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Molecular Mechanisms of Antiseizure Drug Activity at $GABA_A$ Receptors

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Abstract

The $GABA_A$ receptor ($GABA_AR$) is a major target of antiseizure drugs (ASDs). A variety of agents that act at $GABA_A$Rs s are used to terminate or prevent seizures. Many act at distinct receptor sites determined by the subunit composition of the holoreceptor. For the benzodiazepines, barbiturates, and loreclezole, actions at the $GABA_AR$ are the primary or only known mechanism of antiseizure action. For topiramate, felbamate, retigabine, losigamone and stiripentol, $GABA_AR$ modulation is one of several possible antiseizure mechanisms. Allopregnanolone, a progesterone metabolite that enhances $GABA_AR$ function, led to the development of ganaxolone. Other agents modulate GABAergic "tone" by regulating the synthesis, transport or breakdown of GABA. $GABA_AR$ efficacy is also affected by the transmembrane chloride gradient, which changes during development and in chronic epilepsy. This may provide an additional target for "GABAergic" ASDs. $GABA_AR$ subunit changes occur both acutely during status epilepticus and in chronic epilepsy, which alter both intrinsic $GABA_AR$ function and the response to $GABA_AR$-acting ASDs. Manipulation of subunit expression patterns or novel ASDs targeting the altered receptors may provide a novel approach for seizure prevention.

Keywords

inhibition; epilepsy; antiepileptic drugs; GABA receptor; seizures; chloride channel

Seizures frequently result from an imbalance of excitation and inhibition due to a failure of inhibitory neurotransmission. Most agents that enhance $GABA_A$ receptor ($GABA_AR$) function have antiseizure properties due to their ability to increase inhibitory neurotransmitter tone. The evidence linking epilepsy with dysfunction of GABAergic inhibition is substantial, and has been extensively reviewed[1-4].

$GABA_A$Rs and Epilepsy

$GABA_A$Rs are pharmacologically complex, with binding sites for benzodiazepines (BZs), barbiturates, neurosteroids, general anesthetics, loreclezole, and the convulsant toxins, picrotoxin and bicuculline. Protein subunits from seven different subunit families[5] assemble to form pentameric[6] transmembrane chloride channels (Fig. 1). In mammals, 16 subunit subtypes have been cloned, including 6 α, 3 β and 3 γ subtypes, as well as δ, π[7], ε[8] and θ[9]

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and alternatively spliced variants of the β2 and γ2 subtypes. Subunit expression is regulated by region, cell type[10] and developmental stage[11], reducing the number of isoforms expressed in specific brain regions and individual neurons. The most common composition includes two α1, two β2 and a single γ2 subunit; the δ subunit is found instead of γ in receptors expressed extrasynaptically. The subunits are arranged around a central water-filled pore, which gates to conduct Cl^- ions when GABA is bound (Fig. 1). Individual subunit subtypes confer different sensitivities to $GABA_AR$ modulators including BZs[12], loreclezole[13] and zinc ions[14].

$GABA_ARs$ are the target not only of the BZs, but other ASDs including barbiturates and agents like tiagabine and vigabatrin[1] that increase GABA concentration at the synapse. Bicuculline and picrotoxin, which are $GABA_AR$ antagonists, induce seizures in animals and epileptiform activity in brain slice preparations. Several animal models of epilepsy have altered $GABA_AR$ number or function[1,2,15]. $GABA_AR$ subunit expression is altered in the hippocampi of experimental animals with recurrent seizures[16] and in patients with temporal lobe epilepsy[17,18]. . Angelman's syndrome is a human neurodevelopmental disorder associated with severe mental retardation and epilepsy, which is linked to a deletion mutation on chromosome 15q11-13[20] in a region encoding the $GABA_AR$ β3 subunit[21]. Two mutations in the γ2 subunit that impair $GABA_AR$ function[22], K289M[23] and R43Q[24], have been linked to a human syndrome of childhood absence epilepsy and febrile seizures, and a loss-of-function mutation in the α1 subunit causes autosomal dominant Juvenile Myoclonic Epilepsy[25]. The R43Q mutation in the γ2 subunit reduces BZ sensitivity[26] by altering $GABA_AR$ assembly[27-29] and trapping the receptor in the endoplasmic reticulum[30]. Other point mutations in $GABA_AR$ subunits have also been associated with generalized epilepsies (see Fig. 2). Hence, $GABA_AR$ modulation by GABAergic ASDs is likely critical to their antiseizure activity.

Benzodiazepines

BZs were initially developed as anxiolytic agents in the 1950's. Chlordiazepoxide was introduced in 1960, followed by diazepam[31] and nitrazepam.[32] In 1965, diazepam was first used to treat status epilepticus in humans.[33] Clonazepam was introduced in the 1970's primarily as an ASD,[34] and clobazam, a 1,5 benzodiazepine, was later developed as an ASD with less sedative effect.[35] However, the induction of tolerance limits their use as ASDs. The 1,5 BZs may produce less tolerance than the 1,4 BZs, but tolerance to 1,5 BZs does occur.[36]

BZ activity at $GABA_ARs$ is a function of the drug's affinity for the BZ binding site and its intrinsic allosteric effect on the $GABA_AR$. The efficacy of individual compounds varies widely. Most BZs in clinical use are full agonists that maximally enhance $GABA_AR$ activity. Flumazenil, a competitive antagonist used to reverse BZ-induced sedation,[37] binds to the BZ site without affecting GABA R function. Abecarnil,[39] imidazenil,[40] and bretazenil[41] are "partial agonists" at the BZ site which have antiseizure efficacy in animal models and appear less prone to tolerance.[42] Several β-carbolines are "inverse BZ agonists" that inhibit GABA binding[43] and can induce seizures or anxiety.[44]

Antiseizure Activity

BZs are effective against most experimental seizure types, but individual drugs vary in their potency/efficacy in specific seizure models and their other clinical effects.[45] BZs are particularly effective against convulsive seizures induced by pentylenetetrazol[46] and less effective against tonic seizures induced by maximal electroshock.[47] BZs also slow the development of kindling.[48]

$GABA_AR$ Subunits and BZ Pharmacology

BZ augmentation of $GABA_AR$ currents requires a γ subunit, and the selectivity of BZ responsiveness is determined by which α subunits are present.[5,49] The BZ binding site is located in a cleft between the extracellular amino termini of the α and γ subunits.[51] The α1 subunit results in a receptor with high affinity for the hypnotic, zolpidem, defining the "BZ-1" (or Ω-1) receptor type.[50] The α2 and α3 subunits result in receptors with moderate zolpidem affinity, termed BZ-2 receptors. $GABA_ARs$ containing the α5 subunit and/or the γ3 subunit are sensitive to diazepam but not to zolpidem and are termed BZ-3 receptors. $GABA_ARs$ with the α4 or α6 subunits are insensitive to most BZs.

