

Mechanisms of Action of Currently Prescribed and Newly Developed Antiepileptic Drugs

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Summary: Clinically available antiepileptic drugs (AEDs) decrease membrane excitability by interacting with neurotransmitter receptors or ion channels. AEDs developed before 1980 appear to act on sodium (Na) channels, γ -aminobutyric acid_A (GABA_A) receptors, or calcium (Ca) channels. Benzodiazepines and barbiturates enhance GABA_A-receptor-mediated inhibition. Phenytoin, carbamazepine and, possibly, valproate (VPA) decrease high-frequency repetitive firing of action potentials by enhancing Na channel inactivation. Ethosuximide and VPA reduce a low threshold (T-type) Ca-channel current. The mechanisms of action of recently developed AEDs are less clear. Lamotrigine may decrease sustained high-

frequency repetitive firing of voltage-dependent Na action potentials, and gabapentin (GBP) appears to bind to a specific binding site in the CNS with a restricted regional distribution. However, the identity of the binding site and the mechanism of action of GBP remain uncertain. The antiepileptic effect of felbamate may involve interaction at the strychnine-insensitive glycine site of the *N*-methyl-D-aspartate receptor, but the mechanism of action is not yet proven. **Key Words:** Anticonvulsants—Phenytoin—Carbamazepine—Barbiturates—Benzodiazepines—Valproate—Ethosuximide—Lamotrigine—Gabapentin—Felbamate.

A limited number of antiepileptic drugs (AEDs) currently are available for use in treatment of patients with epilepsy: phenytoin (PHT), carbamazepine (CBZ), barbiturates and primidone (PRM), benzodiazepines (BZDs), valproate (VPA), ethosuximide (ESM), and trimethadione (TMO). Recently, several additional AEDs have or will soon become available, the most promising of which are lamotrigine (LTG), gabapentin (GBP), and felbamate (FBM). It is likely that these standard and new AEDs have as their primary targets of action neurotransmitter receptors or ion channels, primarily γ -aminobutyric acid_A (GABA_A)-receptor channels, voltage-dependent sodium (Na) channels, and voltage-dependent calcium (Ca) channels. The basic action of the identified AEDs on neurotransmitter receptor channels or ion channels may be responsible for their clinical actions; these interactions are the subject of this review.

BZDs AND BARBITURATES

BZDs and barbiturates enhance GABAergic inhibition at free-serum concentrations found in ambulatory patients (Macdonald, 1989). Both BZDs and barbiturates interact with the GABA_A receptor (GABAR), which is a macromolecular protein containing binding sites for, at least, GABA, picrotoxin, neurosteroids, barbiturates, and BZDs, and with a chloride (Cl⁻) ion-selective channel (DeLoorey and Olsen, 1992). GABARs appear to be composed of combinations of different subtypes (α 1– α 6, β 1– β 4, γ 1– γ 3, δ , and ρ 1– ρ 2) of polypeptide subunits (Schofield et al., 1987; Pritchett et al., 1989a; Shivers et al., 1989; Cutting et al., 1991). Table 1 contains a summary of some biochemical properties of the different GABAR subunit subtypes, which all share similar structural features and basic functional properties. There is a differential regional expression in the CNS and spinal cord of various subunit subtype messenger RNAs (mRNAs) (Laurie et al., 1992; Wisden et al., 1992). Some subtype mRNAs were expressed only in specific cell types (α 6 mRNA was demonstrated only in cerebellar granule cells), whereas other subtype mRNAs, such

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TABLE 1. GABAR subunit subtype biochemistry

Subunits	α	β	γ	δ	ρ
No. of subtypes	6	4	3	1	2
No. of splice variants	0	1	1	0	0
AA sequence size range (kDa)	48–64	51	48	48	52
% AA homology intrafamily	70–80	70–80	70–80	NA	70–80
% AA homology interfamily	30–40	30–40	30–40	30–40	30–40

GABAR, γ -aminobutyric acid_A receptor; kDa, kilodalton; AA, amino acid; NA, not applicable. From Macdonald and Olsen, 1994.

as for the $\beta 2$ subtype, had a more widespread distribution. Therefore, differential expression and assembly of various subunit subtypes could produce a multitude of GABAR isoforms.

