The Inhibitory Neural Circuitry as Target of Antiepileptic Drugs

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Abstract: Impairments and defects in the inhibitory neurotransmission in the CNS can contribute to various seizure disorders, i.e., γ-aminobutyric acid (GABA) and glycine as the main inhibitory neurotransmitters in the brain play a crucial role in some forms of epilepsy. Recent advances in deciphering the molecular basis of the GABAergic and glycinergic systems has been achieved by means of cloning techniques and gene targeting strategies in animals, contributing to the understanding of drug action. As well, several anticonvulsive substances emerged which target key molecules of the inhibitory systems. Employment of recombinant expression systems, including, but not restricted to the inhibitory circuitry, will further facilitate drug screening and rational approaches to design novel specific antiepileptic drugs, which act highly efficiently to prevent or reduce generation and spread of seizures.

INTRODUCTION

During the last years the understanding of the generation and the spread of epileptic seizures has been largely advanced. As well, the pharmacokinetic profile of several antiepileptic drugs has been elucidated. About forty different seizure disorders in humans have been identified and classified depending on pathophysiological features and clinical manifestations. However, insights in the etiology of epileptogenesis are limited. Genetic predisposition has been implicated in a vast number of epilepsies, and some genes have been identified underlying distinct forms of seizure disorders, but defects in these genes account for only a minority of patients afflicted with epilepsy. However, mutations or knock-out of genes often unexpectedly result in an epilepsy-like phenotype in mice and lead to the discovery of a large number of candidate genes, which might also contribute to epilepsy in humans (reviewed in [1]). It is thus likely that the number of distinct epileptic disorders will rise, as our insight in the cellular and molecular mechanisms of this diseases emerges. From this perspective, it appears questionable whether antiepileptic drug treatment can ever defeat the causes of epileptogenesis or remains limited to the mitigation of seizures, which is the mode of action of all current antiepileptic drugs.

In general, seizures are associated with abnormal and sometimes massive electrical discharges in the brain. The underlying mechanisms are only fragmentarily understood, however, on the cellular level seizures are characterised by hyperexcitability and extensive burst-firing of neuronal populations. It was theorised that a deficit in inhibitory synaptic transmission in the brain may contribute to the generation and spread of seizures. The major inhibitory neurotransmitters in the brain are γ-aminobutyric acid (GABA) and glycine. The GABAergic system (Fig. (**1**)) has long been implicated in epilepsy, evidenced by a change in GABAergic function in the brains of epileptics as well as in animal models of epilepsy, and by the observation that some antiepileptic drugs increase GABA levels in the CNS (reviewed in [2-4]). A defect in any one of the several steps in GABAergic transmission may affect seizures, implying that neurotransmitter metabolism, release, and transport, as well as activation of specific receptors are all important as putative drug targets.

Here we illustrate the molecular basis of inhibitory transmission by GABA, summarise experiments which support the 'GABA hypothesis' of epilepsy, and briefly excurse to the glycinergic system. Several recently developed substances and drugs exert anticonvulsive effects via key molecules of the GABAergic system. We do not discuss in detail drugs which interact with GABAergic synaptic transmission in a yet undefined mode such as valproate, gabapentin, felbamate, topiramate, retigabine, or pregabaline. The reader is referred to several recent references for further discussion of these substances [5-8]. Established antiepileptic drugs clearly acting on specified targets are presented, and we will describe some of the developments which may lead to anticonvulsive drugs in the future or serve as lead designs in further approaches.

METABOLISM OF GABA

GABA is metabolised via the glutamate decarboxylation pathway, in which two enzymes play a central role. The GABA-synthesising enzyme glutamate decarboxylase(GAD) catalyses the decarboxylation of L-glutamate to GABA. GABA is converted via GABA-aminotransferase (previously called GABA transaminase, hence the abbreviation GABA-T) to succinic semialdehyde, which is finally oxidised to

