CHAPTER 6

Epilepsy

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1. Introduction

Epilepsy, a condition of recurrent, paroxysmal seizures, is a major, worldwide, health problem. The epileptic seizure represents an abnormal synchronized discharge among a large population of central neurons. Although the cause of this synchronized activity is largely a mystery, the occurrence of seizures in an epileptic patient can be diminished or eliminated by drugs that target the basic excitability mechanisms of neurons. Most presently used antiepileptic medications were developed by empirical drug screening or serendipity. However, in the past several decades, as understanding of the functional activity of brain circuits has increased, there has been an intense effort to develop new antiepileptic medications on a rational basis. Although progress has been measured, some rationally developed antiepileptic drugs are now entering the market.

The focus of attention for the rational design of antiepileptic drugs has been to target, either directly or indirectly, ion-conducting channels—the fundamental mediators of electrical excitability in neurons. There are two major classes of neuronal ion channels: voltage-dependent channels that shape the subthreshold electrical behavior of the neuron, allow it to fire action potentials, and regulate...
its responsiveness to synaptic signals; and neurotransmitter-regulated channels that mediate the synaptic signals. Each of these two classes of channel participates in the electrical events of the seizure. Voltage-dependent channels contribute to the paroxysmal depolarizing shift, the single cell correlate of the interictal (between seizure) network discharge. Voltage-dependent channels also mediate action potentials that allow transmission of the seizure discharge along axons to neighboring cells and distant brain sites. The neurotransmitter-regulated channels allow the abnormal discharge to propagate between neurons within the neuronal aggregate and also participate in the paroxysmal depolarizing shift. The voltage-dependent ion channels include channels selective for \( \text{Na}^+ \) and \( \text{K}^+ \), that shape the subthreshold behavior of neurons and mediate action potentials as well as channels selective for \( \text{Ca}^{2+} \) that appear to play a critical role in absence seizures (see Section 7.). Neurotransmitter receptor channels include the fast-acting channels for the major excitatory and inhibitory neurotransmitters, glutamate and GABA, as well as for regionally specific transmitters such as monoamines (catecholamines, serotonin, and histamine) and neuropeptides. In addition to rapid actions, the transmitters can mediate slower “modulatory” signals, where the receptor is coupled to an ion channel via G-proteins or other second messengers.

In principle, it may be possible to prevent the occurrence of seizures by targeting any one or a combination of these systems, so that there is an enormous variety of possible anticonvulsant strategies. The ultimate goal is, of course, to prevent the generation or propagation of seizure discharges without interfering with the normal functioning of the nervous system. (An additional and perhaps more satisfying goal is to prevent the development of epilepsy; there are some important leads along these lines as discussed in Section 3.1.) Given the critical role of the neuronal excitability mechanisms mediating seizures for normal brain function, it would seem improbable that an anticonvulsant drug could be developed which suppressed seizures without producing side effects. Nevertheless, several presently available anticonvulsant drugs approach this goal, indicating that it is attainable.
In this chapter, I consider a variety of approaches that are currently under investigation for the rational development of new antiepileptic drugs. The mechanisms of presently available antiepileptic drugs has been reviewed previously (Rogawski and Porter, 1990), and are not considered in detail here.

2. Potentiation of Synaptic Inhibition

Enhancement of synaptic inhibition mediated by GABA$_A$ receptors is an important anticonvulsant strategy. This can occur by targeting of the GABA$_A$ receptor itself, or by enhancement of synaptic GABA levels. Drugs that enhance GABA$_A$ receptor-mediated inhibition show a distinctive spectrum of activity in animal seizure models. Such drugs typically protect very effectively against pentylenetetrazol seizures, but have far weaker activity in the maximal electroshock test. Despite the selectivity of their effects in animal seizure models, GABA$_A$-receptor potentiating anticonvulsants may have a broad spectrum of activity in human seizure disorders. However, they are generally ineffective in absence seizures. (Benzodiazepine receptor agonists that do have anti-absence activity are a notable exception to this rule.)

2.1. GABA$_A$ Receptors as Targets for Antiepileptic Drugs

Presently available drugs that potentiate GABA$_A$ receptor responses fall into two broad classes: benzodiazepine-like and barbiturate-like modulators. In addition, there are a variety of agents, including neuroactive steroids and loreclezole, that act similarly to barbiturates, but probably interact with distinct sites on the GABA$_A$ receptor complex.

2.1.1. Novel Benzodiazepine Receptor Ligands

Benzodiazepine receptor agonists, such as diazepam and lorazepam, have powerful anticonvulsant properties as a result of their ability to enhance GABA$_A$ receptor-mediated neurotransmission in the central nervous system (CNS). Although such benzodiazepine agonists have an important role in the acute treatment of status epilepticus (continuous seizures without return to consciousness),
two significant limitations largely preclude their chronic use in epilepsy therapy. The first limiting factor is that these benzodiazepines produce undesirable side effects, including sedation and muscle relaxation, at doses comparable to those that protect against seizures. The second, and probably more important, limitation is that tolerance develops to the anticonvulsant activity of benzodiazepines receptor agonists with chronic use. In recent years, considerable effort has been directed toward developing strategies for overcoming these limitations. A variety of benzodiazepines and nonbenzodiazepines have been identified that interact with the benzodiazepine binding site of the GABA<sub>A</sub> receptor complex in novel and potentially more favorable ways. As yet, however, there is no convincing evidence that any of these benzodiazepine receptor ligands will have practical utility in the chronic treatment of epilepsy.

2.1.1.1. Benzodiazepine Receptor Partial Agonists. Partial agonism at the benzodiazepine receptor has been proposed by Haefely (see Haefely et al., 1990) as a promising approach to overcome the undesirable side effects and the propensity to the development of tolerance of full benzodiazepine agonists. Partial agonists have low intrinsic efficacy and induce smaller responses than do full agonists at the same fractional receptor occupancy. Thus, at full receptor occupancy, partial agonists produce submaximal biological responses. However, the degree of partial agonism may vary depending on the subunit composition of the GABA<sub>A</sub> receptor (Knoflach et al., 1993). At anticonvulsant doses, partial benzodiazepine receptor agonists may produce less sedation and muscle relaxation than full agonists, and may also exhibit less tolerance and physical dependence.

Benzodiazepine receptor partial agonists have been identified within several structural classes, including true benzodiazepines (bretazenil, FG 8205; Martin et al., 1988; Tricklebank et al., 1990), β-carbolines (abecarnil, ZK 91296; Stephens et al., 1990; Petersen et al., 1984), pyrazoloquinolines (CGS 9896; Bernard et al., 1985), imidazopyrimidines (divaplon; Gardner, 1988), and quinolizinones (Ro 19-8022; Jenck et al., 1992). Some partial agonists—for example, Ro 19-8022—have anticonvulsant potency and efficacy in animal seizure models comparable to full benzodiazepine receptor agonists
(Jenck et al., 1992; Facklam et al., 1992a; Jensen et al., 1984). However, Ro 19-8022 has lower efficacy for sedation and muscle relaxation, and may also have reduced physical dependence liability. Moreover, Ro 19-8022 does not exhibit proconvulsant activity (like benzodiazepine inverse agonists); nor is an abstinence syndrome (characterized by tremors and seizures) precipitated when chronically treated animals are exposed to a benzodiazepine receptor antagonist. Interestingly, in line with the efficacy of conventional benzodiazepines in human absence epilepsy, Ro 19-8022 has been reported to be effective in a rodent genetic model of absence epilepsy. Other benzodiazepine receptor partial agonists, including bretazenil (Haigh and Feely, 1988; Facklam et al., 1992a) and divaplon (Feely et al., 1989; Deacon et al., 1991) similarly display potent anticonvulsant activity, but less sedation and anticonvulsant tolerance than diazepam. Studies using recombinant GABA\(_A\) receptor subunits have demonstrated that bretazenil and divaplon possess 35–58% and 21–28%, respectively, of the intrinsic efficacy of the full agonist flunitrazepam (Knoflach et al., 1993; see also Facklam et al., 1992b).

How can we understand the low propensity of benzodiazepine receptor partial agonists to produce sedation, muscle relaxation, and tolerance? It is now recognized that full benzodiazepine receptor agonists, such as diazepam, produce anticonvulsant effects with low receptor occupancy (Fig. 1). For example, it has been estimated that full benzodiazepine receptor agonists protect against sound-induced seizures in mice at occupancy levels of 2–5% and against pentylenetetrazol seizures at occupancy levels of 25% (Braestrup and Nielsen, 1986). In contrast, 70–90% occupancy is needed for protection against tonic hindlimb extension in the maximal electroshock test (Petersen et al., 1986). Similarly, sedative effects as assessed with various tests of motor impairment also require high levels of occupancy (50–76%; Petersen et al., 1986). Thus, full benzodiazepine receptor agonists exhibit anticonvulsant activity (against sound-induced and pentylenetetrazol seizures) at low doses, and sedation at higher doses. For partial agonists to elicit the same response as a full agonist, higher levels of receptor occupancy are
Fig. 1. The various actions of the full benzodiazepine receptor agonist diazepam occur at different levels of receptor occupancy. Thus, partial agonists may not produce behavioral effects requiring high levels of occupancy, such as impairment of motor function in the rotarod test. In vivo radioligand method for determination of receptor occupancy is described in Petersen et al. (1984). Audiogenic = protection against sound-induced seizures in DBA/2 mice; MES = maximal electroshock test; DMCM = protection against seizures induced by the benzodiazepine receptor inverse agonist methyl 6,7-dimethoxy-4-ethyl-b-carboline-3-carboxylate. Data obtained in the mouse (except as noted) are from Braestrup and Nielsen (1986), Petersen et al. (1984), Jensen et al. (1984), and Petersen et al. (1986).

required. The requirement for higher occupancy is presumably because the potentiation of each GABA_A receptor is smaller and more GABA_A receptors must be recruited to obtain an equivalent response. For example, in one study, 50% protection in the pentylenetetrazol test was reported to occur with 37% receptor occupancy by diazepam and 84% receptor occupancy by ZK 91296, a partial agonist (Petersen et al., 1986). For benzodiazepine agonist effects that require a high degree of GABA_A receptor potentiation (e.g.,
muscle relaxation, ataxia, amnesia, and respiratory depression), it may not be possible to achieve the pharmacological effect with a partial agonist, even with full occupancy. Thus, partial agonists can have anticonvulsant activity and yet not show adverse side effects at any dose. There is substantial evidence that benzodiazepine receptor tolerance occurs as a result of a downregulation in the expression of certain GABA<sub>A</sub> receptor subunits, particularly the γ<sub>2</sub> subunit that confers benzodiazepine sensitivity (Zhao et al., 1994). Nevertheless, since the mechanism coupling chronic receptor activation to changes in the expression of the γ<sub>2</sub> subunit gene is not well understood, it is not yet possible to explain the reduced tendency of partial agonists to induce tolerance. Despite the very favorable properties of benzodiazepine receptor partial agonists, as yet there is no clinical data supporting the utility of these compounds in epilepsy therapy.

Certain benzodiazepine receptor partial agonists, such as abecarnil, may have a distinct spectrum of anticonvulsant activity in comparison with conventional benzodiazepine receptor agonists such as diazepam (Turski et al., 1990). For example, abecarnil is highly active in a variety of chemoconvulsant models (Serra et al., 1992), but, unlike diazepam, is inactive in the maximal electroshock test. The differences in the spectrum of anticonvulsant activity may relate to the unique GABA<sub>A</sub> receptor subunit specificity of abecarnil. Recent studies with cloned GABA<sub>A</sub> receptor subunits indicate that abecarnil can exhibit partial or full agonist properties at different GABA<sub>A</sub> receptor subtypes, mainly dependent on the molecular form of the α-subunit (Knoflach et al., 1993; Pribilla et al., 1993). Thus, the unique pharmacological profile of abecarnil could arise from its actions as a partial agonist at some GABA<sub>A</sub> receptors and as a full agonist at others. Animals treated chronically with abecarnil do not exhibit a diazepam-like withdrawal syndrome on discontinuation of the drug (Löscher et al., 1990; Steppuhn et al., 1993; Löscher, 1993). Moreover, the development of anticonvulsant tolerance to abecarnil is dependent on the model used. Indeed, in some experimental paradigms no anticonvulsant tolerance is exhibited at the same time that tolerance develops to the motor impairing activity of the drug (Löscher et al., 1991b; Serra et al., 1994).
2.1.1.2. Benzodiazepine Receptor Subtype-Selective Agonists

Abecarnil is an example of a benzodiazepine receptor ligand that acts as a partial agonist at some GABA_A receptor subunit combinations and not others. It therefore exhibits some subtype selectivity. The imidazopyridines alpidem and zolpidem, in contrast, show nearly complete subtype selectivity. These compounds exhibit GABA potentiating activity at, for example, GABA_A receptors of composition α1β2γ2, α2β1γ2, α1β1γ2, but not α5β2γ2 (Faure-Halley et al., 1993; Wafford et al., 1993; Horne et al., 1992). In binding to brain tissue, alpidem and zolpidem show selectivity for the ω1/BZ1 subtype of receptor (Benavides et al., 1988; Benavides et al., 1993). Alpidem has anticonvulsant activity comparable to benzodiazepines, but exhibits much-reduced sedative and muscle relaxant activity (Garreau et al., 1992). In addition, the drug fails to induce tolerance or dependence (Perrault et al., 1993). Human trials of alpidem as an anxiolytic have been promising, with a very favorable side-effect profile in comparison with conventional benzodiazepines. Alpidem binding exhibits a differential regional localization in brain that correlates roughly with the presence of ω1/BZ1 binding sites. It has been suggested that the regional selectivity of alpidem for ω1/BZ1 binding sites, which is dependent on the unique subunit specificity of the drug, could account for its favorable properties (Benavides et al., 1993). On the other hand, the possibility that alpidem is a partial agonist at certain GABA_A receptor subtypes could also explain its favorable profile (Perrault et al., 1993). However, recent studies with cloned subunits have demonstrated that alpidem (Im et al., 1993) and zolpidem (Horne et al., 1992) are full agonists at some, if not all, GABA_A receptor subtypes.