GABA binds to GABARs to regulate gating (opening and closing) of the Cl⁻ ion channel. The single-channel gating properties of the main-conductance state of the native GABAR in murine spinal cord neurons in culture have been characterized (Macdonald et al., 1989a; Weiss and Magleby, 1989; Twyman et al., 1990). Binding of GABA increases the probability of channel opening, and the open channel can close and rapidly reopen to create bursts of openings. To explain this complex gating behavior, the single-channel activity of the main conductance state has been modeled using a reaction scheme that incorporates 2 sequential GABA binding sites, 3 open states, and 10 closed states (Macdonald et al., 1989a; Twyman et al., 1990).

Barbiturates and BZDs can modulate GABAR current by regulating the single-channel properties of the receptor. To enhance the current, a drug may increase the channel conductance, increase the channel open-and-burst frequencies, and/or increase the channel open-and-burst durations. The kinetic model of the GABAR has been used to study the mechanisms of action of AEDs that act on the GABAR.

Barbiturates enhance the GABAR current by binding to an allosteric regulatory site on the receptor (Olsen, 1987). Results from fluctuation analysis suggest that phenobarbital and pentobarbital increase the mean channel-open duration of GABAR currents without altering channel conductance (Study and Barker, 1981). Single-channel recordings of barbiturate-enhanced single GABAR currents directly demonstrate that barbiturates increase mean channel-open duration but do not alter receptor conductance or opening frequency (Macdonald et al., 1989b; Twyman et al., 1989).

For $\alpha 1\beta 1$ receptors expressed in *Xenopus* oocytes or Chinese hamster ovary (CHO) cells, currents were increased by pentobarbital (Moss et al., 1990; Verdoorn et al., 1990). Furthermore, the concentration dependence for the effect was the same

for receptors with different α and β subunits coexpressed with $\gamma 2$ and with $\beta 2\gamma 2$ alone in *Xenopus* oocytes (Verdoorn et al., 1990). These results directly demonstrate that the α and β subunits contain the allosteric regulatory site for barbiturates.

GABARs have a high-affinity binding site for BZDs, and BZD and GABAR binding sites have been demonstrated to be allosterically coupled (Olsen, 1987). BZDs increase GABAR current. Results from fluctuation analysis suggest that the BZD diazepam increases GABAR current by increasing opening frequency without altering channel conductance or open duration (Study and Barker, 1981). Single-channel recordings have confirmed that BZDs increase receptor-opening frequency without altering mean open time or conductance (Vicini et al., 1987; Rogers et al., 1989).

The GABARs expressed in *Xenopus* oocytes and CHO cells formed from $\alpha 1\beta 1$ subunits are insensitive to BZDs (Pritchett et al., 1989b; Moss et al., 1990). The basis for this insensitivity was determined when two forms of a third GABAR subunit, the γ and $\gamma 2$ subunits, were isolated from a human fetal brain complementary DNA (cDNA) library (Pritchett et al., 1989b). When the $\gamma 2$ subunit was transiently coexpressed with $\alpha 1$ and $\beta 1$ subunits in human embryonic kidney cells, fully functional GABARs were formed that were sensitive to BZDs, β -carbolines, barbiturates, and picrotoxin.

Analysis of binding of various BZDs to purified GABARs from brain regions revealed the existence of two subclasses of BZD binding sites, type I and type II (Klepner et al., 1978; Braestrup et al., 1981; Lipa et al., 1981; Garret et al., 1985; Eichinger and Sieghart, 1986). The basis for this heterogeneity was clarified after the identification of multiple GABAR subunit families. The molecular basis of types I and II BZD-binding sites was determined using transient expression of $\alpha x(x = 1-6)\beta 1\gamma 2S$ subunit combinations in human embryonic kidney 293 cells (Pritchett et al., 1989a; Pritchett and Seeburg, 1990). The combination of $\alpha 1\beta 1\gamma 2S$ GABAR subtypes produced type I BZD-binding sites, and expression of $\alpha 2\beta 1\gamma 2S$, $\alpha 3\beta 1\gamma 2S$, or $\alpha 5\beta 1\gamma 2S$ GABAR subtype combinations produced type II