The subunit composition not only determines the affinity for particular BZs, but also the clinical/behavioral effect of the BZ at that receptor. The role of the α subunits in BZ pharmacology was revealed by the discovery of a single histidine (H) residue found in all BZ-sensitive α subunits (H101 in the rat α1 subunit), but not in the BZ-insensitive α4 or α6 subunits, which instead have a charged arginine (R) residue. This H residue was discovered in a strain of "alcohol-non-tolerant" rats, which were found to have a spontaneous point mutation in the α6 subunit (R100Q) that made their α6-containing $GABA_ARs$ (found mostly in the cerebellum) diazepam-sensitive, accounting for their ethanol and BZ intolerance.[53] Mutation of R100 to H in α6 dramatically increased BZ binding in this normally insensitive subunit, while mutation of H101 to R in α1 reduced BZ sensitivity.[52] BZ-insensitive α subunit mutations were subsequently "knocked-in" to identify BZ actions at receptors containing that subunit. In homozygous α1(H101R) knock-in mice, the anxiolytic effect was intact, but BZs were not protective against pentylenetetrazol-induced convulsions and did not produce sedation or amnesia, suggesting that binding to the (wild type) α1 subunit is responsible for sedative, amnestic and antiseizure actions.[54] Moreover, the sedative-hypnotic, zolpidem, showed no sedative effect in α1(H101R) mice.[55] Unfortunately, these findings underscore the association between sedative and antiseizure efficacy at α1-containing $GABA_ARs$. Similarly, the anxiolytic[56] and myorelaxant[57] properties of BZs appear to derive from α2- and α3-containing $GABA_ARs$, while the α5 subunit was critical for amnestic effects.[58] BZs may also have a true analgesic effect independent of their sedative and anxiolytic actions, associated with the α2 and α3 more than α5 subunits.[59] Since there is no evidence of biophysically distinct effects of BZs on receptors composed of different α subunits, the different behavioral effects are likely due to the brain regions and neuronal populations expressing these specific $GABA_AR$ isoforms. New α2/α3 subunit-selective BZs appear to be anxiolytic but not sedating,[60] and non-sedating antiseizure BZs that do not induce tolerance[61] may also be possible.

BZ Actions at $GABA_ARs$

BZs increase the amplitude[62] or decay time[63] of GABA-mediated inhibitory post-synaptic potentials (IPSPs). Current noise fluctuation analysis[64] and single channel studies[65] demonstrated that the BZs increased the opening frequency of the $GABA_AR$ chloride channel. In patch-clamp recordings of CNS neurons, BZs produce a leftward shift of the concentration-response curve for GABA,[66] due to an increase in the affinity for GABA at its binding site, with no change in the kinetics of channel gating[65] or single channel conductance. In contrast, an inverse agonist at the BZ site reduced the channel opening frequency for a given GABA concentration. These findings are consistent with binding studies showing the allosteric interaction between the BZ and GABA binding sites. By increasing the affinity of the receptor for GABA through slowing the unbinding rate, the BZs increase the current produced by low GABA concentrations, but not by high GABA concentrations at which receptor binding is saturated, as observed in the synaptic cleft[67]. Thus, BZs generally do not increase the amplitude of miniature inhibitory postsynaptic currents (mIPSCs) from individual synapses, but instead prolong the mIPSC decay phase[68]

by slowing the dissociation of GABA from the receptor.[69] Prolongation of the mIPSC increases temporal and spatial summation of multiple synaptic inputs, which in turn increases the amplitude of stimulus-evoked polysynaptic IPSCs. The BZs thus increase the inhibitory "tone" of GABAergic synapses, which reduces the hypersynchronous firing of neuron populations that underlies seizures.[1]

An alternative mechanism for BZ enhancement of $GABA_AR$ currents has been proposed based on the controversial finding that BZs progressively increase $GABA_AR$ "single channel" conductance.[70] Unlike prior reports of a 27 pS main conductance level and a 19 pS subconductance level,[65] Eghbali et al.[70] found conductance levels ranging from 8 to 53 pS in response to GABA alone, and 70-80 pS in the presence of diazepam, with up to 7-fold increase in conductance observed when diazepam was added. The increases in conductance were seen predominantly in cell-attached patches onto neurons expressing native receptors, but were also observed in outside-out patches. They found similar increases in channel conductance induced by pentobarbital,[71] neuroactive steroids,[72] propofol,[73] and GABA itself.[74] The same group[75] subsequently found that high conductance (>40 pS) $GABA_AR$ channels were not observed in recombinant $\alpha1$-$\beta1$-$\gamma2$ $GABA_ARs$ expressed in L929 cells, nor was conductance increased by diazepam unless the $GABA_AR$-associated protein (GABARAP) was also co-expressed, apparently facilitating clustering of $GABA_AR$ proteins through its interaction with the cytoplasmic loop of the $\gamma2$ subunit as occurs at synapses with native receptors. Since peptides mimicking the intracellular $\gamma2$ loop ($\gamma2$ 381-403) self-associate, and application of this peptide to the cytoplasmic surface of inside-out patches prevented the diazepam-induced increase in conductance, they hypothesized that the apparent single channel conductance changes induced by BZs are due to synchronized openings of multichannel clustered $GABA_ARs$ via concerted action through the interacting cytoplasmic loops of conjoined receptors. Such interactions might occur through shared transmembrane domains between interacting receptors, as observed in G-protein-coupled receptors.[76] This hypothesis could be addressed using concatenated subunits, chimeras and other strategies. However, synchronization of multiple identical channels by such a mechanism should still require a discrete 9 pS unitary conductance of which the larger conductance states are integer multiples, which has not been reported.