2.1.2. Barbiturate-Like Drugs

Barbiturates have fallen out of favor as anticonvulsant agents largely because of their propensity to cause sedation, behavioral impairment, and deleterious effects on cognitive performance (Farwell et al., 1990). Nevertheless, barbiturates are highly effective, broad-spectrum anticonvulsants. Barbiturate-like compounds with improved side-effect profiles would provide interesting candidates for clinical development.
The sedative and anticonvulsant activity of barbiturates is believed to be mainly due to their potentiation of CNS GABA-mediated inhibition (Scholfield, 1978). This occurs by prolongation of burst openings of single GABA<sub>A</sub> receptor channels (Macdonald et al., 1989). However, effects on voltage-activated Ca<sup>2+</sup> channels also appear to play a role in the sedative-hypnotic activity of the barbiturates and could participate in their therapeutic activity as anticonvulsants (ffrench-Mullen et al., 1993). In addition, direct activation of the GABA<sub>A</sub> receptor could account for the sedative-hypnotic side effects of certain barbiturates (Rho et al., 1994b). Finally, barbiturates appear to have actions on excitatory amino acid receptors, with particular selectivity for AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Taverna et al., 1994; Donevan and Rogawski, unpublished). In line with the diversity of barbiturate cellular actions, there are wide differences in the pharmacological properties of structurally similar barbiturates. Pentobarbital is a powerful sedative-anesthetic, whereas the less sedative phenobarbital is used mainly as an anticonvulsant. Although still incompletely understood, the basis for the reduced sedation with phenobarbital may relate to relative affinities for different target sites and also to the magnitudes of effects produced at clinically relevant concentrations. For example, it has been proposed that the strongly depressant action of pentobarbital could relate to its activity both as a GABA potentiator and a Ca<sup>2+</sup> channel blocker. Phenobarbital, in contrast, has a higher affinity for GABA<sub>A</sub> receptors than for Ca<sup>2+</sup> channels (ffrench-Mullen et al., 1993). At clinically relevant concentrations, it may mainly act as a GABA potentiator, but the absolute magnitude of the potentiation may be smaller than that produced by pentobarbital. The lack of effect on Ca<sup>2+</sup> channels and the less robust potentiation could account for its lower toxicity. As the concentration of phenobarbital moves into the supratherapeutic (toxic) range, it becomes more strongly sedative, possibly because effects on Ca<sup>2+</sup> channels become prominent. The types of Ca<sup>2+</sup> channels relevant to the action of barbiturates have not been well defined. One hypothesis is that these are presynaptic channels involved in the regulation of neurotransmitter release, and that the depressant action is largely due to a reduction of glutamate release.
It may be possible to obtain even greater reductions in the sedative-hypnotic activity of barbiturate-like GABA modulators. Thus, the newly introduced anticonvulsant felbamate has a very favorable side effect profile (causing agitation and insomnia more frequently than sedation; Faught et al., 1993), and appears to act, in part, as a barbiturate-like modulator of GABA<sub>A</sub> receptors (Rho et al., 1994c). (Felbamate produces certain severe idiosyncratic toxicities, unrelated to its anticonvulsant action, that limit its clinical utility.) Felbamate is a dicarbamate structurally related to the sedative-anxiolytic meprobamate. Meprobamate has also recently been shown to have barbiturate-like activity. However, it is much more potent and, unlike felbamate, can directly stimulate the opening of GABA<sub>A</sub> receptor coupled Cl<sup>-</sup> channels in the absence of GABA (Rho et al., 1994a). This latter activity could contribute to the propensity of meprobamate to cause greater CNS depression. In addition to its activity as a barbiturate-like modulator of GABA<sub>A</sub> receptors, felbamate also can inhibit NMDA receptors as discussed in Section 3.1.5. This effect could produce behavioral activation that would counterbalance the CNS depression resulting from GABA potentiation. (NMDA antagonists typically have behavioral activating effects at low doses; Willetts et al., 1990.)

2.1.4. Neuroactive Steroids

It has been known for over five decades that the steroids progesterone and deoxycorticosterone acetate have anticonvulsant activity against pentylenetetrazol seizures (Selye, 1942; Craig, 1966). In the intervening years, other structurally related steroids, including 3,20-pregnenediones and 3,20-pregnanediones (Craig and Deason, 1968), 2β-morpholino-5α,3α-pregnanolones (Hewett et al., 1964), and the steroid anesthetic alphaxalone (Peterson, 1989), have been reported to have similar activity. In 1984, Harrison and Simmonds made the seminal discovery that alphaxalone could selectively potentiate central neuron responses to GABA (but not glycine). The 3β-hydroxy isomer betaxalone was inactive, indicating a high degree of structural specificity. More recently, it has been recognized that certain endogenous metabolites of progesterone and deoxycorticosterone are also
highly potent modulators of the GABA<sub>A</sub> receptor (Majewska et al., 1986). These metabolites have anticonvulsant activity (Belelli et al., 1989, 1990; Högskilde et al., 1988), and their anticonvulsant potencies correlate strongly with their ability to modulate GABA<sub>A</sub> receptor current (Kokate et al., 1994). Studies using fluctuation (noise) analysis and single channel recording have demonstrated that the steroids produce a barbiturate-like prolongation of burst openings of GABA<sub>A</sub> receptors (Simmonds, 1991; Twynan and Macdonald, 1992). In addition, they may cause an increase in channel-opening frequency, an effect not observed with barbiturates. Like barbiturates, neuroactive steroids can directly activate the GABA<sub>A</sub> receptor in the absence of GABA.

Do neuroactive steroids have potential in the treatment of seizure disorders? Although highly active against seizures induced by pentylentetrazol and other GABA receptor antagonists including bicuculline and picrotoxin, GABA-potentiating neuroactive steroids are inactive in the maximal electroshock test. This contrasts with phenobarbital which shows some activity in the maximal electroshock test (Belelli et al., 1990). The profile of the steroids in animal seizure models suggests that their primary utility may be in the treatment of absence epilepsy (Rogawski and Porter, 1990). Indeed, there is an anecdotal report of the effectiveness of deoxycorticosterone acetate in six of eight absence seizure patients (Aird and Gordon, 1951).

The extent to which neuroactive steroids can protect against seizures at nontoxic (nonsedating) doses is not clear. All GABA-potentiating neurosteroids cause impairment of motor performance. However, there may be differences among structurally similar neuroactive steroids in relative toxicity. Progesterone and certain pregnene and pregnane 3,20-diones exhibit a degree of separation between their anticonvulsant and toxic doses, but are relatively weak anticonvulsants (Craig and Deason, 1968). However, alphaxalone, a more potent anticonvulsant, protects against seizures only at doses that produce neurological impairment (Peterson, 1989). In a comprehensive study of progesterone and deoxycorticosterone metabolites, there was up to a several-fold separation between the protective and toxic doses. Thus the therapeutic index (ratio of the ED<sub>50</sub> values for motor impairment and for protection in the pentylentetrazol seizure
test) ranged from 1.2–3.8 (Kokate et al., 1994). However, the therapeutic indices of all of the steroids were less than that of the benzodiazepine clonazepam (8.9). Recently, steroid-like substituted ben[e]indenes (tricyclic molecules containing only a portion of the steroid A-ring) have been described which potentiate GABA responses and may have toxicity comparable to the most favorable neurosteroids (Rodgers-Neame et al., 1992). Further structure–activity studies may lead to the identification of steroids with improved toxicity profiles. However, a key—but as yet unanswered—question is whether neurosteroids will exhibit tolerance as do benzodiazepines. If so, like benzodiazepines, they will have limited utility in the chronic therapy of seizure. (A recent study failed to observe any tolerance to the anticonvulsant activity of pregnanolone in the pentylentetrazol test; Kokate et al., 1995b.) However, even if tolerance does develop over time, steroids may still be of value in the acute treatment of status epilepticus, as are benzodiazepines. In fact, the steroids appear to be highly effective in the kainate and pilocarpine models of status epilepticus in the mouse (Kokate and Rogawski, 1994; Kokate et al., 1995a). Because the solubility of steroids is poor, selection of an appropriate vehicle or identification of water soluble prodrug forms will be critical.

Recently, the synthetic 3β-methyl analog of epiallopregnano-lone (3α-hydroxy,3β-methyl-5α-pregnan-20-one; CCD 1042) has been demonstrated to have anticonvulsant potency that is only slightly weaker than its parent. However, the 3β-methylation confers good metabolic stability and oral activity. In Phase I clinical trials, CCD 1042 was well-tolerated at levels substantially higher than those associated with seizure protection in animals (K. W. Gee, personal communication, 1995).

2.1.5. γ-Butyrolactones and γ-Thiobutyrolactones

An additional series of GABA_A receptor modulating compounds has recently been described that appear to act at a site distinct from benzodiazepines, barbiturates, and steroids (Holland et al., 1993). This novel recognition site, referred to as the γ-butyrolactone (GBL) site, is the locus of action of a variety of substituted γ-butyrolactones
and γ-thiobutyrolactones. Certain ligands of the GBL site antagonize GABA<sub>A</sub> receptor response and are convulsant, whereas others potentiate GABA responses and have anticonvulsant properties (Holland et al., 1990). Anticonvulsant GBL site ligands, such as α-ethyl,α-methyl-thiobutyrolactone (α-EMTBL), are protective in the pentylenetetrazol seizure model as are other GABA-potentiating drugs. However, GBL site ligands may also be effective in the maximal electroshock test and other seizure models (Ferrendelli et al., 1989; Holland et al., 1992). α-EMTBL has a therapeutic index that is low relative to other GABA modulating anticonvulsants, but is similar to valproate. Preliminary results indicate that α-EMTBL may demonstrate less tolerance than clonazepan, suggesting that GBL site ligands may be of utility in chronic epilepsy therapy (Ferrendelli et al., 1993).

2.1.6. Loreclezole

Loreclezole is a novel triazole derivative that is protective against pentylenetetrazol seizures but is less active in the maximal electroshock test (Wauquier et al., 1990; Kulak and Sobaniec, 1994). In addition, at low, nontoxic doses, the drug has anti-absence activity in a genetic model of generalized absence epilepsy (Ates et al., 1992). Consequently, loreclezole has a profile of activity similar to that of other benzodiazepines. A potential benzodiazepine-like interaction with GABA<sub>A</sub> receptors is suggested by the observation that the anticonvulsant effects of loreclezole can be reversed by benzodiazepine receptor inverse agonists (Vaught and Wauquier, 1991; Ashton et al., 1992). The benzodiazepine antagonist flumazenil, however, failed to alter the anticonvulsant activity of loreclezole, indicating that loreclezole is not a benzodiazepine receptor agonist (Wauquier et al., 1990). Moreover, on chronic (5–7 d) administration, tolerance did not develop to the anticonvulsant effects of loreclezole as it does with benzodiazepines.

Loreclezole can indeed enhance the activity of GABA<sub>A</sub> receptors, but it interacts with a novel allosteric modulatory site distinct from that of benzodiazepines, barbiturates, or neuroactive steroids (Wafford et al., 1994). Using native rat and cloned human GABA<sub>A</sub> receptors, loreclezole strongly potentiated GABA-activated Cl<sup>-</sup> current. However, activity of the drug did not require the presence of the
γ-subunit and was not blocked by flumazenil, confirming that loreclezole does not interact with the benzodiazepine recognition site. Interestingly, loreclezole had >300-fold activity on receptors containing the β2- or β3-subunit in comparison with those containing the β1 subunit. Using chimeric β-subunits and site-directed mutagenesis, it was demonstrated that a single amino acid, at the carboxyl-terminal end of the β-subunit putative channel-lining domain TM2, confers sensitivity to loreclezole (Wingrove et al., 1994). The affinity for GABA and benzodiazepines was unaltered by amino acid substitutions at this site.

Loreclezole reduced seizure occurrences in several small clinical trials and was relatively free of side effects (Rentmeester and Hulsman, 1992), demonstrating the potential of drugs that modulate GABA_A receptors in novel ways.

2.2. Vigabatrin, a GABA Transaminase Inhibitor

In addition to the focus on postsynaptic GABA_A receptors, there has been considerable interest and, indeed, success in the development of new antiepileptic drugs that modify GABA disposition in the brain. Vigabatrin (γ-vinyl-GABA), perhaps the most notable achievement in this respect, is an enzyme-activated irreversible inhibitor of GABA transaminase, the main degradative enzyme for GABA. The drug elevates brain GABA levels, and exhibits anticonvulsant activity in a variety of animal seizure models. Moreover, there is now substantial clinical data supporting its efficacy in the treatment of human seizure disorders (Reynolds, 1992). Although the precise way in which vigabatrin prevents seizures remains unclear (Bernasconi et al., 1988), GABA transaminase inhibition may increase neuronal GABA in a releasable pool, so that larger amounts of GABA are discharged with stimulation (Gale, 1992). Thus, increased amounts of GABA are released in a use-dependent fashion, as needed, during seizures. It had been believed that tolerance develops only to the sedative side effects of vigabatrin, but not to its anticonvulsant activity (Remy and Beaumont, 1989). Now, however, there is emerging evidence that tolerance may also develop to the therapeutic effects of the drug (Martin and Millac, 1993).
2.3. GABA Uptake Blockers

An alternative approach to increasing synaptic GABA levels is inhibition of GABA reuptake. A variety of cyclic amino acids, including nipecotic acid and guavacine, inhibit glial or neuronal GABA uptake carriers. In general, however, these compounds have only modest potency in vitro and do not enter into the CNS after parenteral administration. In recent years, a variety of lipophilic analogs of nipecotic acid and guavacine have been described (Braestrup et al., 1990; Suzdak, 1993; Andersen et al., 1993). These compounds—which include tiagabine, CI-966, NNC-711, and SKF 100330A—are highly potent and specific inhibitors of GABA uptake into glia and neurons, but have essentially no activity on other uptake transporters. Unlike nipecotic acid with its moderate selectivity for neuronal GABA uptake, lipophilic GABA uptake inhibitors such as tiagabine show a modestly greater affinity for glial uptake, but they do inhibit uptake in both cell types. These compounds are not transported via the GABA uptake carrier nor do they stimulate GABA release. Recently, it has been demonstrated that tiagabine, CI-966, and related lipophilic GABA uptake inhibitors are highly selective for the GAT-1 cloned GABA transporter, the predominant form in rat brain (Borden et al., 1994). Using in vivo microdialysis in rodents and primates, the systemic administration of CI-966 (Taylor et al., 1990) or tiagabine (Fink-Jensen et al., 1992; Sybir ska et al., 1993) was shown to increase extracellular GABA overflow. Similar results were obtained in awake human subjects with hippocampal microdialysis probes following acute oral administration of tiagabine (During et al., 1992). Experiments in brain slice preparations have demonstrated that lipophilic GABA reuptake blockers can prolong the action of applied GABA and also increase the amplitude and duration of inhibitory GABA-mediated synaptic potentials (Reckling et al., 1990; Thompson and Gahwiler, 1992). In addition, these compounds inhibit epileptiform activity in the slice.