BZD-binding sites. These receptor isoforms were differentiated based on binding of the type I BZD-selective compounds, zolpidem and CL 218872. Expression of the $\alpha 4$ or $\alpha 6$ subtype with $\beta 1$ and $\gamma 2S$ subtypes produced less-characterized BZD-binding sites, thought previously to be an artifact (Sieghart et al., 1987; Wisden et al., 1991). These receptor isoforms did not bind the prototypical BZDs, diazepam and flunitrazepam, or β -carbolines, but did bind the "alcohol antagonist" Ro 15-4513 and the BZD antagonist flumazenil (Ro 15-1788). Binding of GABA agonists (muscimol) was not impaired. BZD receptor pharmacology was not altered by substituting any other β subtype. Therefore, BZD pharmacology of recombinant GABARs appeared to depend on the α subtype. The original types I and II BZD classification was further segregated into type I ($\alpha 1$), type IIA ($\alpha 2$ and $\alpha 3$), type IIB ($\alpha 5$), and type III ($\alpha 4$ and $\alpha 6$) BZD sites (Pritchett et al., 1989a; Doble and Martin, 1992). Therefore, despite the finding that the γ subunit confers BZD sensitivity to GABARs, the α subunit appears to determine the type of BZD receptor expressed.

PHT AND CBZ

In patients being treated for epilepsy, PHT and CBZ have been shown to interact with voltage-dependent Na channels at concentrations found free in plasma (Macdonald, 1989). These AEDs were demonstrated to reduce the frequency of sustained repetitive firing of action potentials in neurons in cell culture (McLean and Macdonald, 1983, 1986b). Their characteristic property was no reduction of the amplitude or duration of single action potentials but reduction of the ability of neurons to fire trains of action potentials at high frequency. The limitation of high-frequency repetitive firing was voltage dependent, with limitation of firing increased after depolarization and reduced after hyperpolarization. Once developed, the limitation of firing was prolonged, lasting several hundred milliseconds. AED action appeared to be caused by a shift of Na channels to an inactive state that was similar to the normally occurring inactive state but from which recovery was delayed.

The actions of PHT and CBZ also have been studied on mammalian myelinated nerve fibers (Schwarz and Grigat, 1989). Both drugs produced a voltage-dependent block of Na channels that could be removed by hyperpolarization. PHT produced a shift of the steady-state Na-channel inactivation curve to more negative voltages. PHT and CBZ both reduced the rate of recovery of Na channels from inactivation. In control solutions, Na channels

recovered from complete inactivation in a few milliseconds after a 500-ms depolarization to 25 mV. In the presence of 100 μM PHT or CBZ, recovery was prolonged to 90 or 40 ms, respectively. At 50 μM , PHT and CBZ each produced a frequency-dependent block. At 50 μM , PHT produced an initial block of 50%. With repetitive stimulation at 10 Hz, the block increased to about 80% over 2.5 s. Recovery from this block required approximately 2.5 s. At 100 μM , CBZ also produced a frequency-dependent block that was somewhat less pronounced than that produced by PHT. Thus, PHT and CBZ produced voltage- and frequency-dependent block of Na channels. Because the concentration-response curves could be fitted assuming a first-order reaction, it was suggested that one drug molecule binds to one receptor near or at the Na channel. The data are also consistent with PHT and CBZ binding with higher affinity to inactivated Na channels than to open or resting Na channels. Of interest was the finding that PHT had a longer time dependence for frequency-dependent block and for recovery from block than did CBZ, resulting in a more pronounced frequency-dependent block for PHT than for CBZ. Therefore, although PHT and CBZ have qualitatively similar actions on Na channels, the actions are quantitatively somewhat different. This may explain, at least in part, differences in efficacy for these two AEDs in different patients.