Barbiturates

Phenobarbital was synthesized by Emil Fischer at Bayer in 1911 and introduced as an ASD by Alfred Hauptmann in 1912. Its tendency to produce sedation and cognitive slowing or confusion, as well as paradoxical hyperactivity in children, has curtailed its use in favor of more modern alternatives.[77] In addition to their enhancement of $GABA_AR$ currents, barbiturates also inhibit repetitive action potential firing at neuronal sodium channels[78] by reducing fractional open time and shifting the potential of half-maximal opening towards hyperpolarized potentials.[79] These actions contribute to both their antiseizure action and their adverse effect profile.

When co-applied with low concentrations of GABA, barbiturates increased the mean open time, but had no effect on the lengths of 3 distinct open state durations.[80] The increase in mean open time resulted from fewer openings in the shorter two durations (O1 and O2) and more long duration (O3) openings. There was also an increase in long burst durations, but no change in the single channel opening frequency or in the closed frequency duration histogram. The increase in channel open time results in greater chloride current flux and increased likelihood that channel openings will summate, producing larger inhibitory currents. In the absence of GABA, high concentrations of pentobarbital (EC_{50} 0.33 mM) and phenobarbital (EC_{50} 3 mM) directly activated $GABA_AR$ chloride currents with lower efficacy (smaller maximal currents) than GABA[82]. Barbiturate-activated $GABA_AR$ currents were blocked by bicuculline and picrotoxin, and at high concentrations both phenobarbital

and pentobarbital produced open channel block that rapidly terminated the induced currents. The concentrations involved in both direct activation and open channel block are far higher than usual therapeutic levels, thus the main antiseizure mechanism is likely the $GABA_AR$ allosteric effect in concert with synaptic and extrasynaptic GABA.

Unlike the BZs, barbiturate sensitivity does not require a specific subunit composition. Homomeric β1 receptors expressed in *Xenopus* oocytes were directly activated by pentobarbital even though they were not responsive to GABA; these currents were blocked by picrotoxin or penicillin but not bicuculline.[83] Pentobarbital also induced current in both α1-β3 and β3 homomeric GABA Rs.[84] A At saturating GABA concentrations, pentobarbital markedly potentiated α1-β3-δ currents, increasing desensitization and single channel open duration,[85] suggesting that barbiturates may be particularly effective at enhancing tonic $GABA_AR$ currents at extrasynaptic sites activated by low GABA concentrations. The ε subunit, which increases spontaneous $GABA_AR$ channel openings, significantly reduced[86] but did not completely eliminate[87] barbiturate responsiveness when co-expressed with α and β subunits. These findings suggest that barbiturate sensitivity does not require either an α or a γ subunit as it is present in β homomeric receptors; however, presence of the δ or ε subunits that substitute for γ can dramatically modify barbiturate responsiveness by altering the efficacy of GABA for activating the channel.

Chimera and mutagenesis studies have identified domains and residues that contribute to barbiturate action, though in less detail than for the BZs. Mutation of the proline (P228A) in the first transmembrane domain of the β1 subunit reduced barbiturate enhancement of GABA-evoked currents in α1-β1-γ2L $GABA_ARs$ while increasing apparent GABA affinity, without otherwise altering single channel kinetics.[88] A chimera study using constructs created from the amino terminal end of the β3 subunit and the carboxyl terminal end of the ρ1 subunit, which forms homomeric $GABA_C$ receptors that are insensitive to barbiturates, found that residues of the β3 subunit involved in pentobarbital binding to $GABA_ARs$ are located downstream from the middle of the M2 region.[89] Similarly, mutation of a ρ1 tryptophan (W328) in the third transmembrane domain to a hydrophobic residue produced both allosteric and direct channel activation by pentobarbital.[90]

Loreclezole

Loreclezole has antiseizure activity in a variety of seizure models, acting more like a barbiturate than a BZ in that the increase in seizure threshold produced by loreclezole was potentiated rather than blocked by the BZ antagonist, flumazenil.[91] In the hippocampal slice, loreclezole, potentiated paired pulse inhibition[92] and inhibited epileptiform discharges induced by low Ca^{2+} or low Mg^{2+}. Loreclezole strongly potentiated recombinant $GABA_ARs$ containing a β2 or β3 subunit but did not enhance currents from β1-containing receptors.[93] A single asparagine residue (β2(N289) or β3(N290)) at the cytoplasmic end of the 2nd transmembrane domain confers sensitivity to loreclezole; this amino acid is a serine in the β1 subunit.[94] Mutation of β1S290 to N conferred loreclezole sensitivity to β1-containing $GABA_ARs$, while mutation of β2N289 or β3N290 to S eliminated loreclezole enhancement. When both β1 and β3 were co-expressed in the same receptor, loreclezole sensitivity was abolished, suggesting a dominant effect of the β1 subunit.[95] Coexpression with different alpha subunits altered the degree of loreclezole potentiation (α1=α2=α3>α5>α4) ;[96] expression of α5 and β3 with the π subunit reduced loreclezole potentiation,[97] while α1-β3-ε receptors showed normal loreclezole enhancement.[87]