Lipophilic GABA uptake inhibitors have a spectrum of anticonvulsant activity in animal seizure models that is similar to other drugs which act on brain GABA systems, such as the benzodiazepines. These compounds are highly effective in protecting against pentylenetetrazol seizures and may also have activity against sei-
zures induced by other chemoconvulsants that interfere with GABA-ergic neurotransmission, such as the benzodiazepine receptor inverse agonist DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate). They are far less potent (tiagabine: Nielsen et al., 1991) or inactive (SKF 89976A and SKF 100330–A: Swinyard et al., 1991) in the maximal electroshock test. In addition, lipophilic GABA uptake inhibitors have shown activity in various reflex seizure models (including the photosensitive baboon Papio papio), and in the amygdala and corneal kindling models (Suzdak, 1993; Swinyard et al., 1991; Smith et al., 1995). At doses higher than those required to protect against seizures, lipophilic GABA uptake inhibitors produce neurological impairment, as do other drugs that enhance GABAergic neurotransmission. However, there is evidence that tolerance may develop to these side effects, but not to the anticonvulsant activity (Suzdak, 1994). Lipophilic GABA uptake inhibitors have a spectrum of anticonvulsant activity in animal seizure models that is distinct from the important presently marketed antiepileptic drugs (Swinyard et al., 1991). Therefore, it is difficult to predict from animal studies which forms of human epilepsy would be most amenable to therapy with these agents. However, their potency and relatively broad spectrum of activity suggest that they are interesting candidates for further development.

In human clinical trials, tiagabine has shown activity against complex partial seizures, with an overall incidence of side effects no different from placebo at therapeutic doses (Mengel, 1994). (However, adverse side effect such as headache, tiredness, and dizziness were observed with increased doses.) Importantly, there was no evidence of tolerance to the drug’s anticonvulsant activity with chronic dosing for up to 12 mo. In contrast, in an initial trial in healthy volunteers, CI-966 induced a variety of severe neurological and psychiatric symptoms (Sedman et al., 1990). There are differences between the pharmacological properties of tiagabine and CI-966 that could account for the striking differences in the clinical responses to the two drugs (see Suzdak, 1993). Alternatively, it may simply be that the initial doses chosen for CI-966 were excessive. It is uncertain whether the favorable safety record of tiagabine will be maintained in larger tri-
als. The available information suggests that cautious optimism about the potential of lipophilic GABA uptake inhibitors is warranted.

2.4. Potentiation of GABA Release

Although drugs that elevate synaptic GABA levels by interfering with GABA removal mechanisms have anticonvulsant activity, the continuously elevated GABA levels may have untoward effects. A theoretically more attractive approach would be to increase the amount of GABA released with each synaptic stimulus. At present, no selective GABA releasing agents have been described. However, there is some preliminary evidence that the clinically effective antiepileptic drug gabapentin could act by such a mechanism.

Although gabapentin was originally synthesized as a GABA analog, it does not interact with GABA_A or GABA_B receptors, is not metabolically converted to GABA or a GABA receptor agonist, and does not inhibit GABA uptake or degradation (Taylor, 1994). However, gabapentin does increase GABA turnover in some brain regions (Löschler et al., 1991a) and may increase the release of GABA from brain slices in vitro (Götz et al., 1993). Recently, it has been observed that gabapentin can potentiate electrophysiological responses to released GABA in rat neonatal optic nerve, presumably by increasing release from glia (Kocsis and Honmou, 1994). A similar effect has been reported in the hippocampus (Honmou et al., 1994). Although these studies with gabapentin and GABA release are intriguing, they are as yet inconclusive. Nevertheless, they serve to focus attention on the possibility that drugs that enhance GABA release could have potential as antiepileptic agents.

2.5. Potentiation of GABA and Glycine: Propofol and Chlormethiazole

The anesthetic propofol (2,6-diisopropylphenol) is a structurally novel barbiturate-like modulator of GABA_A receptors (Peduto et al., 1991). However, in contrast to barbiturates such as pentobarbital, several recent reports have suggested that subanesthetic doses of propofol can protect against seizures in animal models, with minimal behavioral side effects (Hasan et al., 1992; al-Hader et al., 1992; Hasan and Wolley,
In humans, propofol may be effective in the treatment of status epilepticus (Wood et al., 1988), although the drug has also been reported to cause seizures under certain circumstances (Makela et al., 1993). Propofol produces an enhancement of GABA-activated Cl\(^{-}\) current and, at high doses, can directly activate GABA\(_A\) receptors (Hales and Lambert, 1991). In addition to these barbiturate-like properties, propofol is also able to potentiate glycine-evoked currents (Hales and Lambert, 1991; but see, Dolin et al., 1992), an action not shared by barbiturates, neuroactive steroids, or benzodiazepines.

Another combined GABA\(_A\)/glycine modulator for which there is even more clinical information is chlormethiazole, a thiamine derivative with sedative, hypnotic, anxiolytic, and anticonvulsant properties (Ögren, 1986). Chlormethiazole is particularly effective in the treatment of status epilepticus (even in cases failing to respond to benzodiazepines and barbiturates). Chlormethiazole is also used in preeclampsia (toxemia of pregnancy), eclampsia (toxemia of pregnancy with seizures), and the alcohol withdrawal syndrome. In both animals and humans, chlormethiazole has anticonvulsant activity at doses that do not produce sedation. Like propofol, chlormethiazole potentiates responses to both GABA and glycine, and can also directly activate GABA\(_A\) receptors (Hales and Lambert, 1992). Propofol and chlormethiazole uniquely potentiate GABA and glycine, and both appear to be effective anticonvulsants with low behavioral toxicity at anticonvulsant doses. The favorable toxicity could be explained if the potentiation of GABA and glycine were additive (or even synergistic) for seizure protection, but were not additive for sedative side effects. Indeed, it is not apparent that a glycine potentiating drug would exhibit sedative-hypnotic activity.

### 3. Interactions with Excitatory Amino Acid Receptor Systems

A complementary approach to facilitation of synaptic inhibition is blockade of synaptic excitation. The bulk of fast synaptic excitation in the CNS is mediated by the excitatory amino acid glutamate, acting on AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic
acids) and NMDA (N-methyl-D-aspartic acid) receptors. In recent years, there has been intense interest in the medicinal chemistry of excitatory amino acid antagonists because of their potential in the treatment of neurological disorders, including epilepsy. The conviction that excitatory amino acid antagonists have a role in epilepsy therapy is based on extensive in vitro and in vivo evidence that NMDA and AMPA receptors participate in the expression of many types of epileptic seizures (Rogawski, 1995). Recently, it has been elegantly shown using microdialysis that glutamate levels are elevated prior to the onset and during the expression of complex partial seizures in human subjects (During and Spencer, 1993). There is thus strong theoretical support for the potential utility of excitatory amino acid antagonists in the treatment of human seizure disorders. However, as yet, this has not been verified in clinical trials.

3.1. NMDA Receptor Antagonists

NMDA receptor antagonists have a broad spectrum of activity in animal seizure models. NMDA antagonists are particularly potent in the maximal electroshock test in rodents and against reflex seizures in rodents and primates (Chapman and Meldrum, 1993). They are also protective against pentylenetetrazol and other chemoconvulsants. Thus, the results from animal testing suggest that NMDA antagonists may have utility in the treatment of generalized tonic-clonic (convulsive) and partial seizures. NMDA antagonists have lower potency against spontaneous absence-like seizures in rodents, so that they are less likely to be of utility in human absence seizures. A particularly interesting feature of NMDA antagonists is their powerful ability to protect against the induction of kindled seizures in vivo and against kindling-like phenomena in the in vitro hippocampal slice preparation (see Rogawski, 1995). This suggests that NMDA antagonists may prevent the development or progression of some types of seizures. This property could be of value in certain clinical situations, such as during the critical period after brain injury or in progressive childhood epilepsies. The status of efforts to develop a safe and effective NMDA antagonist for use in epilepsy therapy has been recently reviewed (Rogawski, 1992).
3.1.1. Competitive NMDA Recognition Site Antagonists

In 1982, Croucher et al. reported that phosphonic acid competitive NMDA recognition site antagonists were protective against sound-induced and minimal pentylenetetrazol seizures in mice. This important observation stimulated an intensive research effort into the medicinal chemistry of NMDA receptor antagonists (Rowley and Leeson, 1992). Numerous structural classes of NMDA antagonists have been investigated. Perhaps the most mature line of investigation is that evolving from the straight chain ω-phosphono-α-amino acid compounds that were the basis of the study by Croucher et al. (see Watkins, 1991). The original compounds are very polar and as a consequence have poor brain penetration. However, cyclic analogs with the amino nitrogen in a six-member ring, as in CGS 19755 (cis-4-phosphonomethyl-2-piperidine carboxylate; Hutchison et al., 1989; Lehmann et al., 1988), or in a piperazine ring, as in CPP (3-[2-carboxypiperazin-4-yl]propyl-1-phosphonate; Davies et al., 1986; Lehmann et al., 1987), had improved systemic activity, and were even effective orally (Patel et al., 1990). It was subsequently found that unsaturation of the chain, as in CGP 37849 ([E]-2-amino-4-methyl-5-phosphono-3-pentanoic acid; Fagg et al., 1990; Schmutz et al., 1990; De Sarro and De Sarro, 1992), or addition of a ketone as in MDL 100453 ([R]-4-oxo-5-phosphonovaline; Whitten et al., 1990) also resulted in compounds with powerful systemic anticonvulsant activity. Moreover, the carboxyethylester of CGP 37849 (CGP 39551) showed particularly prolonged oral anticonvulsant activity (De Sarro and De Sarro, 1992). (The reduced analog MDL 10453 also is a potent NMDA antagonist; Bigge et al., 1992.) Unsaturation of the side chain of CPP to form CPPene (Aebischer et al., 1989) similarly produced an anticonvulsant compound with a long duration of action. The D(-)-enantiomer is the active species of CPPene and has been used in several studies. D(-)-CPPene has protective activity in a variety of reflex epilepsy models (Patel et al., 1990; Smith and Chapman, 1993; Smith et al., 1993; De Sarro and De Sarro, 1993) and, as is the case for other NMDA antagonists, protects against the development of kindled seizures (Durmüller et al., 1994). Most competitive NMDA recognition site antagonists have a phosphonic
acid group as in the original structures produced by Watkins (1991). However, this is not essential to activity as demonstrated by LY233053 and LY275059, in which the phosphonic acid group is replaced by the bioisosteric tetrazol functionality. LY233053 offers good protection against NMDA and maximal electroshock seizures, but its duration of action is short (Schoepp et al., 1990a,b; Ornstein et al., 1992). The structurally novel bicyclic phosphonic acid LY274614 has long lasting oral anticonvulsant activity (Schoepp et al., 1991), whereas the corresponding tetrazol LY233536 (Leander and Ornstein, 1990; Ornstein et al., 1990), like LY233053, has a more rapid time course.

Competitive NMDA recognition site antagonists exhibit a broad spectrum of activity in animal seizure models. Not unexpectedly, these compounds are highly effective in protecting against seizures and lethality induced by systemic or intracerebroventricular NMDA. They are also protective in the maximal electroshock test with comparable or slightly lower potency (Ferkany et al., 1989). Protection is also conferred against pentylenetetrazol-induced clonic seizures, but typically at somewhat higher doses. However, a structurally unique cyclic phosphonic acid NPC 12626 (2-amino-4,5-[1,2-cyclohexyl]-7-phosphonoheptanoic acid) has been described which is less potent in the maximal electroshock test than against NMDA or pentylenetetrazol seizures (Ferkany et al., 1989). Recently, the highest potency isomer of NPC 12626 has been identified as the 2R,4R,5S form (Hamilton et al., 1993). It too shows a reverse ordering of potencies in animal seizure models (Ferkany et al., 1993). Whether this unique profile is related to an unusual selectivity for NMDA receptor subtypes or some other property remains to be determined.

Although diverse NMDA recognition site antagonists with good bioavailability and favorable pharmacokinetic properties are now at hand, the problem of side effects has not been so readily solved. Since NMDA receptors are critical to numerous brain functions, NMDA antagonists can produce a variety of behavioral and neurological toxicities. There are, however, certain encouraging features of the preclinical profile of competitive NMDA recognition site antagonists. Anticonvulsant effects typically occur at doses below those
that produce motor impairment (De Sarro and De Sarro, 1993; Ferkany et al., 1993). However, the separation between anticonvulsant activity and motor toxicity is typically smaller than for other anticonvulsant drugs. Drug discrimination studies indicate that the subjective effects of competitive NMDA recognition site antagonists in rodents and primates are distinct from those of dissociative anesthetic-like NMDA antagonists (Gold and Balster, 1993; Bobelis and Balster, 1993). While competitive antagonists do not seem to have the same propensity for producing certain behavioral side effects such as stereotypies and disturbances of locomotor behavior common with dissociative anesthetic-like NMDA antagonists (see Section 3.1.2.), these still occur. The side effects may be especially prominent in kindled animals (Löschner and Hönack, 1991a,b). Tolerance does not seem to develop to either the therapeutic activity or the side effects (Smith and Chapman, 1993).