Similar voltage-clamp experiments were performed on isolated mammalian brain neurons (Wakamori et al., 1989). Hippocampal pyramidal neurons from the CA1 region were obtained from 1- and 2-week-old rats. At 200 μM , PHT produced a 20-mV negative shift in the steady-state inactivation curve for Na channels and produced frequency-dependent block of Na channels. Frequency-dependent block was shown at frequencies as low as 1 Hz; the block increased to 50% at 10 Hz. Therefore, the ability of PHT to enhance inactivation in neurons in cell culture and in mammalian myelinated nerve fibers also was present in isolated mammalian neurons.

The effect of PHT on human Na channels has also been examined (Tomaselli et al., 1989). Total mRNA was extracted from human brain and injected into *Xenopus* oocytes. The human brain Na channels expressed in oocytes were also blocked by PHT in a voltage-, frequency-, and time-dependent fashion. The effects of PHT on human Na channels were very similar to those on cultured mouse neurons, rat myelinated nerve, and rat hippocampal pyramidal neurons.

Evidence from voltage-clamp experiments has

therefore confirmed the basic mechanism of action of PHT and CBZ. Both appear to stabilize the inactive form of the Na channel in a voltage-dependent fashion, the effect being lessened at large negative membrane potentials and increased at less negative membrane potentials. Both AEDs slow the rate of recovery from Na-channel inactivation and shift the steady-state Na-inactivation curve to more negative voltages. This stabilization of the inactive form of the receptor results in a frequency-dependent block of Na channels and in the blockade of sustained high-frequency repetitive firing of action potentials evoked from reduced membrane potentials. It is notable that PHT has a stronger slowing effect than CBZ; therefore, these AEDs have slightly different actions under different conditions of repetitive firing.

ESM AND TMO

A number of AEDs have been demonstrated to modify the properties of voltage-dependent Ca channels (Macdonald, 1989*b*). PHT, barbiturates, and BZDs reduce Ca influx into synaptic terminals and block presynaptic release of neurotransmitter. However, these actions have been demonstrated only at concentrations that are above therapeutic free-serum concentrations in patients treated for epilepsy. Therefore, it has been concluded that the primary actions of AEDs are not on Ca channels. Ca channels, however, have been shown to be heterogeneous (Snutch et al., 1990, 1991; Hui et al., 1991; Mori et al., 1991; Starr et al., 1991; Williams et al., 1992). At least four different types of voltage-dependent Ca channels have been described: L-type, T-type, N-type, and P-type (Nowycky et al., 1985; Mintz et al., 1992). These four Ca channels have different voltage ranges for activation and inactivation and different rates of activation and inactivation. Each channel type has been cloned and shown to be composed of several subunits: L-type channel (Campbell et al., 1988; Catterall, 1988); N-type channel (Williams et al., 1992; Fujita et al., 1993); P-type channel (Mori et al., 1991); and T-type channel (Soong et al., 1993). In addition, many subtypes of channels have been identified. It is also likely that these are not the only types of Ca channels present on neurons.

In view of the finding that neurons express multiple Ca channels, it may be that AEDs act on specific types of channels. Indeed, this has been demonstrated for ESM, TMO, and VPA, which are effective in the treatment of generalized absence seizures. Generalized absence epilepsy is characterized clinically by brief periods of loss of con-

sciousness and electrically by a generalized 3-Hz spike-and-wave EEG discharge. It has been suggested that thalamic relay neurons play a critical role in the generation of the abnormal thalamocortical rhythmicity that underlies the 3-Hz spike-and-wave discharge. Whole-cell voltage-clamp recordings from acutely dissociated relay neurons from rat thalamus have demonstrated the presence of low-threshold (T-type) and high-threshold Ca currents (Coulter et al., 1989*a*). The T-type currents had properties such that T-channel activation was necessary and sufficient to cause the generation of low-threshold Ca spikes in thalamic relay neurons. It was demonstrated that both ESM and dimethadione, the active metabolite of TMO, reduced the T-type current of thalamic neurons isolated from guinea pigs and rats (Coulter et al., 1989*b,c*). The reduction of the T-type current was produced at clinically relevant concentrations of ESM and dimethadione. PHT and CBZ, which are ineffective in the control of generalized absence seizures, had minimal effects on T-type current. The ESM-induced reduction of the T-type current was voltage-dependent. The reduction was most prominent at negative membrane potentials and less prominent at more positive membrane potentials. ESM did not alter the voltage dependency of steady-state inactivation or the time course of recovery from inactivation. Dimethadione reduced T-type current by a mechanism similar to that of ESM. Another antiepileptic succinimide, α -methyl- α -phenylsuccinimide, also reduced T-type currents, whereas an epileptic succinimide, tetramethylsuccinimide, reduced the T-type current only at very high concentrations (Coulter et al., 1990). These results suggest that anticonvulsant succinimides and dimethadione, compounds effective in the treatment of generalized absence epilepsy, may have their primary action in the reduction of the T-type Ca current in thalamic relay neurons.