At higher concentrations (> 6 μM) loreclezole caused concentration-dependent inhibition of $GABA_AR$ currents by enhancing the rate of apparent desensitization.[98] The effect was inconsistent with open channel block as it was voltage independent, non-competitive, and increased with increasing GABA concentration. The BZ site was not involved as the

inhibition was not antagonized by flumazenil and did not require a γ subunit. This finding has important clinical implications, since inadvertent high levels of drug could inhibit rather than enhance $GABA_AR$ function and potentially trigger seizure activity. Loreclezole inhibited α1-β1-γ2L $GABA_AR$ currents that were not potentiated by low concentrations of loreclezole, suggesting separate sites for enhancement and inhibition. At the single channel level, high loreclezole concentrations decreased α1-β1-γ2L mean open time by decreasing the average durations of the open states, and also increased the occurrence of a 20 ms closed state. Loreclezole inhibition was equally effective when applied to the intracellular side of the receptor, suggesting that its inhibitory binding site was accessible from both sides of the membrane, and pre-application of loreclezole prior to GABA inhibited the subsequent $GABA_AR$ current, indicating that binding did not require an open channel. Loreclezole was also found to inhibit ρ1 homomeric $GABA_C$ receptors with an IC_{50} of 0.5 μM, which was proposed as a rapid means of pharmacological identification of these receptors.[99]

Ganaxolone

Ganaxolone (3α-OH-3β-methyl-5α-pregnan-20-one) is the 3β-methylated synthetic analog of the neurosteroid, allopregnanolone, a natural metabolite of progesterone that allosterically enhances $GABA_AR$ current. It has a broad range of antiseizure activity in animal epilepsy models[100] including seizures induced by bicuculline, t-butylbicyclophosphorothionate (TBPS, a high affinity ligand for the $GABA_AR$ picrotoxin site), aminophylline and corneal kindling, and is well tolerated and effective against seizures in humans. Ganaxolone is currently under evaluation for partial onset seizures. It may have special utility in women with catamenial epilepsy who have increased seizures during periods of low progesterone in the menstrual cycle, as well as in children with infantile spasms.[101]

The antiseizure effect of neurosteroids is not due to action at the progesterone receptor (PR), as exogenous allopregnanolone prevented seizures in PR knockout mice.[102] Moreover, progesterone's antiseizure effect requires metabolism to allopregnanolone, as it was blocked by finasteride, a 5α-reductase inhibitor that blocks allopregnanolone synthesis from progesterone. Hence, the antiseizure effect of allopregnanolone (and ganaxolone) is mediated by $GABA_ARs$.

Specific neurosteroids can allosterically modulate $GABA_AR$ activity, either positively or negatively, via distinct binding sites on $GABA_ARs$.[104] Since a variety of $GABA_AR$-interacting steroid hormones are synthesized in the brain,[105] these agents represent endogenous modulators of $GABA_AR$ function. Allopregnanolone is synthesized in two steps from progesterone: 5α-reductase converts progesterone to 5α-dihydroprogesterone, then 3-α-hydroxysteroid oxidoreductase reversibly converts 5α-dihydroprogesterone to 3α-OH-5α-pregnan-20-one (allopregnanolone). Addition of the 3β-methyl group on ganaxolone does not alter binding to $GABA_ARs$, but prevents further metabolism of the 3α-OH group and thus prolongs its $GABA_AR$-modulating actions.[100] Ganaxolone enhanced GABA and BZ binding via positive allosteric modulation of $GABA_AR$ activity, and at nanomolar concentrations potentiated GABA-evoked currents at α1-β1-γ2L, α2-β1-γ2L or α3-β1-γ2L $GABA_ARs$ expressed in *Xenopus* oocytes, while direct activation of chloride flux occurred to a limited extent only at micromolar concentrations.[100]

The effects of $GABA_AR$-enhancing neurosteroids on single channel $GABA_AR$ currents were studied using androsterone (5α-androstan-3α-ol-17-one) and pregnanolone (5β-pregnan-3α-ol-20-one) but likely apply to other positive steroid $GABA_AR$ modulators including ganaxolone. Like the barbiturates and loreclezole, these agents increased the proportion of openings to the two longer open states (O2 and O3) without altering the intrinsic durations of those states, and produced longer burst durations.[106] There was no change in single channel conductance. Unlike the barbiturates, however, the neurosteroids

also increased single channel opening frequency and reduced closed time durations in all but the shortest closed time distributions (thought to represent intraburst closures). At high concentrations (10 μM), both agents reduced open channel durations consistent with open channel ("flickering") block.

The subunit selectivity of $GABA_AR$-enhancing neurosteroids was initially controversial. An early study[107] of recombinant receptors expressed from combinations of α1, α6, β3, γ2 and δ showed loss of neurosteroid responsiveness with combinations containing the delta subunit. There was also reduced sensitivity of cerebellar granule neurons to neurosteroids later in *in vitro* development, when δ subunit expression increases. These findings were interpreted as showing inhibition of neurosteroid responsiveness by inclusion of the δ subunit. However, mice in which the δ subunit was knocked out showed decreased behavioral sensitivity to neurosteroids.[108] Moreover, in receptors composed of α1 or α6, β3 and either γ2L or δ, the greatest potentiation by tetrahydrodeoxycorticosterone (THDOC) was seen in α1-β3-δ $GABA_ARs$.[109] At high concentrations (1 μM), THDOC inhibited this isoform. There is currently consensus that δ subunit-containing receptors are more sensitive to neurosteroid enhancement, though other subunits are also involved in mediating neurosteroid actions. Presence of the α6 subunit reduced neurosteroid sensitivity.[110] THDOC (1 μM) enhanced α1-β3-δ more than α6-β3-δ currents, but increased the extent of desensitization and prolonged deactivation for both receptor isoforms; α1-α6 and α6-α1 chimeras (spliced in transmembrane domain M1) suggested that differences in deactivation rate and its voltage-dependence correlated with N-terminal domains, while the extent of desensitization and its voltage-dependence correlated with C-terminal domains.[111] In dentate granule cells from epileptic animals, an increase in α4 subunit expression was associated with decreased neurosteroid enhancement.[112] In receptors composed of one α subunit (from α2 through α5), β2 and γ2S, neurosteroid potentiation was dependent on the conserved glutamine residue (α1(Q241)) in the first transmembrane domain of the respective α subunit.[113] The δ subunit did not affect neurosteroid binding but likely influenced the efficacy of neurosteroid potentiation. Mutation of α1Q241 to L abolished neurosteroid potentiation, while mutation to W mimicked the effect of steroids and prevented further augmentation; the neighboring S240 residue also participated in steroid binding.[114] A subsequent study using concatenated subunits demonstrated that a single functional binding site (not disrupted by the Q241 mutation) was sufficient for neurosteroid modulation.[115]

$GABA_AR$ effects of other ASDs

Topiramate

Topiramate is a heterotricyclic sulfamate with several mechanisms of action including inhibition of sodium and calcium channels, inhibition of carbonic anhydrase, and augmentation of GABA-evoked currents. Topiramate's effects on $GABA_ARs$ may contribute both to its antiseizure efficacy and its side effect profile including memory problems, fatigue and psychomotor slowing. It is approved for partial and generalized seizures, the Lennox Gastaut syndrome, and migraine.