Despite the various favorable properties of NMDA recognition site antagonists, reports of clinical trials have been disappointing. In an open-label study of D-CPPene in eight patients with intractable complex partial seizures, all patients withdrew prematurely because of side effects. The side effects included poor concentration, sedation, ataxia, depression, dysarthria (impairment of speech due to motor impairment), and amnesia (Sveinbjörnsdottir et al., 1993). None of the patients experienced a reduction in seizure frequency. Interestingly, healthy volunteers tolerated much higher doses than the patients. Thus, as in kindled animals, patients with epilepsy may have greater sensitivity to the side effects of NMDA antagonists. A final problem is that competitive antagonists (like the dissociative anesthetic-like compounds discussed in Section 3.1.2.) can produce neuronal vacuolization and morphological damage in certain brain regions (Olney et al., 1991).

Until recently, it was not appreciated that competitive NMDA recognition site antagonists exhibit differing activities at NMDA receptors composed of different subunit combinations. The information now available indicates that this is the case. Thus, CPP appears to have modestly higher affinity for NMDA receptors composed of NMDAR1-1b subunits that contain a 21-amino acid insert generated
by alternative splicing of exon 5 (see McBain and Mayer, 1994). In addition to sensitivity to competitive antagonists, this N-terminal variation determines a variety of functional properties of the NMDA receptor, including potentiation by Zn$^{2+}$ and polyamines and inhibition by protons (Hollmann et al., 1993). In the future, it may be possible to more selectively target specific NMDA receptor subunit combinations, thus potentially providing for the development of less toxic anticonvulsants.

3.1.2. Channel-Blocking NMDA Receptor Antagonists

The NMDA receptor channel blockers are often referred to as "uncompetitive" antagonists, because their binding and blocking action requires the receptor-channel to be gated in the open state. Occupancy of a binding site within the ionophore of the NMDA receptor prevents cation flux through the channel, thus producing a functional block of NMDA receptor responses (MacDonald et al., 1991). Unlike competitive NMDA recognition site antagonists, uncompetitive antagonists share with other noncompetitive antagonists of the NMDA receptor the theoretical advantage that their blocking action would not be overcome by high synaptic levels of glutamate as may occur during seizures. In addition, uncompetitive antagonists have the additional theoretical advantage of use-dependence, implying that their inhibitory action may specifically be potentiated at sites of excessive receptor activation. The current status and potential clinical utility of channel-blocking NMDA receptor antagonists have recently been reviewed (Rogawski, 1992, 1993).

Channel-blocking NMDA receptor antagonists fall into two broad categories: dissociative anesthetic-like agents and low affinity antagonists. Shortly after the discovery of selective competitive NMDA recognition site antagonists in the early 1980s, the dissociative anesthetics phencyclidine and ketamine were demonstrated to be effective NMDA receptor antagonists (Anis et al., 1983). The structurally dissimilar dissociative anesthetic-like agent dizocilpine (MK-801) is also an extremely potent NMDA receptor antagonist. These compounds exert their block in a use-dependent and voltage-dependent fashion, indicating that they act by an open-channel mechanism
(Huettnerr and Bean, 1988). Like competitive NMDA recognition site antagonists, dissociative anesthetics are potent broad spectrum anticonvulsants in a wide variety of animal seizure models (see Rogawski, 1992). However, at anticonvulsant doses, they produce severe neurobehavioral side effects—including cognitive, sensory, and various schizophrenia-like deficits (Krystal et al., 1994)—that limit their potential clinical utility. In addition, dissociative anesthetics have been reported to cause reversible vacuolization in rat cortical neurons at low doses (Olney et al., 1989, 1991), and frank necrosis at higher doses (Fix et al., 1993). The propensity of dissociative anesthetics to cause neuronal injury is shared by many NMDA antagonists, raising doubts as to overall safety of such compounds. However, caution must be exercised in extrapolating the histopathological findings obtained in rats to other species. Indeed, NMDA antagonist-induced pathomorphological changes have not been documented in primates. Moreover, it is now apparent that some NMDA antagonists may produce only transitory vacuolization with no necrosis, or no changes at all.

The anticonvulsant activity of dissociative anesthetics is shared by a variety of channel-blocking NMDA antagonists, some of which are analogs of the dissociative anesthetics and others that are structurally distinct. Certain of these compounds have substantially reduced behavioral toxicity in animals. For several, there is human clinical experience documenting an acceptable level of safety. These reduced-toxicity channel-blocking NMDA antagonists have been referred to as "low affinity" uncompetitive antagonists. However, low affinity per se may not be the only factor that accounts for their more favorable toxicity characteristics (see below).

The existence of low-toxicity, channel-blocking NMDA antagonists was first recognized with the discovery that certain 1-phenylcycloalkylamines such as 1-phenylcyclohexylamine (the analog of phencyclidine substituting a primary amine for the piperidine ring) and 1-phenylcyclopentylamine were effective anticonvulsants, but had a reduced propensity to produce motor impairment at anticonvulsant doses (Rogawski et al., 1988, 1989; Thurkauf et al., 1990; Blake et al., 1992). Later, an analog of dizocilpine ADCI (5-amino-
carbonyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) was observed to have similar properties, and also to retard the development of kindled seizures (Rogawski et al., 1991). These compounds displace binding of dissociative anesthetic receptor ligands ([3H]1-[1-(2-thienyl)cyclohexyl]piperidine and [3H]dizocilpine) to brain membranes and block NMDA receptor currents in a use-dependent and voltage-dependent manner, indicating that they are channel-blocking NMDA antagonists (Rogawski et al., 1992; Jones and Rogawski, 1992). However, the binding affinities of these reduced-toxicity uncompetitive NMDA receptor antagonists are lower than that of phencyclidine and dizocilpine. At equieffective concentrations, these lower affinity ligands would exhibit faster apparent rates of block and unblock, and this may, at least in part, account for the lower toxicity (see Rogawski, 1993). However, reduced binding affinity alone cannot completely explain the lower toxicity since there is an imperfect correlation between binding affinity and therapeutic index (Rogawski et al., 1989). Moreover, ketamine, a drug with strong dissociative anesthetic properties, has a binding affinity comparable to many of the less toxic channel-blocking agents. In fact, there is now evidence that lower toxicity ligands may bind selectively to a different population of NMDA receptors than do dissociative anesthetics (Porter and Greenamyre, 1995).

Recent work with cloned NMDA receptor subunits suggests that there may be differences among subunit combinations with respect to their sensitivity to channel-blocking agents (McBain and Mayer, 1994). For example, assembly of the NMDAR1 subunit with NR2C generates receptors with lower affinity for some channel blockers than is the case with NR2A or NR2B. In addition, the presence of exon 5 in NMDAR1 (the NMDAR1-1b splice variant; see Section 3.1.1.) confers higher sensitivity to block by dizocilpine and ketamine (Hollmann et al., 1993; Rodriguez-Paz et al., 1995). More subtle variations in the structure of NMDA receptors could also modify the actions of channel blockers. A particularly critical site of the NMDAR1 subunit is the neutral asparagine at position 598. This corresponds to the edited arginine/glutamine (Q/R) site in membrane segment 2 (M2) of AMPA receptors, where a positively charged
arginine residue in GluR2 confers low Ca\textsuperscript{2+} permeability (see Section 3.2.2.). Similarly, mutations in NMDAR1 that switch the asparagine to a positively charged arginine generate receptors resistant to block by dissociative anesthetics (Kawajiri and Dingledine, 1993; Mori et al., 1992; Sakurda et al., 1993). Although there is currently no evidence for RNA editing of NMDA receptors at this site (as occurs for AMPA receptor subunits), variations in channel structure in this or a nearby region of the receptor could have important consequences for the sensitivity to channel blockers. Of course, it remains to be determined which, if any, structural variation in the NMDA receptor accounts for the selective binding of low-affinity channel-blocking NMDA antagonists, and, moreover, whether this contributes to their more favorable toxicity characteristics. Factors other than binding affinity and subunit selectivity might also contribute to the more favorable side-effect profiles of certain low-affinity NMDA antagonists (Rogawski, 1993). In particular, synergism between effects on NMDA receptors and other anticonvulsant targets could be a factor in some circumstances. For example, ADCI has Na\textsuperscript{+} channel blocking activity similar to carbamazepine (see Section 4.), although the extent to which this occurs at concentrations relevant to the anticonvulsant activity is not clear (White, 1994).

In recent years, several anticonvulsants of potential clinical importance have been recognized as low-affinity NMDA receptor channel-blockers. These include dextromethorphan and its metabolite dextrorphan; ARL 12495 (formerly FPL 12495; 1,2-diphenyl-2-propylamine), the metabolite of the anticonvulsant remacemide; and the adamantane analog memantine. In contrast to dissociative anesthetics, low-affinity channel-blocking NMDA antagonists substitute poorly, or not at all, for dizocilpine in drug discrimination testing. This indicates that they may not produce dissociative anesthetic-like subjective effects (Grant, Colombo, Grant, and Rogawski, unpublished). Moreover, for dextromethorphan, dextrorphan, remacemide, and memantine, substantial clinical experience indicates that the compounds can be well tolerated in humans. However, adverse experiences with all of these compounds have been reported (see Rogawski, 1993). Controlled therapeutic trials will be necessary to
verify the efficacy and safety of these low-affinity, uncompetitive NMDA antagonists in the treatment of seizure disorders. Nevertheless, there is cause for optimism with this class of compounds.

In addition to their potential in chronic epilepsy therapy, NMDA antagonists may have utility in the treatment of seizures that occur during ethanol withdrawal (Grant et al., 1990; Liljequist, 1991; Morrisett et al., 1990). This may be in part because of a compensatory upregulation in the expression of NMDA receptors after chronic ethanol exposure (Sanna et al., 1993). ADCI is particularly effective in this regard (Grant et al., 1992).

3.1.3. Glycine Site Antagonists

It is now well recognized that glycine is a required co-agonist for activation of the NMDA receptor (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). Consequently, agents that competitively antagonize the action of glycine would produce a functional block of the receptor and could, therefore, have anticonvulsant activity. Several classes of antagonists are now known which more or less selectively block the glycine site (Huettner, 1991; Carter, 1992). With intracerebroventricular administration, several of these compounds protect against various types of seizure activity (Singh et al., 1991; Baron et al., 1990; Kock and Colpaert, 1990; Palfreyman and Baron, 1991; Bisaga et al., 1993). Recently, Rundfeldt et al. (1994) observed that intraventricular injection of glycine site antagonists and partial agonists increases seizure threshold in amygdala-kindled rats, an effect not observed with other types of NMDA antagonists. A similar positive effect of a glycine site partial agonist (D-cycloserine) was observed against kainate-induced seizures (Baran et al., 1994). These authors suggested that agents active at the glycine site of the NMDA receptor may be of utility in the treatment of seizure types that are refractory even to other NMDA antagonists.

A significant impediment to the evaluation of glycine site antagonists in conventional seizure models has been their lack of CNS accessibility. This problem now seems to have been largely overcome, but complete studies of the activity of available compounds in animal seizure models have not yet been reported. Initial
clues to the development of systemically active glycine site antagonists came from the recognition that HA-966 (3-amino-1-hydroxypryridin-2-one) could be resolved into enantiomers with distinct pharmacological activities. One enantiomer was a partial, low-affinity antagonist of the glycine site (Singh et al., 1990; Pullan et al., 1990). This $R(\text{+})$-enantiomer has activity in the electroshock seizure test when administered intravenously, but its therapeutic index is poor (Vartanian and Taylor, 1991). However, several analogs have been synthesized with greater potency, selectivity, and more favorable toxicity properties, such as L-687,414 (3$R$-amino-1-hydroxy-4$R$-methylpyrrolidine-2-one) (Leeson et al., 1990, 1993; Smith and Meldrum, 1992). Nevertheless, despite intense effort, it was not possible to produce a compound in this series with sufficiently favorable properties to warrant clinical development.

Another glycine site partial agonist with anticonvulsant activity is 1-aminocyclopropanecarboxylic acid (ACPC), a particularly high affinity ligand. ACPC is protective in several seizure models (Skolnick et al., 1989; Witkin and Tortella, 1991; Smith et al., 1993; Bisaga et al., 1993), but may not be active in the maximal electroshock test. Unlike other glycine site partial agonists with anticonvulsant properties, ACPC has nearly full efficacy (about 85%; Watson and Lanthorn, 1990), thus perhaps accounting for its unusual spectrum of activity. Indeed, it is surprising that the drug is anticonvulsant at all.

Recently, certain 5-nitro quinoxaline-2,3-diones were demonstrated to have highly potent ($K_b < 10 \text{ nM}$) glycine site blocking activity and good central availability when administered systemically. These compounds also block AMPA receptors, but do so with several hundred-fold lower potency (Woodward et al., 1993). Two such compounds, 5-nitro-6-methyl-7-chloro-2,3-quinoxalinedione (NMCQX) and 5-nitro-6,7-dimethyl-2,3-quinoxalinedione (NMDX), have been reported to have activity in the maximal electroshock test at relatively low systemic doses (Tran et al., 1994). However, the extent to which the weak AMPA receptor blocking activity contributes to the compounds' anticonvulsant effects is unclear. The related quinoxalinedione ACEA-1021 (5-nitro-6,7-dichloro-1,4-dihydro-
2,3-quinoxalinedione) has been reported not to substitute for phencyclidine in drug discrimination testing, indicating that this class of compounds may not produce dissociative anesthetic-like subjective effects (Balster et al., 1993). It is possible that selective actions of these compounds at different NMDA receptor subtypes could, in part, account for the favorable behavioral profile. However, to date, glycine site antagonists have not been reported to have substantial subunit selectivity.