VPA

The effect of VPA on Na channels has been studied less extensively. Although VPA blocked sustained high-frequency firing of neurons in culture (McLean and Macdonald, 1986*a*), detailed voltage-clamp experiments of VPA actions on Na currents have not been performed. It cannot be determined whether VPA has a mechanism of action similar to that of PHT and CBZ until these studies have been performed.

VPA is one of the most effective AEDs against generalized absence seizures. Interestingly, initial studies of VPA did not demonstrate any effect on

the T-type Ca current, but subsequently VPA was shown to reduce T-type currents in primary afferent neurons (Kelly et al., 1990). The effect was produced over a concentration range of 100–1,000 μ M. However, the magnitude of the effect was modest: a 16% reduction was seen at 1 mM VPA. It is unclear whether this modest reduction in T-type Ca current is sufficient to explain the effect of VPA on generalized absence seizures. Furthermore, the basis for the discrepancy between the results obtained in rat thalamic neurons and rat primary afferent neurons remains uncertain. It may be that different neuron types have different sensitivities to these AEDs or that the small effect is difficult to characterize. Whether this a relevant mechanism of action for VPA will have to be determined by future investigation.

FBM

FBM, 2-phenyl-1,3-propanediol dicarbamate, is a dicarbamate with a structure similar to that of meprobamate, an antianxiety agent. In experimental animals, it was effective in blocking seizures induced by maximal electroshock (MES), pentylene-tetrazol (PTZ), and picrotoxin (Swinyard et al., 1986). FBM inhibited bicuculline-induced seizures at high concentrations but was ineffective against strychnine-induced seizures (Sofia et al., 1991). These results suggest that FBM increases seizure threshold and prevents the electrical spread of seizure activity (Swinyard et al., 1986). In clinical studies, FBM has been effective for treatment of partial seizures with and without becoming secondarily generalized in adults and for partial and generalized seizures associated with the Lennox-Gastaut syndrome in children.

FBM has been tested for interaction with the GABAR complex as a possible mechanism of anti-epileptic activity. In rat brain cortical membranes, FBM did not affect ligand binding to the GABA-, BZD-, or picrotoxin-binding sites of the GABAR complex. In addition, in radiolabeled Cl^- influx studies in cultured mouse spinal cord neurons, FBM did not affect GABA-induced ^{36}Cl influx (Ticku et al., 1991). These results suggest that FBM may not have a direct effect on the GABAR complex. However, FBM in subprotective doses enhanced the protective effects of diazepam against seizures induced by MES, PTZ, and isoniazid, but not by bicuculline, suggesting that it has indirect effects on the GABAR complex or is involved in other mechanisms of action (Gordon et al., 1991). FBM reduced sustained repetitive firing of voltage-dependent Na channels in mouse spinal cord neu-

rons, producing 50% inhibition at 67 μ g/ml compared with a control population of neurons, of which 72% responded with sustained repetitive firing (White et al., 1992). It remains to be determined whether these results indicate a direct interaction of FBM with voltage-dependent Na channels.

