persistent increase in neuronal excitability associated with kindling is uncertain, there is overwhelming evidence that NMDA receptors are required for the development of kindled seizures since NMDA antagonists delay or prevent kindling. The large body of literature addressing this issue includes studies with competitive NMDA recognition site antagonists, noncompetitive channel-blocking compounds, and even glycine site antagonists (see, e.g., References 95, 128–135). While such antagonists are highly effective in blocking the development of kindling, they have only weak anticonvulsant activity against fully kindled seizures; and at doses that protect against seizures, they may produce neurological impairment,[95,136,137] adverse behavioral effects,[137] and epileptiform EEG changes.[138]

NMDA antagonists have also been demonstrated to prevent the development of a kindling-like phenomenon that can be induced in the *in vitro* hippocampal slice with repeated electrical stimulation.[139,140] Regenerative, all-or-none seizure-like discharges can be evoked in such kindled slices under various conditions. The development of these epileptiform events is blocked by NMDA antagonists. However, as in the *in vivo* situation, NMDA antagonists do not inhibit the seizure-like discharges once the epileptic state has been established. Recently, it was demonstrated that *in vitro* kindling is associated with a marked increase in CA3 axon terminal hyperexcitability exhibited by the appearance of spontaneous (ectopic) antidromic action potentials. The induction of terminal hyperexcitability, as is the case for the other manifestations of the epileptic-like state, is prevented by APV, whereas the drug has no effect on the ectopy itself.[141] Taken together, the results obtained with the *in vitro* kindling model strongly support the concept that NMDA receptors, while critical to the induction of the seizure-prone state, are not required for its maintenance or expression (see Section V.A).

B. NON-NMDA RECEPTORS

In contrast to the situation for NMDA receptors, non-NMDA receptors are important in the expression and generalization of kindled seizures, but do not appear to play a role in the kindling induction.[142] Thus, the non-NMDA antagonists GYKI 52466 and NBQX have been found to reduce behavioral kindled seizures and electrographic afterdischarges, but not to retard the development of kindling, which upon discontinuation of the drugs, is expressed at the same level as controls not receiving drug treatment.[142a] An analogous situation is seen in the hippocampal slice preparation where non-NMDA receptor antagonists are involved in the expression of kindled burst firing, but are not required for the development of the kindled state.[143] Indeed, as long as afterdischarges mediated by NMDA receptor activation are evoked by the stimulation protocol, kindling epileptogenesis can occur in the presence of non-NMDA antagonists.

VII. EXCITATORY AMINO ACID RECEPTORS AS MEDIATORS OF SEIZURE-INDUCED NEURONAL PATHOLOGY

In patients with epilepsy, prolonged seizures may cause neuronal cell loss, particularly in the hippocampus.[144-146] Excitotoxicity produced by excessive glutamate release (see Section III and Reference 147) perhaps in the setting of metabolic compromise[148,149] could contribute to the pathology. Seizure-induced pathological damage to neurons has been observed in several model systems both *in vivo*[150,151] and *in vitro*.[37,152] The cytopathological changes occurring in the hippocampus following prolonged seizure activity resemble those produced by excitotoxins.[153,154] Vulnerable neurons in the CA1 and CA3 areas and hilus of the fascia dentata show dendritic swelling with distended mitochondria and somal necrosis, but there is sparing of axons as with excitotoxins.[155] Despite the strong suggestion that these seizure-induced pathological changes are due to activation of EAA receptors, the areas of vulnerability are not specifically correlated with the presence of high densities of EAA receptor subtypes, indicating that factors other than receptor density account for the sensitivity of certain cell populations to seizure-induced damage.[156]

As discussed in Section II.A.1, intense seizure-like activity occurs in hippocampal neuron cultures when withdrawn from chronic excitatory amino acid antagonist treatment. In these cultures, persistence

of the epileptiform activity for more than a brief period of time results in extensive neuronal death. Although the seizure-like electrical events appear to be dependent on activation of both NDMA and non-NMDA receptors, survival can be enhanced by exposure to the NMDA antagonist APV alone.[37] Thus, in this *in vitro* system, there is a dissociation between the anticonvulsant activity of NMDA antagonists and their ability to protect against seizure-induced neurotoxicity. On the basis of experiments with a different but generally comparable culture system (low Mg^{2+}, glycine supplemented), it has been demonstrated that the neuronal death is produced by abnormal elevations of intracellular Ca^{2+} consequent to NMDA receptor activation.[152]