A series of related structures—the 3-nitro-3,4-dihydro-2-quinolones—have been described with varying degrees of glycine site or AMPA receptor selectivity. These compounds are based on 7-chlorokynurenic acid, a potent glycine site antagonist in vitro, but eliminate the carboxylic acid group that seems to prevent blood–brain barrier penetration. One of these, L-698,544, exhibited good systemic activity as an anticonvulsant (ED$_{50}$, 13.2 mg/kg, ip) in the DBA/2 mouse (Carling et al., 1993). Although L-698,544 interacts with the NMDA ($K_b$, 6.7 μM) and AMPA ($K_b$, 9.2 μM) recognition sites, these interactions occur with somewhat lower affinity than for the glycine site (IC$_{50}$, 0.41 μM). A prodrug ester of the potent glycine site antagonist 5,7-dichlorokynurenic acid has also been reported to have systemic anticonvulsant activity in the DBA/2 mouse (ED$_{50}$, 62 mg/kg, ip; Moore et al., 1993). Similarly, another quinolinone glycine site antagonist MDL 104,653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2[1H]-one; McQuaid et al., 1992) has been observed to have anticonvulsant activity, when administered either intraperitoneally or orally, in DBA/2 mice (Chapman et al., 1993). Interestingly, high doses of the compound produced ataxia and sedation, and the therapeutic ratio was no better than for other NMDA antagonists.

Study of a further series of noncarboxylic acid quinolinones resulted in the identification of L-701,273 (3-cyclopropyl-ketone-4-hydroxyquinolin-2[1H]-one), with high potency against audiogenic seizures in DBA/2 mice (4.1 mg/kg, ip; Rowley et al., 1993). This compound has moderately high activity at the glycine site (IC$_{50}$, 0.42 μM), but also interacts with the NMDA recognition site ($K_b$, 3.4 μM), although it is inactive at AMPA receptors. More recently, a series of 3'-substituted-3-phenyl analogs of the 4-hydroxyquinolinones were
discovered with nanomolar binding affinities for the glycine site (Kulagowski et al., 1994). As is the case for the parent 3-ketones, the 3-phenyl substituted analogs show activity at the NMDA recognition site but do not block AMPA receptors. These compounds appear to be the most potent systemically active glycine site antagonists described to date, with ED$_{50}$ values <1 mg/kg in the DBA/2 mouse model when administered either intraperitoneally or orally. It would appear that these compounds have favorable toxicity characteristics, producing motor impairment only at doses that are substantially higher than those that protect against seizures, but this will require further confirmation. Thus, a variety of potent, and more or less selective, glycine site antagonists are now available, some of which have high systemic potency and excellent oral bioavailability. It remains to be determined if these compounds will be superior to other types of NMDA antagonists for seizure therapy. Of particular interest is the possibility that the additional AMPA receptor blocking activity of some of the glycine site antagonists may actually provide enhanced anticonvulsant activity. As discussed in Section 3.2., AMPA receptor blockade can confer a powerful anticonvulsant effect.

3.1.4. Polyamine Site Antagonists

The demonstration by Ransom and Stec (1988) that the polyamines spermine and spermidine enhanced binding of [3H]dizocilpine to NMDA receptors in brain homogenates suggested that polyamines could facilitate gating of the NMDA receptor. Although polyamines have multiple effects on NMDA receptors (Rock and Macdonald, 1992; Benveniste and Mayer, 1993), a major action is to increase channel opening frequency via an interaction with a distinct recognition site on the NMDA receptor complex (Williams et al., 1991). The polyamine facilitatory effect appears to be mediated by an increase in the affinity for glycine and also by a distinct glycine-independent mechanism (Williams et al., 1994; Zhang et al., 1994b). There has been interest in developing polyamine site antagonists, but success to date has been limited.

Two related compounds proposed as polyamine site antagonists are the neuroprotective agents ifenprodil and eliprodil (SL 82.0715)
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(Scatton et al., 1994). Ifenprodil and eliprodil are now well recognized to be potent noncompetitive NMDA antagonists both in vitro and in vivo. Electrophysiological studies in cultured hippocampal neurons have demonstrated that ifenprodil produces a complex effect on gating of the NMDA receptor channel (Legendre and Westbrook, 1991). This action occurs in a manner that is distinct from that of other NMDA antagonists, but is otherwise poorly characterized. Although there is considerable evidence that ifenprodil and eliprodil can functionally interact with polyamine effects on the NMDA receptor, it is unlikely that this occurs at a common recognition site (Ogita et al., 1992). It has instead been proposed that the polyamine and ifenprodil/eliprodil binding sites may share overlapping domains on the NMDA receptor complex or that the apparent antagonism occurs because of a functional interaction at distinct sites.

Recently, it has been observed that the distribution of [3H]ifenprodil binding sites in brain closely matches the expression of NMDAR-2B subunit mRNA (Dana et al., 1991). Moreover, ifenprodil appears to selectively antagonize NMDA receptors composed of NR1A and NR2B subunits, although it can also block NR1A/NR2A receptors at substantially higher concentrations (Williams, 1993). Therefore, at ordinary doses, ifenprodil may interact with only a subpopulation of NMDA receptors. Interestingly, it now appears that polyamine facilitation is variably expressed depending on the subunit composition of the NMDA receptor. The 2B subunit is a key subunit in conferring the receptor with polyamine sensitivity (Zhang et al., 1994b).

Despite their relatively potent NMDA receptor blocking activity, ifenprodil and eliprodil appear to have more favorable toxicity profiles than other NMDA antagonists (Scatton et al., 1994). At neuroprotective doses, they do not produce neurological impairment and do not substitute for phencyclidine in drug discrimination studies or induce amnestic effects in passive avoidance testing. Moreover, clinical trials have indicated that the drugs do not produce psychostimulant, amnestic, or psychotomimetic effects in humans. In addition, eliprodil does not appear to produce pathomorphological changes, that, as has been noted in Section 3.1.2., are characteristic
of other NMDA antagonists (Duval et al., 1992). It has been suggested that the unique subunit selectivity of ifenprodil and eliprodil may account for their favorable toxicity characteristics.

As is the case for other NMDA antagonists, ifenprodil and eliprodil have a broad spectrum of anticonvulsant activity in animal seizure models (De Sarro and De Sarro, 1993; Scatton et al., 1994). These effects, however, occur at doses that are higher than the neuroprotective doses and are nearer to the doses that cause toxic effects. For example, in the case of ifenprodil, there is little separation in the doses that protect against seizures (mouse maximal electroshock test ED₅₀, 29 mg/kg, ip) and induce motor impairment. For eliprodil the therapeutic index is somewhat better (electroshock ED₅₀, 15 mg/kg, ip; rotarod ED₅₀, 59 mg/kg). (Interestingly, however, eliprodil has been shown to potentiate the anticonvulsant activity of the competitive NMDA recognition site antagonist CGP 37849; Deren-Wesolek and Maj, 1993.) Thus, it may be that the NMDA receptor subtype selectivity of ifenprodil/eliprodil is not optimally suited for seizure protection, at least in the animal models used for screening. Whether this is a characteristic of all functional polyamine antagonists, remains to be determined.

A variety of other compounds have been proposed as polyamine antagonists, including several polyamines (Williams et al., 1990) and certain peptide components of marine snail toxins (Skolnick et al., 1994). Nevertheless, few of these proposed antagonists are suitable for evaluating the idea that polyamine antagonism is a viable anticonvulsant strategy. Indeed, several putative polyamine site antagonists have complex effects on the NMDA receptor, including channel-blocking activity, which confound any interpretation of their specific polyamine site effects (Donevan et al., 1992; Subramaniam et al., 1992). However, one interesting candidate antagonist is N-(3-aminopropyl)cyclohexylamine (APCHA), a cyclohexyl diamine. It has been demonstrated to block the facilitation by spermidine of NMDA-induced seizures in mice (Chu et al., 1994). Unfortunately, APCHA may not be active with systemic administration and it has yet to be demonstrated that this compound acts via a specific interaction with the polyamine site.
There is some evidence that brain polyamine concentrations may increase with kindling, either produced electrically (Hayashi et al., 1989) or by repeated treatment with pentylenetetrazol (Hayashi et al., 1993). However, it remains undetermined whether these increased levels contribute to the hyperexcitability of the kindled state. Should this be the case, the argument supporting the potential use of polyamine site antagonists in seizure therapy would be strengthened.

3.1.5. Antagonists at Other Sites on the NMDA Receptor Complex and the Combined Actions of Felbamate on NMDA/GABA<sub>A</sub> Receptors

In recent years, a class of NMDA antagonist has been identified in which inhibition is exerted at sites that are distinct from those of known NMDA receptor coagonists or facilitatory modulators. Indeed, ifenprodil and eliprodil are probably best considered among this class of NMDA antagonist. Other examples include a series of n-alkyl diamines that inhibit NMDA receptors by acting at a nonvoltage dependent, hydrophobic binding site as well as in a channel-blocking fashion (Subramaniam et al., 1994). Similarly, the anticonvulsant remacemide may also block NMDA receptors in part by such an allosteric action (Subramaniam et al., 1995a). Felbamate too is an example of an anticonvulsant that may antagonize NMDA receptors by an allosteric effect on channel gating, although a fast channel-blocking action of the drug may be more important. Although glycine and the glycine site agonist d-serine are able to functionally reverse the anticonvulsant effects of felbamate in vivo (Harmsworth et al., 1993; Coffin et al., 1994), there is strong evidence that felbamate does not act directly at the glycine site (Rho et al., 1994d; Subramaniam et al., 1995b).

As discussed in Section 2.1.2., felbamate also produces a modest potentiation of GABA<sub>A</sub> receptor responses within the same range of concentrations that it blocks NMDA responses (Rho et al., 1994b). Both of these effects occur at concentrations of felbamate likely to be present in the brain during antiepileptic therapy. Felbamate exhibits a broader spectrum of anticonvulsant activity than conventional antiepileptic agents such as phenytoin and carbamazepine. (It con-
fers protection in the maximal electroshock test and also against seizures induced by pentylentetrazol; Swinyard et al., 1986.) Clinically, the drug has activity in the treatment of partial and secondarily generalized tonic-clonic seizures and it is also effective against refractory seizures of the Lennox-Gastaut syndrome in children, which are notoriously difficult to treat. The combination of actions of felbamate on excitatory and inhibitory synaptic transmission could account for its broad spectrum of activity and favorable side-effect profile. More specifically, it can be postulated that the distinct actions on NMDA and GABA<sub>A</sub> receptors are by themselves below the threshold for side effects, but that the actions at the two sites are additive or even synergistic against seizures. That a drug such as felbamate with low neurological toxicity may exert its therapeutic activity via low-affinity interactions with more than one receptor system contradicts the conventional view that side-effect reduction is best achieved by high selectivity and potency. However, for anticonvulsant drugs that modulate excitability mechanisms critical to normal brain function, agents with high potency and efficacy may have unacceptable toxicity. (See Section 2.1.1.1. for a more detailed discussion with reference to partial benzodiazepine receptor agonists.) Therefore, the search for drugs that exert low efficacy effects on more than one receptor seems to be a worthwhile strategy.

3.2. AMPA Receptor Antagonists

AMPA (non-NMDA) receptors play a key role in CNS integration as the prime mediators of fast synaptic excitation. The critical nature of AMPA receptors is hard to overstate inasmuch as they participate in virtually every central circuit and, of particular importance here, mediate epileptiform activity in a variety of model systems (Rogawski, 1994). Consequently, the observation that AMPA receptor antagonists have anticonvulsant activity is not unexpected. However, at present, it is unclear if AMPA receptor antagonists will be sufficiently free of toxicity to be clinically useful anticonvulsants. Nevertheless, the possibility of selectively targeting the various AMPA receptor subtypes provides enormous opportunities for drug development.
3.2.1. Competitive AMPA Receptor Antagonists

During the early period of excitatory amino acid receptor research, AMPA receptor antagonists were unavailable. However, in the late 1980s the situation changed: Honoré et al. (1988) developed a series of quinoxalinediones as selective and relatively potent competitive AMPA recognition site antagonists (see Davies and Collingridge, 1990). Although the first quinoxalinediones to be described were not systemically active, compounds with good systemic bioavailability are now available. These include the benzo-fused quinoxalinedione NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[F]quinoxaline; Sheardown et al., 1990) and the 6-imidazol substituted quinoxalinedione YM90K (6-[1H-imidazol-1-yl]-7-nitro-2,3-[1H-4H]-quinoxalinedione; Ohmori et al., 1994) that may have improved CNS penetration in comparison with NBQX. A series of isatin oxamines has also been described which are highly selective AMPA receptor antagonists (Wätjen et al., 1993). These compounds have anticonvulsant activity against AMPA-induced seizures following intravenous and oral administration. In addition, a substituted decahydroisoquinoline-3-carboxylic acid has been recently described with good systemic AMPA receptor blocking activity (Ornstein et al., 1993). This compound, LY215490 ([3SR,4aRS, 6RS,8aRS]-6-[2-(1H-tetrazol-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid), also interacts with the NMDA recognition site, but with 5- to 10-fold lower potency.

Competitive AMPA recognition site antagonists are protective in the maximal electroshock test and against seizures induced by various chemoconvulsants (Yamaguchi et al., 1993; Ornstein et al., 1993). In addition, these compounds have activity against reflex seizures in rodents and primates (Chapman et al., 1991; Smith et al., 1991; Meldrum et al., 1992; Ohmori et al., 1994). In the kindling model, NBQX has been reported to have protective activity, although it is less potent than in other seizure models (Löschler et al., 1993). This is in sharp contrast to NMDA antagonists which are weak or ineffective against established kindled seizures. Despite its activity against kindled seizures, NBQX has no effect on the development of
kindling (Dürmüller et al., 1994). This is another important difference from NMDA antagonists, which, as has already been noted (Section 3.1.), do protect against kindling development.