Topiramate inhibited voltage-gated sodium channels, with a left shift of the steady state inactivation curve[116] resulting in intermittent blockade of sustained repetitive action potential firing during prolonged depolarization.[117] It also blocked repetitive firing in hippocampal CA3 neurons from spontaneously epileptic rats, and reduced excitatory post-synaptic potentials and responses to bath-applied glutamate, suggesting blockade of post-synaptic glutamate receptors,[118] affecting kainate-evoked but not NMDA-evoked currents.[119] Topiramate also inhibited both L-type and non L-type high voltage activated

calcium currents[120] and reduced bicarbonate production via inhibition of carbonic anhydrase,[121] which may contribute to its antiseizure effect.

Topiramate (10 μM) enhanced chloride flux into cerebellar granule neurons stimulated by 10 μM GABA, but did not significantly increase chloride influx alone.[122] It also enhanced GABA-evoked currents in cultured cortical neurons that were insensitive to the BZ, clonazepam, and clonazepam potentiated GABA currents in topiramate-insensitive neurons, confirming that topiramate's site of action on $GABA_A$Rs is independent of the BZ site.[123] Subunit selectivity studies have been inconsistent. Topiramate (1-100 μM) reversibly inhibited Cl^- currents evoked by 1-10 μM GABA in *Xenopus* oocytes expressing α1-β2-γ2S and α2-β2-γ2S $GABA_A$Rs, and reduced the apparent desensitization ("current-fading rate") in α1-β2-γ2S-expressing oocytes, but potentiated GABA-evoked Cl^- currents and increased the desensitization rate in α6-β2-γ2S $GABA_A$Rs, with no effect on α4-β2-γ2S receptors or mixed population $GABA_A$Rs expressed from rat brain mRNA.[124] In contrast, another study found that topiramate could both potentiate and directly activate β2 or β3-containing heteromeric $GABA_A$Rs, with greatest effect on α4-β3-γ2S;[125] positive or negative effects on β1-containing $GABA_A$Rs depended on the co-expressed alpha subunit. Additional studies are needed to clarify the site and mechanism of action.

Felbamate

Felbamate was launched as a promising novel ASD in 1993 with an uncertain mechanism of action, but its use was curtailed after early reports of aplastic anemia[126] and hepatic failure.[127] Felbamate inhibited binding of the GABA antagonist [^{3}H]T-BOB with a regional pattern different from that produced by GABA agonists, bicuculline, zinc or neurosteroids,[128] and enhanced GABA-elicited Cl^- currents in cultured cortical neurons. Felbamate enhancement was not blocked by flumazenil, and felbamate did not affect pentobarbital potentiation or PTX inhibition of GABA-evoked currents, suggesting an independent site of action. It prolonged the mean burst duration of GABA-activated single channel currents, suggesting a barbiturate-like effect.[129] Felbamate also blocked N-methyl-D-aspartate (NMDA) receptor currents, an alternative possible antiseizure mechanism.[130] Derivative compounds including fluorofelbamate and carisbamate that are not associated with hematopoietic or hepatic toxicity may revive interest in felbamate–like agents.[131]

Ezogabine

Ezogabine (formerly retigabine) is a novel ASD effective in a variety of animal models. Its primary effect is enhanced activation of heteromeric potassium channels composed of the KCNQ2 and KCNQ3 subunits[132] which underlie the "M current" that is a major determinant of resting membrane potential and neuronal excitability. However, ezogabine also dose-dependently and reversibly potentiated $GABA_A$R-dependent IPSC peak amplitude, decay times and total charge transfer.[133] EPSCs were unaffected, and paired pulse depression was unchanged, suggesting a post-synaptic effect at $GABA_A$Rs. Ezogabine potentiated GABA-induced currents in rat cortical neurons in a concentration-dependent fashion at 10 μM and above.[132] This action was not antagonized by flumazenil, indicating a non-BZ site of action. Subunit dependence and binding site are not known.

Losigamone

Losigamone is a novel ASD which inhibited the persistent component of sodium currents in hippocampal neurons at depolarized potentials, suppressed sustained repetitive firing[134] and decreased the frequency of spontaneous action potentials without affecting miniature post-synaptic current amplitudes.[135] Losigamone stimulated $^{36}Cl^-$ influx into spinal cord neurons in the absence of GABA, and potentiated $^{36}Cl^-$ influx stimulated by submaximal GABA concentrations; both effects were blocked by bicuculline or picrotoxin.[136] Losigamone did

not affect the specific binding of [^{3}H]GABA, [^{3}H]flunitrazepam, or [^{35}S]t-butyl-bicyclophosphorothionate (TBPS) to their receptors, and there was no difference in the effect of the + or – stereoisomers or the racemic mixture. The site of action at $GABA_AR$s is unknown.

Stiripentol

Stiripentol is as an adjunct ASD which was thought to act by inhibiting cytochrome P450 enzymes involved in metabolism of conventional ASDs. Recently, stiripentol was found to enhance recombinant $GABA_AR$ currents, with greater potentiation of α3-containing receptors and reduced potentiation with the β1 or ε subunits.[137] It caused a leftward shift in the GABA concentration-response relationship without increasing maximal GABA-evoked currents, and did not involve sites associated with neurosteroid or loreclezole potentiation.[137] Saturating barbiturate sites with pentobarbital occluded stiripentol enhancement, and stiripentol increased the duration but not the frequency of opening of $GABA_AR$ channels, suggesting a barbiturate-like mechanism.[138]

GABA-enhancing agents

An additional mechanism for enhancing $GABA_AR$-mediated inhibition involves increasing the concentration or duration of GABA in the synaptic cleft and perisynaptic/extrasynaptic sites. Enhancing GABA synthesis or blocking its reuptake or catabolism could prolong GABA IPSPs and increase perisynaptic spill-over, increasing tonic/extrasynaptic GABA currents which likely play a major role in seizure prevention.