FBM has also been tested for a possible effect on excitatory amino acid receptors. It inhibited *N*-methyl-D-aspartate (NMDA)- and quisqualate-induced seizures in mice but did not significantly inhibit MK-801 binding (Sofia et al., 1991). However, FBM has been shown to inhibit the binding of [^3H]-5,7-dichlorokynurenic acid, a competitive antagonist, at the strychnine-insensitive glycine site of the NMDA receptor (McCabe et al., 1993). FBM also reduced the ability of glycine to enhance NMDA-induced Ca currents in cerebellar granule cells measured by the fluorescent probe indo-1. In other studies, D-serine, a glycine-site agonist, was administered cerebroventricularly in audiogenic seizure-susceptible mice and produced a parallel right shift in the FBM anticonvulsant dose-response curve (Harmsworth et al., 1993). Although not conclusive, these results suggest that FBM activity at the glycine site of the NMDA receptor may be related to the antiepileptic mechanism of action.

GBP

GBP, 1-(aminomethyl)cyclohexaneacetic acid, is a cyclic GABA analogue originally designed to mimic the steric conformation of GABA (Schmidt, 1989), to have high lipid solubility that permits penetration of the blood-brain barrier, and to be a centrally active GABA agonist with potential therapeutic value (Rogawski and Porter, 1990). GBP has been shown to have anticonvulsant activity in a variety of animal seizure models (Bartoszyk et al., 1986) and is effective in the treatment of human partial and generalized tonic-clonic seizures.

Despite its demonstrated efficacy, the anticonvulsant mechanism of action of GBP is not known. Early work suggested that GBP may act on GABAergic neurotransmitter systems; studies showed that it protected mice from tonic extension in chemical convulsion models using inhibitors of GABA synthesis (3-mercaptopropionic acid, isonicotinic acid, semicarbazide) or antagonists acting on the GABA_A-receptor complex (bicuculline, picrotoxin) (Bartoszyk et al., 1983; Bartoszyk and Reimann, 1985). However, subsequent work has not clearly demonstrated a specific effect of GBP on GABAergic neurotransmitter systems. Inhibition of monoamine release by GBP in electrically stimulated rabbit caudate nucleus (Reimann, 1983) and

rat cortex (Schlicker et al., 1985) was not modified by GABA, baclofen, or bicuculline, suggesting that GBP did not act on GABA_A or GABA_B receptors.

Binding experiments in rat brain and spinal cord have shown that GBP has no significant affinity for the GABA_A or GABA_B binding sites measured by [³H]muscimol and [³H]baclofen displacement, respectively. GBP did not significantly inhibit the binding of [³H]diazepam, had only a weak inhibitory effect on the GABA-degrading enzyme GABA-aminotransferase, did not elevate GABA content in nerve terminals, and did not affect the GABA uptake system (Bartoszyk et al., 1986). However, GBP has been shown to increase GABA turnover in several regions of rat brain (Loscher et al., 1991). Recent work has shown that GBP binds to the novel high-affinity site in the CNS (Hill et al., 1993; Suman-Chauhan et al., 1993) and is potently displaced by the anticonvulsant 3-isobutyl GABA (Taylor et al., 1993), but the identity of this binding site remains uncertain. In addition, GBP has been shown to be a substrate for a saturable L-amino acid transport system in rat gut tissues (Stewart et al., 1993). These latter findings raise the possibility of an active transport process of GBP across neuron membranes.

In electrophysiologic studies, GBP did not affect depolarizations elicited by iontophoretic application of GABA on cultured mouse spinal cord neurons (Taylor et al., 1988; Rock et al., 1993). In addition, GBP appeared to act by GABAR-independent mechanisms in studies with rat hippocampal slices (Haas and Wieser, 1986) and the feline trigeminal nucleus (Kondo et al., 1991). GBP has been shown to decrease inhibition evoked by paired-pulse orthodromic stimulation of pyramidal neurons in the hippocampal slice preparation (Dooley et al., 1985; Taylor et al., 1988); however, the specific effect of GBP is not known.

GBP protected mice from convulsions caused by strychnine, a glycine-receptor antagonist, but was unable to displace [³H]strychnine in binding studies at the highest concentrations tested (Bartoszyk et al., 1986). Electrophysiologic studies showed no effect of GBP on the response of spinal cord neurons to iontophoretically applied glycine (Rock et al., 1993).