It has generally been found that AMPA receptor antagonists have neurological toxicity at anticonvulsant doses, although this varies depending on the seizure model and test conditions (see Chapman et al., 1991). Thus, NBQX produced motor impairment at doses that are similar to those that are protective in the maximal electroshock test (Yamaguchi et al., 1993). The LY215490 has only a modestly better therapeutic ratio (=2.2) (Ornstein et al., 1993). Nevertheless, it would be premature to conclude that AMPA receptor antagonists are unlikely to have clinical utility in seizure therapy, since blockade of AMPA receptors is an entirely new therapeutic strategy. Only through clinical trials will it be possible to assess the significance of the therapeutic ratio values determined from animal testing.

Löschler et al. (1993) observed that low doses of NMDA receptor antagonists synergistically enhance the anticonvulsant activity of NBQX in the kindling model, without producing an increase in adverse effects. Although the basis of this phenomenon is poorly understood, it seems reasonable that a combination of NMDA and AMPA receptor blockade may ultimately prove to be the optimal approach for epilepsy therapy. In fact, low levels of NMDA receptor blockade may provide an antiepileptogenic effect and may also protect against seizure-induced brain damage, in a manner not obtained with AMPA receptor antagonists (Berg et al., 1993). As discussed in Section 3.1.3., many of the recently described glycine site antagonists also have various degrees of AMPA receptor blocking activity. It will be of interest to determine if the combined activities provide clinically superior therapeutic activity with acceptable side-effect properties.

Another potential approach to obtaining improved anticonvulsant activity with a reduction in side effects would be to target AMPA receptor subtypes specifically involved in seizure generation or propagation. Such putative seizure-related AMPA receptor subunits have yet to be identified. However, there is evidence that non-NMDA receptors can be selectively targeted with antagonists. Thus, the isatin oxime NS-102 (5-nitro-6,7,8,9-tetrahydrobenzo-
[l]indole-2,3-dione-3-oxime) is a non-NMDA antagonist with selectivity for the kainate binding subunit GluR6 (Johansen et al., 1993; Verdoorn et al., 1994). However, the anticonvulsant profile of this compound remains to be determined.

3.2.2. Noncompetitive AMPA Receptor Antagonists

A novel class of selective AMPA receptor antagonists has recently been identified that exert their blocking action in a mechanistically distinct fashion from competitive AMPA recognition site antagonists. These antagonists—typified by the 2,3-benzodiazepine (homophthalazine) GYKI 52466 (1-[4-aminophenyl]-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine)—offer an alternative approach for blockade of AMPA receptors with potentially distinctive consequences (Tarnawa et al., 1990; Ouardouz and Durand, 1991; Jones et al., 1992). GYKI 52466, unlike the quinoxalinedione AMPA antagonists, does not interact with the AMPA recognition site, nor is it a channel blocker. Rather, it appears to block AMPA receptors in a noncompetitive fashion via a novel allosteric site on the AMPA receptor complex (Donevan and Rogawski, 1993). The blocking action of GYKI 52466 is highly selective in vitro, with essentially no effect on NMDA, metabotropic glutamate, or GABA_A receptor responses. The lack of effect on GABA_A receptors is particularly notable in view of the structural similarity of GYKI 52466 with conventional 1,4-benzodiazepines (see Section 2.1.1.).

Like competitive AMPA recognition site antagonists, GYKI 52466 has anticonvulsant activity in a broad spectrum of animal seizure models (Chapman et al., 1991; Smith et al., 1991; Yamaguchi et al., 1993; Steppuhn and Turski, 1993). In some experimental models, GYKI 52466 has improved anticonvulsant activity compared with NBQX. In others, NBQX appears to have greater selectivity and potency. One situation where the noncompetitive antagonists would theoretically be preferable to competitive antagonists is in protection against seizures associated with high synaptic glutamate levels (see Rogawski, 1993). Under these conditions, the synaptically released glutamate would surmount the blocking action of a competitive antagonist so that high doses of the antagonist would
be required to confer seizure protection. However, these high doses would be more likely to induce side effects. In contrast, minimal doses of drugs like GYKI 52466 may be effective, since equivalent degrees of block are achieved whatever the glutamate level.

A series of N-substituted GYKI 52466 analogs has been described with varying degrees of AMPA receptor blocking activity (Tarnawa et al., 1993). Two of these analogs with greater in vitro AMPA receptor blocking potencies were found to have correspondingly greater in vivo anticonvulsant potencies (Donevan et al., 1994). This supports the view that the anticonvulsant activity of the 2,3-benzodiazepines is due to AMPA receptor blockade.

Recently, it has been demonstrated that AMPA receptors containing the GluR2 subunit have a linear or outwardly rectifying current–voltage relationship and a low permeability for Ca\(^{2+}\). Combinations lacking GluR2 are inwardly rectifying and have a high Ca\(^{2+}\) permeability (Hume et al., 1991; Hollmann et al., 1991; Verdoorn et al., 1991). GYKI 52466 appears to block Ca\(^{2+}\)-permeable and Ca\(^{2+}\)-impermeable AMPA receptors with equal potency (Donevan and Rogawski, unpublished). This is in contrast to the barbiturate pentobarbital that selectively blocks Ca\(^{2+}\)-impermeable AMPA receptors (Taverna et al., 1994) and certain arthropod toxins that are selective for Ca\(^{2+}\)-permeable AMPA receptors (see Section 3.2.3.). Interestingly, GYKI 52466 and its analogs appear to have much higher blocking potency for AMPA-selective non-NMDA receptor subunits than for kainate preferring subunits (Paternain et al., 1995). The implications of these various selectivity differences are not yet well understood.

### 3.2.3. Channel-Blocking AMPA Receptor Antagonists

At present, no selective channel-blocking AMPA receptor antagonists have been identified. However, certain polyamine arthropod toxins could provide clues to the development of such compounds. The toxins (such as argiotoxin 636, philanthotoxin, and Joro spider toxin) act as potent noncompetitive antagonists of vertebrate AMPA receptors (Brackley et al., 1993; Blaschke et al., 1993). These toxins act in a use- and voltage-dependent manner suggesting that they are channel blockers (Herlitze et al., 1993). However, the
toxins are not selective for AMPA receptors, and indeed their block of NMDA receptors is more reliable and often more potent (Priestley et al., 1989; Ragsdale et al., 1989). A likely reason for the inconsistency of the AMPA blocking action is suggested by the recent discovery that the polyamine toxins selectively block inwardly rectifying AMPA receptors (that is, those that lack the GluR2 subunit) (Blaschke et al., 1993; Herlitze et al., 1993).

From the point of view of their use as pharmacological tools, the fact that the toxins block both NMDA and AMPA receptors is a disadvantage. However, this may not be an obstacle to the use of such compounds as therapeutic agents. As has been noted previously, combined NMDA/AMPA receptor blockade may produce a synergistic anticonvulsant effect. Moreover, the selectivity of the toxins for inwardly rectifying AMPA receptors may be a distinct advantage. Since rectifying AMPA receptors are Ca$^{2+}$ permeable, it is not hard to imagine that they could play a role in seizure generation or in the neurotoxicity associated with prolonged seizures. In fact, there is suggestive, but by no means conclusive, evidence in support of both possibilities. Thus, it has recently been reported that amygdaloid kindling is associated with a 25–30% reduction in GluR2 levels in the limbic forebrain and piriform cortex/amygdala (but not in the entorhinal cortex or amygdala; Prince et al., 1995). The decrease—which was specific for the GluR2 subunit—was observed in kindled animals at 24 h after the last seizure; GluR2 levels had returned to normal at 1 wk and 1 mo. Similar changes were not obtained after induction of seizures with the convulsant pentylenetetrazol. It is conceivable that the decrease in GluR2 could be a factor in the induction, but not the maintenance, of kindled seizures. If this is the case, agents such as the polyamine toxins which selectively block AMPA receptors lacking the GluR2 subunit could have interesting antiepileptogenic potential.

An additional line of experimentation is consistent with the possibility that GluR2 subunit downregulation could play a role in seizure-induced neuropathology. Thus, Friedman et al. (1994) recently reported that persistent seizure activity (status epilepticus) can result in reduced expression of the GluR2 subunit in hippocampal regions most vulnerable to neurodegeneration. 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. Polyamine toxin-like agents with specificity for Ca\(^{2+}\)-permeable AMPA receptors might, therefore, be well suited as neuroprotectants to ameliorate seizure-induced pathological changes.

Although only limited information is available, polyamine toxins and a series of small mol wt analogs referred to as "araxins" do have anticonvulsant and neuroprotective activity when administered intracerebroventricularly or systemically (Seymour and Mena, 1989; Mueller, personal communication). At therapeutically effective doses, the compounds do not appear to produce dissociative anesthetic-like behavioral or histopathological effects, and do not generalize to phencyclidine in drug discrimination studies. However, with systemic administration, there is poor separation between the doses that confer seizure protection and induce neurological impairment. In addition, the toxins may produce significant cardiovascular side effects.

**3.3. Metabotropic Glutamate Receptors**

Metabotropic glutamate receptors are a diverse group of G-protein-coupled receptors that are structurally and functionally distinct from other glutamate receptors (Suzdak et al., 1994). In contrast to NMDA and AMPA receptors which are themselves ion channels, metabotropic glutamate receptors act via various second messengers to indirectly regulate a wide variety of ion channels. The potential role of metabotropic glutamate receptors as targets for anticonvulsant drugs is not well defined. Nevertheless, activation of metabotropic glutamate receptors with selective agonists can produce, in adult rodents, limbic-like seizures similar to those observed in kindled animals (Sacaan and Schoepp, 1992; Tizzano et al., 1994). In neonatal rats, metabotropic agonists can produce frank convulsions (McDonald et al., 1993). Interestingly, however, low doses of certain metabotropic glutamate receptor agonists can have weak anticonvulsant effects (see Thomsen et al., 1994). The anticonvulsant effects presumably arise because the metabotropic glutamate receptor agonists reduce glutamate release by a presynaptic action on inhibitory autoreceptors (Glaum and Miller, 1994).
Recently, a novel series of conformationally restricted phenylglycine analogs have been described which act as competitive antagonists of at least some metabotropic glutamate receptors (Birse et al., 1993; Thomsen and Suzdak, 1993; Ferraguti et al., 1994). One of these compounds, (S)-4-carboxy-3-hydroxyphenylglycine ([S]-4C3HPG), has been reported to protect against audiogenic seizures in DBA/2 mice at doses that do not produce neurological impairment (Thomsen et al., 1994). This effect occurred with intraventricular, but not systemic, administration of the drug. In addition, (S)-4C3HPG diminished the epileptiform activity observed in rat cortical slices exposed to Mg^{2+}-free conditions. L-AP3 (L-2-amino-3-phosphonopropionate), a low potency noncompetitive metabotropic receptor antagonist (Schoepp, 1994), has also produced a weak anticonvulsant effect following intraventricular administration (Klitgaard and Jackson, 1993). Both (S)-4C3HPG and L-AP3 can have metabotropic glutamate receptor agonist properties under some circumstances (Birse et al., 1993). It is therefore conceivable that the anticonvulsant activity of these compounds relates, at least in part, to this agonist activity, possibly by presynaptic effects on glutamate release. Indeed, it is not clear at present whether metabotropic receptor agonism, antagonism, or a combination of agonist and antagonist effects at distinct metabotropic receptor types represents the most effective anticonvulsant approach. Nevertheless, targeting of the various metabotropic receptor types with selective agonists and antagonists provides interesting opportunities for anticonvulsant drug development.

3.4. Glutamate Release Inhibitors

Metabotropic glutamate receptors are just one example of a host of receptors and ion channels of the glutamatergic nerve terminal that are attractive anticonvulsant targets. It has already been noted that barbiturates might in part exert their anticonvulsant activity via inhibition of glutamate release (through effects on voltage-dependent Ca^{2+} channels). In addition, as discussed in the next section, the therapeutic effects of anticonvulsant drugs that interact with voltage-dependent Na^{+} channels is likely due to some extent to inhibition of glutamate release. It appears that certain toxins including ω-Aga
IVA and ω-Aga MVIIC, which block Ca\(^{2+}\) channels in presynaptic nerve terminals can have anticonvulsant effects when administered intracerebroventricularly (Jackson et al., 1994). Moreover, a series of novel N,N'-diarylguanidines have been reported to be potent, systemically active glutamate release blockers although their mechanism of action is not well understood (Reddy et al., 1994). There are thus a number of interesting leads to glutamate release antagonists of potential clinical utility. The challenge will be to develop agents that have sufficiently low toxicity to be useful in seizure therapy.

4. Interactions with Voltage-Dependent Na\(^+\) Channels

A wide variety of anticonvulsant drugs are believed to act, at least in part, via their effects on voltage-dependent Na\(^+\) channels. Phenytoin is perhaps the best studied example. Phenytoin is a relatively weak Na\(^+\) channel blocker and its ability to inhibit Na\(^+\) channel activity is highly dependent on the state of the channel. Therapeutic concentrations of the drug have little activity on resting Na\(^+\) channels at hyperpolarized membrane potentials. Thus, at a potential of –80 mV, 10 \(\mu\)M phenytoin, a concentration at the high end of the range of free therapeutic plasma levels, would produce no more than 15% block of Na\(^+\) current (Lang et al., 1993). However, there is more significant block when Na\(^+\) channels are repetitively activated or when the initial membrane potential is at a more depolarized level. These properties are believed to reflect the higher affinity of phenytoin for activated and inactivated channels than for resting channels (Ragsdale et al., 1991). During high frequency firing, there is a progressive accumulation of drug binding as Na\(^+\) channels cycle through the activated and inactivated states with each action potential. In addition, a greater proportion of Na\(^+\) channels is in the inactivated state at depolarized membrane potentials, thus accounting for the voltage dependence of block. The rate at which phenytin-bound Na\(^+\) channels recover from inactivation is markedly slowed, particularly at depolarized potentials (Schwartz and Grigat, 1989; Kuo and Bean, 1994). This property accounts for the stabilization of Na\(^+\) channels in the inactivated state.
The precise way in which a modification of Na\(^+\) channel gating results in seizure protection is not well understood. However, the following hypothesis has often been proposed. During seizures, neurons fire more rapidly and are more depolarized than under normal conditions, so that their Na\(^+\) channels would be more susceptible to block. This would allow phenytoin to selectively suppress high frequency firing. Indeed, at low concentrations, phenytoin is well known to produce a selective inhibition of repetitive action potential firing, whereas single action potentials are resistant (McLean and Macdonald, 1983). The effects of phenytoin on Na\(^+\) channels may ultimately translate into reduced glutamate-mediated synaptic excitation, and this could play an important role in its anticonvulsant activity. In fact, phenytoin inhibits the release of various neurotransmitters, including glutamate from brain slices stimulated by the Na\(^+\) channel activator veratrine (Leach et al., 1986).