Gabapentin and Pregabalin

Gabapentin was designed as a GABA analog, and some studies have suggested that it modulates the action of the GABA synthetic enzyme, glutamic acid decarboxylase (GAD) and the glutamate synthesizing enzyme, branched-chain amino acid transaminase, resulting in increased GABA synthesis.[139] Gabapentin increases non-synaptic GABA responses from neuronal tissues *in vitro* and increases GABA levels in brain.[140] Its other (likely primary) mode of action involves binding to the α2-δ binding site on L- or P/Q-type presynaptic voltage-gated calcium channels, presumably inhibiting excessive neurotransmitter release[141] by interfering with calcium channel functional expression or trafficking[142]. Pregabalin, which binds to the α2-δ calcium channel subunit with higher potency, may have similar mechanisms of action. Most studies emphasize the calcium channel as the primary site,[143] though a contributory effect on GABA metabolism is possible.[144]

Valproic Acid

Valproic acid may affect GABA production, among other ASD mechanisms. Valproate caused non-significant increases in cerebral GABA levels but elevated brain GAD activity significantly.[145] Valproic acid increased GABA synthesis and release in brain regions including substantia nigra,[146] which is thought to be involved in the control of seizure generation and propagation.[147] It also reduced the release of gamma-hydroxybutyric acid and attenuated neuronal excitation induced by NMDA-type glutamate receptors.[147] Additional ASD mechanisms include enhancing sodium channel inactivation (like phenytoin) and reducing T-type Ca^{2+} channel currents (like ethosuximide),[148] perhaps explaining its utility against both partial onset and absence seizures.

Tiagabine

Tiagabine is a derivative of nipecotic acid that binds to the presynaptic GAT-1 GABA transporter and blocks GABA reuptake, without acting as a “false neurotransmitter.” In

control hippocampal slices, tiagabine alone induced a significant chloride conductance, suggesting that GAT-1 activity controls the basal level of extracellular GABA.[149] Tiagabine prolonged hippocampal IPSC duration in a lamina-specific fashion, with greater effect in stratum radiatum (167%) than stratum oriens (115%).[150] Tiagabine reduced the frequency of epileptiform discharges in hippocampal slices exposed to low Mg^{2+} or 4-aminopyridine.[151] The ability of tiagabine to prolong GABA currents was preserved in hippocampal slices from pilocarpine-treated epileptic rats, hence the GAT-1 transporter remains a functional target for regulating GABA levels in this model of temporal lobe epilepsy.[152] Tiagabine was effective as an adjunct agent against partial onset seizures, though its prolonged titration period, requirement for multiple daily doses and CNS side effect profile (dizziness, tremor, somnolence, mood disturbances and rare psychotic symptoms) have limited its use.[153]

Vigabatrin

Vigabatrin (γ-vinyl-GABA) has a unique mechanism of action, functioning as a "suicide inhibitor" of GABA transaminase, the primary enzyme for GABA catalysis. This leads to permanent inhibition of the affected enzyme, requiring synthesis of new GAT-1 and hence prolonging the biological half-life beyond its pharmacokinetic half-life of 8 hours.[154] It is effective against complex partial seizures,[155] Lennox-Gastaut syndrome and West Syndrome, for which it has become an alternative to ACTH[156] particularly in children with tuberous sclerosis.[157] Vigabatrin increases the extracellular concentrations of GABA both *in vitro*[158] and *in vivo*.[159] The anti-seizure effects of increasing GABA concentration may be complex. Biphasic responses to vigabatrin administration have been observed, with an early proconvulsant effect.[160] A biphasic effect was also seen on after-discharge duration with an early facilitation of the limbic pattern of epileptiform discharge and later suppression at higher doses.[161] Elevated GABA concentrations may increase neuronal synchronization, particularly of thalamic neurons in absence epilepsy, and there have been several reports of vigabatrin worsening absence seizures or precipitating absence status epilepticus.[162]

A major limitation to the use of vigabatrin is the relatively high incidence of vision disturbance associated with chronic use. In 21 of 30 children treated with vigabatrin who were screened for vision disturbances despite no report of visual symptoms, 4 had visual field constrictions, which did not improve after discontinuation of the drug.[163] One study associated visual disturbances with infantile spasms rather than the drug.[164] The prevalence of vision problems ranged from 10 to 40% of pediatric and adult patients exposed to vigabatrin.[165] This adverse effect may be directly related to elevated GABA concentrations, as hippocampal neurons grown in depolarizing conditions (25 mM KCl) showed 20% loss of MAP-2-positive neurons in the presence of vigabatrin, which was mimicked by GABA (100 μM) and blocked by bicuculline or picrotoxin.[166] The cellular mechanism of this toxicity is not entirely clear, but in situations in which GABA is depolarizing, excessive GABA could promote calcium entry through L-type (slowly desensitizing) voltage-gated calcium channels,[167] which in turn could result in activation of caspases and cell death.[168]