GBP has been tested in animal seizure models where seizures are induced by administration of excitatory amino acids. GBP prolonged the onset latency of clonic convulsions and tonic extension and death in mice after i.p. injections of NMDA, but not of kainic acid or quinolinic acid. GBP did not have a clear effect on convulsions when these compounds or glutamate were injected into the lateral

ventricle of rats (Bartoszyk, 1983). The i.p. injections in mice of GBP or the NMDA-receptor competitive antagonist 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphoric acid (CPP) antagonized tonic seizures. The effect of GBP, but not of CPP, was dose-dependently antagonized by the administration of serine, an agonist at the glycine receptor on the NMDA receptor complex, suggesting an involvement of the strychnine-insensitive glycine site of the NMDA receptor in the anticonvulsant activity of GBP (Oles et al., 1990).

In unpublished studies cited by Chadwick (1992), GBP reportedly antagonized NMDA-induced, but not kainate-induced, depolarizations in thalamic and hippocampal slice preparations, and antagonized NMDA-induced currents in the presence of glycine in cultured striatal neurons, an effect that was reversed by addition of serine or increased glycine. Other studies did not show a significant effect of GBP on neuronal responses to iontophoretic application of glutamate or on membrane depolarizations and single-channel currents evoked by NMDA with or without coapplication of glycine (Rock et al., 1993). These results, in part, are similar to the findings of others, in which GBP had no effect on spinal cord neuron depolarizations elicited by iontophoretically applied glutamate (Taylor et al., 1988) or pressure-ejected NMDA (Wamil et al., 1991a). In addition, in extracellular recordings from rat hippocampal slice preparations, GBP had no effect on long-term potentiation, making it unlike NMDA-receptor antagonists (Taylor et al., 1988).

GBP had no effect on sustained repetitive firing of action potentials in mouse spinal cord neurons (Taylor et al., 1988; Rock et al., 1993). In other experiments using the same neuron preparation, high concentrations of GBP (100 μM) reduced sustained repetitive firing of action potentials. After overnight exposure of the cultures to GBP, sustained repetitive firing of action potentials was reduced by GBP 1 μM (Wamil et al., 1991b). The significance of these findings is not fully established at this time. The different results of the studies done on mouse spinal cord neurons suggest that GBP does not directly interact with voltage-dependent Na channels or limit sustained repetitive firing of action potentials; therefore, the antiepileptic activity of GBP is not attributable to this mechanism of action.

Although most effective in the treatment of human partial and generalized tonic-clonic seizures, the effect of GBP on absence seizures has been studied in both animal models and as add-on therapy in AED-resistant patients with epilepsy. In animal studies using PTZ-induced clonic seizures,

GBP protected mice from clonic convulsions in both the subcutaneous Metrazol test and the intravenous threshold test (Bartoszyk et al., 1986). However, in a rat genetic model of absence epilepsy, GBP increased EEG spike-and-wave bursts in a dose-dependent manner (Foot and Wallace, 1991). In human studies, GBP reduced >50% of absence seizures in half of the patients in one study (Bauer et al., 1989) and in another study reduced absence seizures and generalized spike-and-wave complexes in patients undergoing 24-h EEG monitoring (Rowan et al., 1989). In studies of mouse spinal cord neurons, GBP blocked responses to Bay K 8644, an agonist at the dihydropyridine binding site of the L-type Ca channel (Wamil et al., 1991a). In other electrophysiologic studies, however, GBP did not significantly affect any Ca-channel current subtype (T, N, or L), suggesting that the basic mechanism of action was not on voltage-dependent Ca channels (Rock et al., 1993).

In summary, the results of several studies have not demonstrated that GBP has a major effect on ligand- or voltage-gated channels. Further work on the high-affinity binding site of GBP should contribute significantly to understanding its mechanism of action.