A wide variety of anticonvulsant drugs have a spectrum of anticonvulsant activity in animal seizure models similar to that of phenytoin (Rogawski and Porter, 1990). Many of these may also act via an interaction with Na\(^+\) channels, and in several cases this has been confirmed in vitro. For example, lamotrigine, an anticonvulsant effective in the treatment of refractory partial seizures (Yuen, 1994), has a similar profile to phenytoin; it is protective in the maximal electroshock test but not against pentylenetetrazol-induced clonic seizures (Miller et al., 1986). At clinically relevant concentrations, lamotrigine has been shown to block repetitive firing of CNS neurons. This effect can be reversed by hyperpolarization of the resting membrane potential, in a manner similar to that of phenytoin (Cheung et al., 1992). In addition, the drug suppresses burst firing in cortical neurons (Lees and Leach, 1993), and inhibits binding of \(^{[3]}\)H]batrachotoxinin A 20-α-benzoate, a ligand for Na\(^+\) channels, to rat brain synaptosomes (Cheung et al., 1992). In voltage clamp studies, lamotrigine produced a use-dependent inhibition of Na\(^+\) channels and slowed the rate of recovery from inactivation, effects similar to those produced by phenytoin (Lang et al., 1993; Taylor, 1993). Thus, the similarity between lamotrigine and phenytoin in animal seizure models is paralleled by corresponding actions on Na\(^+\) channels.
Riluzole is another anticonvulsant drug with a phenytoin-like spectrum of activity (Mizoule et al., 1985). (Riluzole also has neuroprotective properties [see, for example, Wahl et al., 1993] as does phenytoin [Stanton and Moskal, 1991; Hayakawa et al., 1994].) Among its various pharmacological actions, riluzole is a blocker of voltage-dependent Na+ channels. Interestingly, however, unlike phenytoin, the block produced by riluzole does not occur in a use-dependent fashion, suggesting that it does not preferentially bind to activated Na+ channels. Rather, riluzole has an exceptionally high (>150–300-fold) selectivity for the inactivated state of the Na+ channel (Benoit and Escande, 1991). These results with riluzole indicate that stabilization of the inactivated state of the Na+ channel may be a particularly critical factor in the anticonvulsant activity of phenytoin.

Several other anticonvulsant drugs with a phenytoin-like spectrum of anticonvulsant activity interact with Na+ channels in a fashion similar to phenytoin. These include carbamazepine (Ragsdale et al., 1991; Lang et al., 1993), ralitoline (Rock et al., 1991; Fischer et al., 1992), and flunarizine (Kiskin et al., 1993). In addition, the anticonvulsant U 54494A (3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzamide) (Zhu and Im, 1992), its two major active metabolites (Zhu et al., 1993); and several other structurally related benzamides with anticonvulsant properties (Zhu et al., 1992) have been shown to interact with Na+ channels in a voltage- and use-dependent fashion, similarly to phenytoin. The success in identifying phenytoin-like anticonvulsants is probably a result of the widespread use of the maximal electroshock test as a screening method (inasmuch as the test is highly sensitive to such agents). Since there are already a large number of safe and effective anticonvulsant drugs of this type, the development of additional phenytoin-like compounds may not seem be a high priority. However, it would be reasonable to search for drugs that produce phenytoin-like effects in combination with other pharmacological activities that result in seizure protection (for example, potentiation of GABA or inhibition of excitatory amino acid transmission). The major acute neurological toxicity of phenytoin is referable to cerebellar dysfunction, possibly because
cerebellar neurons are among the most rapidly firing in the CNS and are therefore particularly susceptible to use-dependent block. Different types of acute toxicities are produced by anticonvulsant drugs acting on other targets. For example, GABA-potentiating drugs typically produce sedation, an effect not usually seen with phenytoin. Thus, by combining Na+ channel blocking activity with another action it may be possible to produce enhanced anticonvulsant effects without a corresponding increase in toxicity.

5. K+ Channel Opener Drugs

Like GABA\(_A\) receptors, the fundamental role of K+ channels is to dampen excitability. Consequently, drugs that enhance the activity of K+ channels are reasonable antiepileptic drug candidates. Activation of K+ channels would be expected either to hyperpolarize neurons and thus inhibit them or to limit action potential firing by increasing the opposing influence that K+ currents have on depolarizing Na+ currents.

In recent years, a diverse group of molecules have been identified that are capable of opening K+ channels in excitable cells. Research in this area began with the discovery that the antihypertensive benzopyran cromakalim relaxed vascular smooth muscle by opening K+ channels (Weston and Edwards, 1992). Subsequently, it was recognized that several other structurally dissimilar antihypertensive agents, including diazoxide, minoxidil, and pinacidil, were also K+ channel openers. These studies with existing compounds led to the synthesis of a large number of novel K+ channel openers (Robertson and Steinberg, 1990).

There are a great variety of K+ channels in neuronal cells and a corresponding diversity in their functional roles. Consequently, in characterizing the pharmacological effects of a K+ channel opener drug, it is of critical importance to define the type of K+ channels modulated by the drug. The range of K+ channel types sensitive to K+ channel opener drugs is still incompletely defined. It may be that the channel types affected depend on the specific drug and tissue examined. For example, the benzimidazolone NS 004 (1-[2-hydroxy-5-chlorophenyl]-5-trifluoromethyl-2-benzimidazolone) is said to
selectively activate large-conductance Ca\(^{2+}\)-dependent K\(^+\) channels in neurons (Olesen et al., 1994). However, in many cases, it appears that ATP-sensitive K\(^+\) channels are the major target.

ATP-sensitive K\(^+\) (K\(_{ATP}\)) channels are K\(^+\) channels that close when intracellular ATP levels rise. Such channels were originally described in cardiac cells (Noma, 1983), but were subsequently identified in a wide variety of other excitable cells (Ashcroft and Ashcroft, 1990) including CNS neurons (Ashford et al., 1990a,b; Politi and Rogawski, 1991). In many cases, ATP-sensitive K\(^+\) channels are blocked by sulfonylurea antidiabetic agents such as glyburide. Indeed, the inference that K\(^+\) channel opener drugs activate K\(_{ATP}\) channels was based on the observation that their functional effects could be antagonized by sulfonylureas (Sanguinetti et al., 1988).

Although sulfonylureas may not be entirely selective for K\(_{ATP}\) channels (Crépel et al., 1991), they have provided a critical pharmacological tool for studying these channels. Moreover, maps of sulfonylurea binding sites in brain have provided clues to the localization of K\(_{ATP}\) channels. The highest densities of these binding sites are in the substantia nigra and globus pallidus, but they are also present in many other brain areas including the hippocampus (Mourre et al., 1989; Treherne and Ashford, 1991). In confirmation of the binding data, there is evidence from biochemical and electrophysiological studies for the existence of K\(^+\) channel opener-sensitive, K\(_{ATP}\) channels in brain regions such as the substantia nigra (Schmid-Antomarchi et al., 1990; Murphy and Greenfield, 1991) and hippocampus (Politi and Rogawski, 1991). These channels could play a role in the generation, maintenance or arrest of seizure activity and could serve as targets for antiepileptic drugs. For example, activation of ATP-sensitive K\(^+\) channels hyperpolarizes substantia nigra neurons and inhibits GABA release from substantia nigra slices (Schmid-Antomarchi et al., 1990; Häusser et al., 1991; During et al., 1995). The ultimate role these channels might play in the regulation of seizure activity is not easy to predict. However, K\(_{ATP}\) channels could participate in the maintenance of status epilepticus or in the generation of hypoglycemic seizures (Amoroso et al., 1990). The scenario envisaged is that intense seizure activity would lead to a reduction in intracellular
ATP levels in substantia nigra neurons that would activate $K_{ATP}$ channels and depress GABA release. The overall effect would be to obviate the critical role played by the substantia nigra in the control of seizure spread (Gale, 1985, 1992). In hypoglycemia, reduced blood glucose would also lead to diminished intracellular ATP levels and a similar inactivation of the substantia nigra seizure-control mechanism, perhaps contributing to hypoglycemic seizures.

In other brain regions such as the hippocampus, $K_{ATP}$ channel activation could serve to inhibit seizure activity. Thus, cromakalim reduced bursting of hippocampal neurons in the in vitro slice preparation (Alzheimer and ten Bruggencate, 1988) and in cell culture (Simon and Lin, 1993). However, in other situations the drug failed to affect hippocampal epileptiform discharges, although it was able to inhibit the response to anoxia (Mattia et al., 1994). There are a few reports that intraventricularly administered cromakalim can protect against seizures in certain in vivo chemoconvulsant models (Gandolfo et al., 1989a,b; Del Pozo et al., 1990; Popoli et al., 1991), but these reports require confirmation with more traditional anticonvulsant screening tests. As of yet, no $K^+$ channel opener drug has been demonstrated to have CNS activity following systemic administration. Recently, however, benzopyrans structurally related to cromakalim have been described with oral anticonvulsant activity in the maximal electroshock test (Evans et al., 1992). Whether this anticonvulsant activity occurs as a result of effects on $K^+$ channels remains to be determined.

The $K^+$ channel opener drugs represent an interesting direction for anticonvulsant drug development. However, there are a number of impediments that must be overcome before this strategy can be considered seriously. As noted, blood–brain barrier permeable analogs are required. Assuming such compounds become available, nonsclective $K^+$ channel opener drugs could theoretically have proconvulsant effects mediated, for example, by the substantia nigra. In addition, there is evidence that some $K^+$ channel openers can block certain voltage-dependent $K^+$ channel types that might predispose to seizures (Politi et al., 1993). At present, the potential utility of $K^+$ channel opener drugs in epilepsy therapy is uncertain.
Interestingly, however, there are a few reports in the literature that suggest that presently available anticonvulsant drugs may act by enhancing $K^+$ channel function. For example, it has been proposed that the tetronic acid derivative losigamone could protect against seizures by such a mechanism (Köhr and Heinemann, 1990). In addition, there is a report indicating that carbamazepine may enhance a $K^+$ current in neocortical neurons (Zona et al., 1990). However, until further confirmatory data is obtained, these observations must be considered preliminary.

6. Adenosine Agonists and Release-Enhancing Agents

Adenosine is a powerful endogenous inhibitory neuromodulator that is normally present in the extracellular environment at a concentration of about 1 $\mu M$ (Zetterstrom et al., 1982; Greene and Haas, 1991). Adenosine exerts its inhibitory action on CNS excitability by direct (postsynaptic) hyperpolarization of neurons and by inhibition of synaptic release, particularly of glutamate, via adenosine receptors on excitatory nerve terminals (Proctor and Dunwiddie, 1987). These actions are primarily mediated by adenosine receptors of the $A_1$ type (McCabe and Scholfield, 1985). During seizure activity, adenosine levels increase dramatically, which has led to the proposal that the adenosine may mediate seizure arrest and postictal refractoriness (Lewin and Bleck, 1981; Dragunow, 1986; Whitcomb et al., 1990; During and Spencer, 1992). A recent study in the hippocampal slice preparation has suggested that glutamate acting on NMDA receptors can stimulate adenosine release from interneurons (Manzoni et al., 1994). However, the extent to which this accounts for the adenosine released during seizures in the hippocampus or elsewhere remains uncertain. Evidence that endogenous adenosine release protects against seizure activity is the well-known proconvulsant activity of adenosine $A_1$ antagonists (Albertson et al., 1983; Murray et al., 1985). Conversely, treatments that increase adenosine levels or its actions on inhibitory mechanisms may be effective in preventing
seizures. For example, studies in a hippocampal slice preparation have demonstrated that adenosine can suppress epileptiform activity (Dunwiddie, 1980; Lee et al., 1984; Ault and Wang, 1986), whereas blockade of adenosine receptors induces sustained interictal discharge and seizure-like activity (Thompson et al., 1992; Alzheimer et al., 1993). There is evidence that adenosine selectively depresses excitatory but not inhibitory synaptic transmission (Yoon and Rothman, 1991; Thompson et al., 1992; Katchman and Hershkowitz, 1993). The selectivity for excitatory transmission provides a mechanism for adenosine's superior anticonvulsant activity in comparison with other presynaptic modulators (for example, GABA<sub>B</sub> agonists) that may reduce inhibitory as well as excitatory synaptic events.