Excitatory $GABA_A$ Currents

Early in CNS development, neurons express the $Na^+/K^+/Cl^-$ cotransporter, NKCC1, rather than the K^+/Cl^- cotransporter, KCC2, which is expressed in adult neurons. NKCC1 increases intracellular Cl^- resulting in a depolarizing Cl^- reversal potential, while KCC2 exports Cl^- yielding the hyperpolarizing Cl^- reversal potential found in adult neurons.[169] As a result, activation of $GABA_A$Rs may be excitatory during early development.[170] Excitatory $GABA_A$R currents may play a trophic role in neuronal migration and connectivity,[171] but may also contribute to epileptogenesis.[172] Endogenous GABA appears to be proconvulsant in early postnatal rat hippocampal slices, as $GABA_A$ antagonists blocked epileptiform

activity induced by depolarization with high external $[K^+]$[173]. However, BZ and barbiturate antiseizure efficacy appear to be intact, likely because persistent opening of $GABA_AR$ channels (in the presence of allosteric agents) may reduce the depolarizing chloride reversal potential, resulting in "shunt" inhibition. Alternatively, subthreshhold GABA-evoked depolarization may inactivate sodium channels and prevent action potential firing.[174] The current through $GABA_AR$ channels can also be altered by changes in intracellular bicarbonate, $[HCO_3^-]$,[169] which can flow through the channel.[175] Changes in $[HCO_3^-]$ may underlie reduced synaptic GABA currents during development of BZ tolerance.[176] Depolarizing $GABA_AR$ currents may also be a source of interictal spike activity, as observed in epileptic subiculum neurons in hippocampal brain slices from patients with temporal lobe epilepsy.[177] Changes in the GABA current reversal potential might also explain why diazepam can be less effective in children with epileptic encephalopathies,[178] and rarely can cause status epilepticus in patients with the Lennox Gastaut syndrome.[179]

Bumetanide, an inhibitor of NKCC-1 used clinically as a potent loop diuretic, has been proposed as an adjunctive agent to reduce intraneuronal chloride accumulation and reverse the depolarizing chloride gradient that makes $GABA_ARs$ excitatory, particularly in the neonatal setting.[170] Bumetanide improved the responsiveness of hippocampal slices to phenobarbital in reducing epileptiform discharges induced by low Mg^{2+},[180] and also suppressed electrographic seizures in neonatal rats.[172] Depolarizing GABA currents may occur in cortical more than subcortical brain regions, possibly explaining the electroclinical uncoupling that occurs with phenobarbital in neonatal seizures.[181]

Bumetanide has been used successfully in a single reported human neonate with seizures,[182] and a clinical trial (NCT00830531) is in progress. However, elimination of the depolarizing Cl^- gradient during development may have adverse consequences; a permanent reduction in AMPAR-mediated excitatory neurotransmission and sensorimotor gating deficits were observed after bumetanide exposure in neonatal rats.[183] Such potential adverse effects will have to be weighed against the benefits associated with seizure termination or prevention.

$GABA_AR$ subunit changes and ASD efficacy in epilepsy and status epilepticus

There is considerable evidence that $GABA_AR$ composition and function change with epilepsy, both acutely in the setting of status epilepticus and in chronic epilepsy. During status epilepticus, there is a rapid change in $GABA_AR$ function that reduces BZ sensitivity,[184] apparently due to activity-dependent internalization of $GABA_ARs$ and replacement with BZ-insensitive receptors.[185] Kainic acid-induced status epilepticus in young (postnatal day 9) rats altered the normal developmental pattern of $GABA_AR$ subunit maturation,[186] preventing the normal switch from α2 to α1-containing $GABA_ARs$. These changes correlate with the finding in animal models[15,184,187] and humans[188,189] that BZs are effective early in the course of status epilepticus but lose their potency and efficacy for termination of status with longer seizure duration. These findings increase the urgency for treatment of status epilepticus early in its course, and also suggest the need to find alternative treatments that retain efficacy in refractory status epilepticus, or methods to prevent or reverse the $GABA_AR$ changes that occur with prolonged seizures.

In the pilocarpine model of temporal lobe epilepsy, the α1 subunit in dentate granule neurons was downregulated and α4 subunit was upregulated,[190] while in younger (postnatal day 20) animals, the α1 subunit was increased after status epilepticus.[191] $GABA_AR$ subunit changes after pilocarpine-induced status are associated with altered physiological properties including reduced neurosteroid sensitivity and an increase in α4-containing receptors colocalized with synapses rather than extracellular sites.[112] Such changes might contribute

to the propensity for spontaneous seizures or alternatively represent a functional adaptation to reduce seizure expression. Substitution of α1 with α4 might reduce synaptic (phasic) but increase extrasynaptic (tonic) inhibition, and BZ sensitivity would be lost in α4-containing receptors. Using an adenovirus vector to insert the α1 subunit gene driven by the α4 promoter resulted in a decrease in seizure frequency, suggesting that the $GABA_AR$ subunit changes were contributory toward epileptogenesis.[192] Hence, altering $GABA_AR$ subunit expression in brain regions critical to seizure development may provide a new therapeutic strategy for seizure prevention. Alternatively, novel ASDs targeting the specific epilepsy-associated subunit composition might provide a less invasive approach toward seizure control.

Long term exposure to the $GABA_AR$ allosteric agents themselves can alter $GABA_AR$ composition and function. In rat pups exposed to therapeutic concentrations of diazepam or phenobarbital from postnatal day 10 through 40, then tapered for 2 weeks and euthanized on postnatal day 90 showed increased mRNA expression in dentate granule neurons for GAT-1, 3 and 4, $GABA_AR$ subunits α4, α6, β3, δ and θ and $GABA_B$ receptor subunit R1, and decreased mRNA expression for GAD65, GAD67 and $GABA_AR$ subunits α1 and α3.[193] Tolerance- and dependence-associated changes in $GABA_AR$ subunit composition with chronic use of BZs[194,195] and barbiturates[196,197] have been reported, and may reflect tolerance to either the antiseizure or adverse CNS effects. However, these findings suggest that not only seizures, but their prolonged treatment with GABAergic agents, can alter inhibitory neuronal function for extended periods or even permanently, with possible cognitive and behavioral consequences that must be considered when contemplating chronic GABAergic ASD use.