Kainic acid, a potent excitotoxin, produces an *in vivo* seizure model that illustrates the potential of prolonged seizures to induce brain damage. Unlike other selective EAA agonists that typically produce explosive seizures followed by death, systemic or intracranial injection of kainic acid induces a characteristic syndrome similar to that exhibited by kindled animals (rearing on hind limbs, peroral frothing, and head and forepaw clonus). However, in contrast to kindled seizures that are self-limited, kainic acid-treated animals exhibit increasingly severe and frequent seizures; thus in the later stages of the syndrome, the seizures may occur continuously producing a state referred to as "limbic status epilepticus".[153,157] Prolonged periods of limbic status epilepticus are associated with widespread neuronal damage, both at the site of the injection (when the toxin is administered intracranially) and also at sites that are anatomically removed. It has been argued that the distant damage is mediated via the propagation of seizure activity along axonal pathways with the resultant killing of certain vulnerable synaptic targets. Given the cytopathological appearance of the affected distant brain regions, it is reasonable to suppose that killing of target neurons occurs via an excitotoxic mechanism, just as in the cell culture models discussed above. However, this has not as yet been definitively demonstrated. In any case, it is interesting to note that the pattern of damage produced by kainic acid is similar to that associated with human temporal lobe epilepsy.

Although it seems logical that EAA antagonists would protect against distant damage occurring during sustained seizures, there are only a few reports specifically addressing this issue. However, in a study of sustained focal motor seizures induced by pial application of bicuculline in rats, NMDA antagonists were found to protect thalamic neurons from degeneration.[158] Interestingly, as in the *in vitro* system discussed above, the NMDA antagonists did not eliminate the persistent electrographic activity recorded from the cortex and thalamus. A similar situation applies in lithium-pilocarpine status epilepticus where NMDA antagonists paradoxically do not prevent the intense electrographic seizure activity seen in this model, but may nevertheless be neuroprotective.[41,159-161] Thus, NMDA antagonists might have neuroprotective actions in status epilepticus apart from their anticonvulsant activity. In contrast, non-NMDA antagonists are more effective in suppressing electrographic seizure activity, but may also have neuroprotective activity in some situations.[162,163] Ultimately, optimal treatment of status epilepticus may require combined use of both non-NMDA and NMDA antagonists.

VIII. CONCLUSION

The field of epilepsy research has been an important beneficiary of the fundamental progress in the understanding of excitatory neurotransmitter systems that has been made possible, to a large extent, by advances during the past decade in the pharmacology of EAA receptors. It is now clear that both NMDA and non-NMDA receptors participate in a fundamental way in the expression of epileptiform activity in *in vitro* models and also in behavioral seizures in the intact animal. Moreover, there is an emerging appreciation of the potentially important role of metabotropic glutamate receptors in epileptic phenomena. At least in the kindling model, NMDA receptor activation appears to be critically important for the development of the tendency to exhibit repeated seizures. This key observation suggests that NMDA receptors may play a central role in the pathogenesis of at least some forms of epilepsy in humans. There is less certainty regarding the role of EAA systems in the maintenance of the epileptic hyperexcitable state. Both pre- and postsynaptic factors seem to be important but the contributions of each may vary depending on the circumstances. Despite the progress made to date, in the final analysis, the cellular basis of human epilepsy remains a mystery. Nevertheless, the effectiveness of EAA antagonists as anticonvulsants in diverse seizure models suggests that EAA receptors represent promising targets for drug therapy. Moreover, the clear fact that NMDA receptor antagonists protect so effectively in the induction of kindling suggests that they may have utility in epilepsy prophylaxis, for example, after head trauma or in certain progressive childhood seizure disorders. Of course, there is the concern that learning, normal

developmental changes, or useful synaptic remodeling such as that occurring during recovery from injury may be depressed by NMDA receptor blockade. Further research is necessary to determine whether these fears are warranted.

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