Much evidence from in vivo experimental models demonstrates that adenosine receptor stimulation can modulate seizure activity. Adenosine itself has weak anticonvulsant properties because it has an extremely short biological half-life (3–6 s) and probably penetrates the blood–brain barrier poorly (Rudolphi et al., 1992). Nevertheless, adenosine can protect against audiogenic seizures in mice (Maitre et al., 1974) and also prolongs the latency to pentylenetetrazol seizures (Dunwiddie and Worth, 1982). Selective adenosine A<sub>1</sub> receptor agonists—such as L-phenylisopropyladenosine, cyclohexyladenosine, and 2-chloroadenosine—have much greater anticonvulsant potency. These compounds have been reported to have activity in the maximal electroshock test (Wiesner and Zimring, 1994). In addition, they protect against seizures induced by pentylenetetrazol (Dunwiddie and Worth, 1982; Murray et al., 1985); bicuculline (Zhang et al., 1994); penicillin (Niglio et al., 1988); DMCM, a benzodiazepine receptor inverse agonist (Petersen, 1991); homocysteine, a putative adenosine sequestering agent (Marangos et al., 1990); and 4-aminopyridine (Jackson et al., 1994). In addition, adenosine agonists prevent the development of kindling induced by electrical stimulation of the amygdala (Dragunow and Goddard, 1984) or by the β-carboline FG 7142 (Stephens and Weidmann, 1989). In contrast, adenosine agonists have variable activity against seizures in fully kindled animals (Barraco et al., 1984; Rosen and Berman, 1985). Conventional anticonvulsant drugs generally do not
interfere with kindling development, although NMDA antagonists have a powerful antiepileptogenic effect (see Section 3.1.). Thus, the ability of adenosine agonists to prevent kindling development could be related to their inhibition of excitatory amino acid release and a consequent reduction in the activation of NMDA receptors.

Despite the effectiveness of adenosine A1 agonists in animal seizure models, it is unlikely that these compounds would be clinically useful anticonvulsants. Adenosine A1 agonists have powerful cardiovascular effects as a result of their actions on peripheral adenosine receptors in the heart and vasculature. Even if these peripheral side effects could be overcome (for example by coadministration of a blood–brain barrier impermeable adenosine antagonist such as 8-p-sulfophenyltheophylline), adenosine agonists at anticonvulsant doses may produce strong sedative effects that would limit their clinical utility (Dunwiddie and Worth, 1982). Consequently, alternative strategies will need to be developed. One approach is to utilize inhibitors of adenosine inactivation. These may specifically target epileptic brain regions since seizure activity is accompanied by local release of adenosine. Such agents would theoretically potentiate the protective effects of adenosine where needed, without producing untoward side effects (since release of adenosine may be less significant under nonepileptic conditions). There are multiple nucleoside transport (uptake) systems in brain that inactivate released adenosine. Electrophysiological studies in the brain slice preparations have indicated that adenosine uptake inhibitors such as dipyridamole, dilazep, nitrobenzylthioguanosine, nitrobenzylthioinosine, hexobendine, and soluflazine can potentiate the inhibitory effects of exogenous adenosine (Sanderson and Scholfield, 1986; Ashton et al., 1987) and reduce epileptiform activity (Ashton et al., 1988). However, adenosine uptake inhibitors generally have little effect on normal synaptic transmission. Moreover, microinjection of several adenosine uptake inhibitors into the rat prepiriform cortex reduced bicuculline-induced seizures (Zhang et al., 1993). Thus, adenosine uptake inhibition is a potentially promising anticonvulsant strategy. However, the available adenosine uptake inhibitors are generally of low potency or do not penetrate the blood–brain barrier. Another
approach to selectively enhance endogenous adenosine levels is to inhibit adenosine metabolism with, for example, inhibitors of adenosine kinase (such as 5'-amino-5'-deoxyadenosine) or adenosine deaminase (such as 2'-deoxycoformycin). Although such enzyme inhibitors have anticonvulsant activity when injected locally (Zhang et al., 1993), there is no evidence that they are effective with systemic administration. Finally, the purine precursor 5-amino-4-imidazole carboxamide riboside (AICAr) (which increases adenosine levels during ATP catabolism, as may occur in a seizure focus), has weak anticonvulsant activity that can be enhanced by the adenosine uptake blocker mioflazine (Marangos et al., 1990). AICAr has low blood–brain barrier permeability; it will be necessary to develop analogs with improved bioavailability before evaluating the potential of this approach. In sum, adenosine release enhancement—whether by adenosine uptake blockade, inhibition of adenosine metabolism, or promotion of adenosine release (as may occur with the xanthine oxidase inhibitor oxypurinal; Rudolphi et al., 1992)—would appear to be a worthwhile investigational approach for anticonvulsant drug development.

7. The Special Problem of Absence Epilepsy

Absence epilepsy, commonly referred to as petit mal, is a childhood neurological disorder characterized by episodes of sudden loss of awareness lasting 3–10 s (Penry et al., 1975). A child exhibiting an absence attack becomes immobile, stares, and may display an automatism such as eyelid fluttering or a slight movement of the face. There is no loss of body tone (falling), as in generalized tonic-clonic seizures. Absence seizures have a characteristic electroencephalographic signature consisting of bilateral, symmetrical, synchronous, 3 Hz spike-wave bursts. The disorder is rare (constituting perhaps 8% of patients with childhood epilepsy) and usually self-limiting (absence seizures typically remit by adolescence). Absence seizures are fundamentally different from other types of human epilepsies in several respects. For the purposes of this discussion, the most important difference is the unique pharmacological responsiveness of absence seizures. Ethosuximide, a drug with little activity in other
seizure types, is highly effective in the treatment of absence seizures. Conversely, absence seizures are worsened by antiepileptic drugs, such as phenytoin and carbamazepine, that are effective in generalized convulsive and partial epilepsies. This pharmacological evidence provides strong support for the concept that the pathophysiological mechanisms underlying absence seizures are distinct from those of generalized convulsive or partial epilepsies. Indeed, absence seizures are believed to represent a disorder of corticothalamic rhythmicity, as originally proposed in the corticoreticular hypothesis of Gloor (1968). Therefore, the brain systems mediating absence seizures may be entirely distinct from those that participate in other seizure types.

Current understanding of absence seizure mechanisms derives from extensive studies of Gloor and his colleagues with the penicillin model of generalized spike-and-wave in the cat (Gloor, 1984). These studies were based on the historic work of Penfield and Jasper in the 1940s and 1950s (see Jasper, 1991) on centrencephalic seizures (i.e., seizures that arise in both hemispheres simultaneously and are associated with immediate loss of consciousness, as in absence seizures). It was hypothesized by Penfield and Jasper that centrencephalic seizures are generated by activity in subcortical structures, including the thalamus, with widespread projections to the cortex. This hypothesis was refined by Gloor, who proposed that the cortex and thalamus acted in concert during generalized seizures. Simultaneous recordings in the cortex and thalamus of cats treated with penicillin demonstrated a pattern of firing during each burst consistent with the circulation of impulse flow within a loop between thalamus and cortex (Avoli et al., 1983; Avoli, 1987). In agreement with this idea was the observation that both cortex and thalamus are required for spike-and-wave discharges: surgical removal or pharmacological inhibition of either eliminates epileptiform activity. The critical involvement of cortex and thalamus has now been confirmed in a brain slice preparation (Coulter and Lee, 1993) and in the genetic absence epilepsy rat from Strasbourg (GAERS), a more realistic model of human absence epilepsy than the penicillin model in the cat (Marescaux et al., 1992b).
A more detailed understanding of the cellular events in generation of the spike-wave discharges by the thalamocortical circuit has come from electrophysiological (voltage clamp) studies of thalamic neurons and, more recently, from modeling of the membrane potential of these cells using parameters derived from voltage clamp experiments (Wang et al., 1991; Huguenard and McCormick, 1992). Burst firing in thalamic neurons is dependent on a slow potential, referred to as the “low-threshold spike” (LTS), mediated by T-type voltage-dependent Ca\(^{2+}\) channels (Jahnsen and Llinás, 1984a, b; Suzuki and Rogawski, 1989; Coulter et al., 1989a). Strong hyperpolarization is required to prime (de-inactivate) T-type Ca\(^{2+}\) channels that are normally inactivated at resting potential. Activation of the LTS in thalamocortical relay neurons is, in turn, dependent on inhibitory drive from thalamic reticular nucleus neurons (Huguenard and Prince, 1992b). These neurons have intrinsic pacemaker activity that generates rhythmic sequences of oscillatory bursts (Bal and McCormick, 1993). Interestingly, this burst firing is also dependent on a T-type Ca\(^{2+}\) current, but with somewhat different properties from that present in relay neurons (Huguenard and Prince, 1992b). Thalamic reticular nucleus neurons are GABAergic. These neurons form recurrent inhibitory connections among themselves, and also provide a powerful inhibitory input necessary to de-inactivate T-type Ca\(^{2+}\) channels of relay neurons. The prolonged inhibitory postsynaptic potential (IPSP) in relay neurons is probably mediated by GABA\(_A\) and GABA\(_B\) receptors (Thomson, 1988; Soltesz et al., 1989). Presently, the role of GABA\(_B\) receptors in regulating the normal firing of relay neurons is poorly defined (McCormick, 1992; von Krosigk et al., 1993). However, during seizure activity (as can be induced with GABA\(_A\) receptor antagonists in thalamic slices), GABA\(_B\) receptors appear to play a critical role in synchronizing the firing (von Krosigk et al., 1993).

### 7.1. T-Type Voltage-Dependent Ca\(^{2+}\) Channel Antagonists

The fundamental insights from electrophysiological studies of the thalamus have led to a mechanistic understanding of the selective antiabsence drug ethosuximide and have also suggested strategies...
for the development of new medications for absence seizures. Thus, Coulter et al. (1989b) demonstrated that ethosuximide produces a modest (up to 40%) reduction in amplitude of the T-type Ca$^{2+}$ current in thalamic neurons (see also Kostyuk et al., 1992; Huguenard and Prince, 1994). The EC$_{50}$ for this effect is 200 μM and there is a roughly 20–35% reduction in Ca$^{2+}$ current at ethosuximide concentrations within the clinically relevant concentration range. Computer simulations of individual thalamic neurons have shown that even smaller reductions in the T-type Ca$^{2+}$ current could result in a diminution of the LTS (Lytton and Sejnowski, 1992). These results support the hypothesis that ethosuximide's effects on the T-type Ca$^{2+}$ current selectively alter the dynamics of slow bursting in thalamic relay neurons that could lead to protection against absence seizures. Additionally, however, ethosuximide probably also acts on the T-type Ca$^{2+}$ current in nucleus reticularis neurons to reduce their burst firing (Huguenard and Prince, 1992a). The effect on reticularis neurons would diminish the hyperpolarizing influence that these cells generate in relay neurons and that is necessary for their burst firing. The dual action of ethosuximide on nucleus reticularis and relay neurons would act in concert to interrupt thalamic bursting, and in turn thalamocortical synchronization during absence seizures.

In addition to ethosuximide, dimethadione (the active metabolite of the anti-absence drug trimethadione) and methylphenylsuccinimide (the active metabolite of another anti-absence drug methsuximide) have been shown to cause a reduction in the T-type Ca$^{2+}$ current in both thalamic relay neurons and nucleus reticularis neurons (Coulter et al., 1990; Huguenard and Prince, 1992a). In contrast, valproate, an agent that is also highly effective in the treatment of absence seizures, has not been found to affect T-type Ca$^{2+}$ currents in thalamic neurons at clinically relevant concentrations. Nonetheless, the drug has been reported to affect a similar current in a peripheral neuron (Kelly et al., 1990). Whether valproate's activity as an absence agent relates to effects on Ca$^{2+}$ channels or to effects on another molecular target is unknown.
7.2. GABA<sub>B</sub> Receptor Antagonists

The critical role of GABA<sub>B</sub> receptors in the generation of absence seizures has been demonstrated in several animal models by the use of selective GABA<sub>B</sub> agonists and antagonists. Thus, the GABA<sub>B</sub> agonist baclofen enhanced, and the GABA<sub>B</sub> antagonist CGP 35348 inhibited, absence-like seizures in γ-hydroxybutyrate-treated rats (Snead, 1992), the GAERS rat (Liu et al., 1992; Marascaux et al., 1992a), and the lethargic mouse (Hosford et al., 1992). Interestingly, in the lethargic mouse there is evidence for a genetic defect resulting in the overexpression of GABA<sub>B</sub> receptors. These animals show a modest increase in GABA<sub>B</sub> receptor density as assessed with radioligand binding and also exhibit evidence of enhanced GABA<sub>B</sub> receptor functional activity in brain slice recordings (Hosford et al., 1992). In the GAERS rat, however, no such alteration in GABA<sub>B</sub> receptors was observed (Spreafico et al., 1993). Thus, while absence-like seizures can occur in animal models as a result of a variety of underlying pathophysiological mechanisms, GABA<sub>B</sub> receptor antagonists appear to be effective in all instances.

The precise site at which GABA<sub>B</sub> antagonists exert their anti-absence activity has not been defined with certainty. However, electrophysiological studies in thalamic slices have supported the hypothesis that, during seizure activity, synaptic activation of GABA<sub>B</sub> receptors generates the hyperpolarization necessary to de-inactivate the T-type Ca<sup>2+</sup> current (von Krosigk et al., 1993). Interruption of this hyperpolarizing influence by antagonists would prevent the seizure activity, but would not affect normal ongoing activity, which seems less dependent on GABA<sub>B</sub> receptor activation. Although there is now extensive experimental evidence supporting the potential clinical utility of GABA<sub>B</sub> antagonists as anti-absence agents, this will need to be confirmed in clinical trials, now in progress.

7.3. Potential Anti-Absence Drugs of Unknown Mechanism

There are a variety of compounds that have an anticonvulsant profile in animal seizure models similar to that of exthosuximide, but whose mechanism of action is unknown. One such compound is the
imidazopyridine AHR-12245 (2-[4-chlorophenyl]-3H-imadazo[4,5-b]pyridine-3-acetamid) (Johnson et al., 1991). Like ethosuximide, AHR-12245 is active orally against pentylenetetrazol seizures in both mice and rats, but is ineffective in the maximal electroshock test. However, AHR-12245 has a substantially greater therapeutic index than ethosuximide and is therefore an interesting candidate for evaluation in the treatment of generalized absence seizures.

As noted in Sections 2.1.4. and 2.1.6., GABA-potentiating neuroactive steroids and ioreclezole have profiles of activity similar to that of ethosuximide. Whether these compounds will prove useful in the treatment of absence seizures remains unknown.

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