LTG

LTG, 3,5,-diamino-6-(2,3-dichlorophenyl)-*s*-triazine, a phenyltriazine with weak antifolate activity, was developed in response to observations that use of phenobarbital, PRM, and PHT resulted in reduced folate levels, and that folates could induce seizures in experimental animals (Reynolds et al., 1966). Initially, antifolate activity was thought to be related to anticonvulsant activity, but this has not been demonstrated by structure-activity studies (Rogawski and Porter, 1990). LTG has anticonvulsant activity in several animal seizure models, including hind-limb extension in MES and maximal PTZ seizures in rodents (Miller et al., 1986). It has been effective as add-on therapy in the treatment of human partial and generalized tonic-clonic seizures.

The action of LTG on the release of endogenous amino acids from rat cerebral cortex slices *in vitro* has been studied. LTG potently inhibited release of glutamate and aspartate evoked by the Na-channel activator veratridine and was much less effective in the inhibition of release of acetylcholine or GABA. At high concentrations, LTG had no effect on spontaneous or potassium-evoked amino acid release. These studies suggest that LTG acts at voltage-dependent Na channels, resulting in decreased presynaptic release of glutamate (Leach et al., 1986).

In radioligand studies, the binding of [³H]batrachotoxinin A 20- α -benzoate, a neurotoxin that binds to receptor site 2 on voltage-dependent Na channels, was inhibited by LTG in rat brain synaptosomes. In electrophysiologic studies, LTG blocked sustained repetitive firing in cultured mouse spinal cord neurons in a dose-dependent manner at concentrations therapeutic for human seizures (Cheung et al., 1992). These results suggest that the anticonvulsant effect of LTG is due to a specific interaction at the voltage-dependent Na channel, which may result in a preferential decreased of presynaptic glutamate release.

CONCLUSION

The currently available AEDs appear to have only three major mechanisms of action (Table 2). Drugs that are effective against generalized tonic-clonic and partial seizures appear to reduce sustained high-frequency repetitive firing of action potentials by delaying recovery of Na channels from activation. AEDs that are effective against generalized absence seizures appear to reduce low-threshold (T-type) Ca currents. Finally, drugs that are effective against myoclonic seizures generally enhance GABAR inhibition. Although the currently available AEDs have been shown to be effective, there are patients, especially those with complex partial seizures, whose seizures are refractory to these AEDs. FBM, LTG, and GBP have shown promise in clinical trials and may help in managing some refractory patients. Although the mechanisms of action of these drugs are unclear, those of at least GBP and FBM may be novel (Table 3). Furthermore, it is probable that AEDs now under development will have actions on new neurotransmitter receptors or channels. For example, considerable effort has been directed toward developing compounds that are antagonists of excitatory amino acid transmission. With new AEDs that act on different neurotransmitter receptors or channels, it is

TABLE 2. Antiepileptic drug actions

	Na channels	GABAR	T-Ca channels
Carbamazepine	++	-	-
Phenytoin	++	-	-
Primidone	+	-	?
Valproate	++	?/+	?/+
Barbiturates	+	+	-
Benzodiazepines	+	++	-
Ethosuximide	-	-	++

Na, sodium; GABAR, γ -aminobutyric acid_A receptor; T-Ca, T-type calcium channel.

TABLE 3. *New antiepileptic drug actions*

	Na channels	GABAR	T-Ca channels	NMDAR
Felbamate	+	-	?	+
Gabapentin	+	-	-	?
Lamotrigine	++	-	?	?

Na, sodium; GABAR, γ -aminobutyric acid_A receptor; T-Ca, T-type calcium channel; NMDAR, *N*-methyl-D-aspartate receptor.

hoped that patients refractory to available AED therapy will become medically treatable.

Approaches to the investigation of the mechanisms of action of AEDs to date have been fairly descriptive. With the recent development of new molecular biologic techniques for the study of CNS function and the cloning of cDNAs for specific neurotransmitter receptors and ion channels that are targets of AEDs, it may be possible to study more closely the interaction of AEDs with their target receptors or channels. This will assist in elucidating channel and receptor structure and may also provide insights into the interactions of AEDs with receptors and channels. Insights gained from these studies may assist in the design of improved AEDs, which may act on the same receptors or channels as standard AEDs but may have more specific or selective actions.

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