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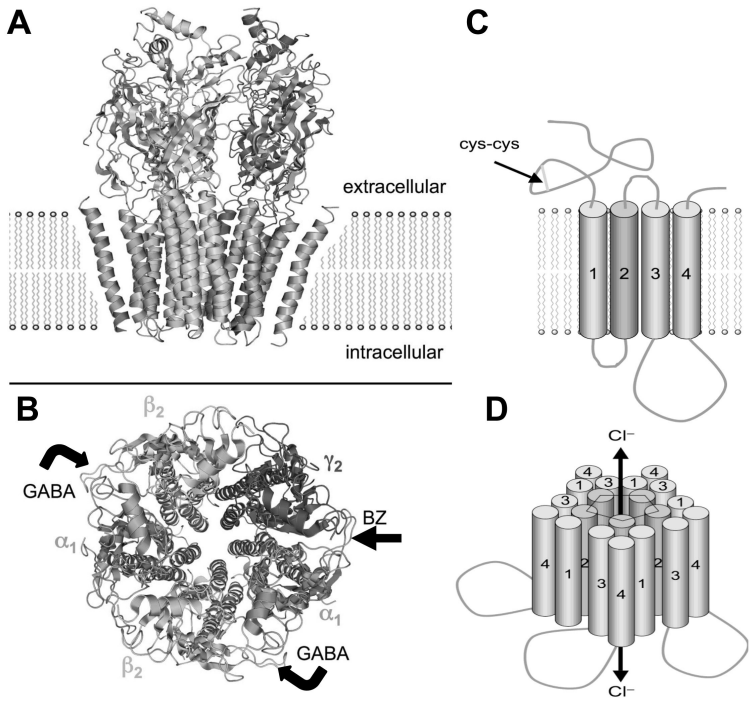

Figure 1.

Model of a $GABA_A$ receptor in the plasma membrane. A. A space-filling model of the pentomer in side view (A) and top view (B) based on the high sequence homology with the nicotinic acetylcholine receptor. There are with two binding sites for GABA, between α and β subunits (bent arrows), and one for BZs between the alpha and gamma subunits (arrow). C. A schematic view shows the topology of each subunit with a large extracellular loop containing a cysteine loop and 4 transmembrane domains (M1-M4), the second of which forms the chloride ion channel. Binding of GABA allows the channel to open and conduct Cl^- ions, resulting in the fast inhibitory post-synaptic potential (IPSP). D. Putative arrangement of 5 subunits to form a pentamer with central chloride channel lined by the M2 subunit. [Derived from the published structure: RCSB PDB Database·PDB ID: 2BG9 from Unwin, N. (2005)[198]. Images modified from en.wikipedia.org/wiki/GABA_A_receptor, used with permission (public domain)].

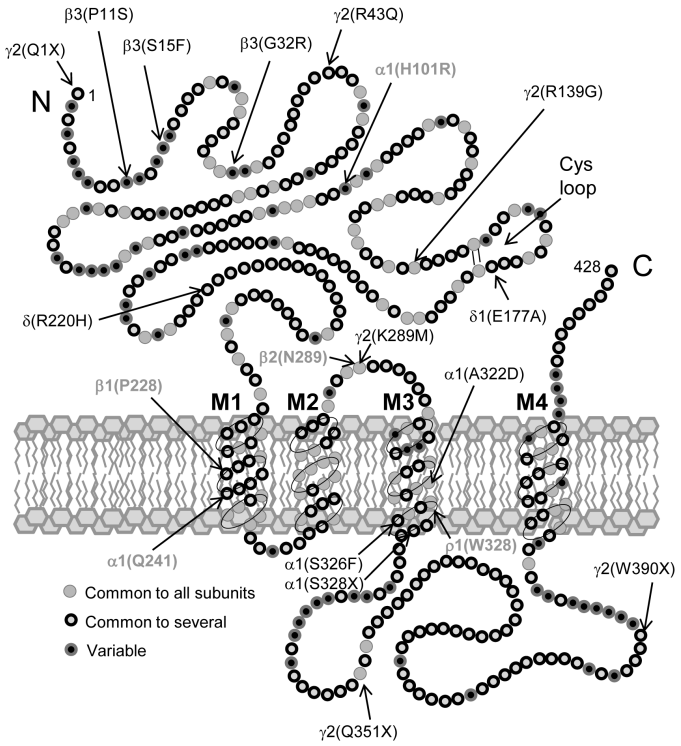

Figure 2.

Model of a prototype $GABA_AR$ subunit (based on α1 subunit diagram from Olsen & Tobin, 1990)[199] showing approximate locations of point mutations associated with generalized epilepsies (see also Macdonald et al., 2012 [200]) in black, and locations of point mutations associated with ASD sites of action. See text for details.

Table 1

Antiseizure Drugs and their $GABA_AR$ Effects

Anti-Seizure Drug	Subunit Specificity	Site of Action	GABAAR Action	Other Mechanisms
Benzodiazepines	α1-3, α5; γ	α/γ interface, α1(H101)	Left shift of GABA C/R curve, ↑ open frequency	Possible increased GABAAR channel conductance
Zolpidem	α1>α2,3; γ	α/γ interface	" "	" "
Barbiturates	β subunits?	M3 residues?	↑ channel open time & burst duration	Use-dependent Na^+ channel block
Loreclezole	β2, β3	B2(N289)	Barbiturate-like?	inhibits GABAAR at ↑ concentration
Ganaxolone	α, β, δ, ↓ with ε	α1(Q241)	Barbiturate-like, also ↑ open frequency	Open channel block at high conc.
Topiramate	α6 >α4> α1-α2?	unknown	Enhanced GABA currents	Na^+ /Ca^{2+} channel block, AMPA/kainate receptor block
Felbamate	unknown	unknown	Barbiturate-like	Blocks NMDA receptor currents
Retigabine	unknown	unknown	Increased GABA IPSCs	Opens KCNQ2/3 potassium channels
Losigamone	unknown	unknown	Enhanced GABA receptor current	Use-dependent Na^+ channel block
Stiripentol	α3, ↓ with β1, ε	unknown	Barbiturate-like	CYP450 inhibition
Vigabatrin	GABA transaminase	–	increases [GABA]	–
Gabapentin, pregabalin	Glutamic acid decarboxylase	–	↑ GABA synthesis?	α2-δ subunit of voltage-gated Ca^{2+} channel
Valproic Acid	Glutamic acid decarboxylase	–	↑ GABA synthesis?	block of Na^+, T-type Ca^{2+} channels
Tiagabine	GAT-1 (GABA transporter)	–	Blocks GABA reuptake	–