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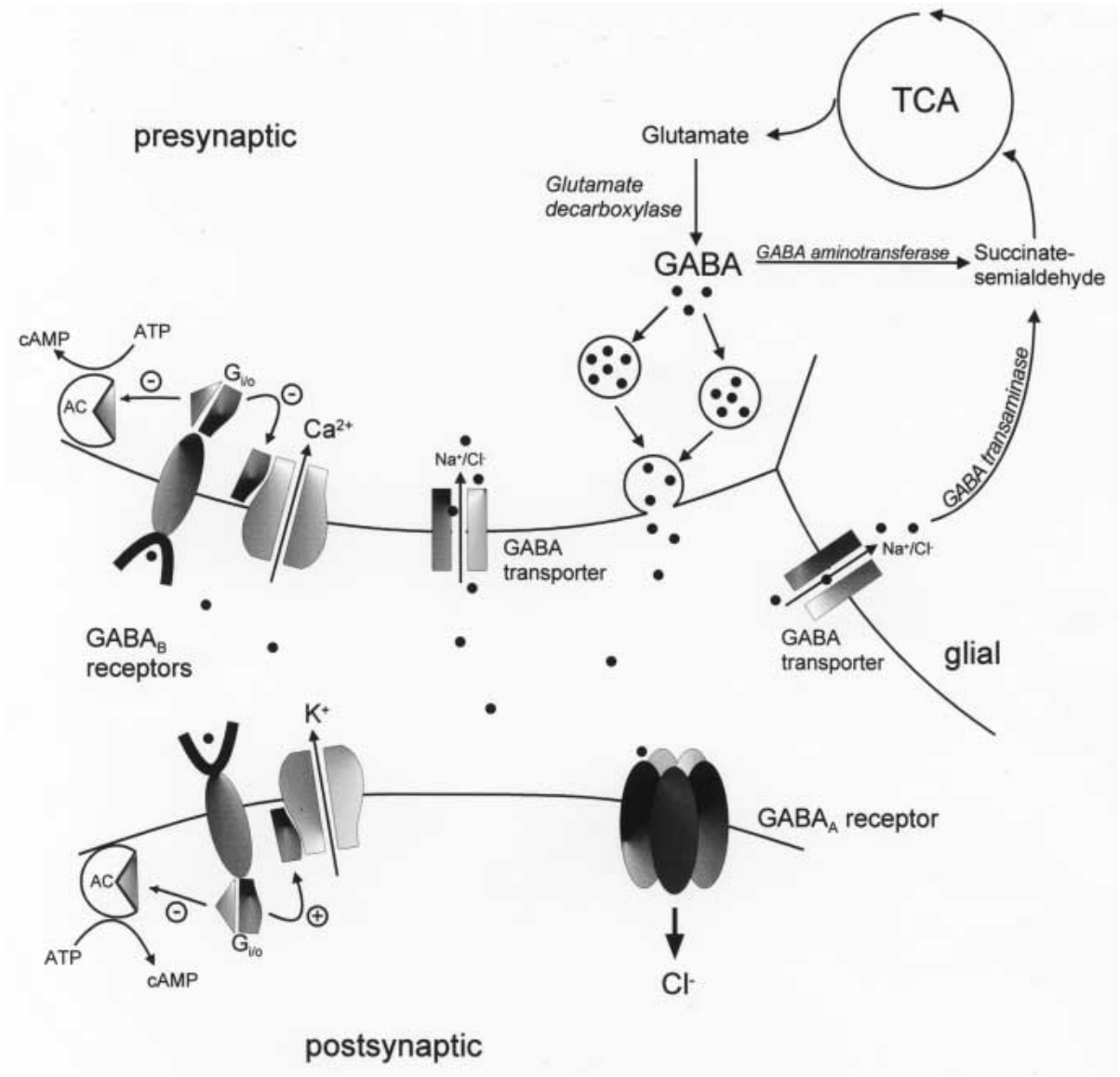


Fig. (1). Schematic overview illustrating the processes at inhibitory GABAergic synapses.

GABA metbolism, neuronal and glial uptake, and pre- and postsynaptic GABA receptors are shown. See the text for tetails and explanations. AC=adenylate cyclase; TCA=tricarbonic acid cycle; $G_{i/o}$=$G_{i/o}$ type G proteins.

succinate (Fig. (**2**)). Because the GABA metabolism intersects the tricarboxylic acid cycle twice, i.e. L-glutamate arises from 2-ketoglutarate and GABA is catabolised to succinate, the glutamate decarboxylation pathway is also called the GABA shunt (reviewed in detail in [9]).

65- and 67 kDa isoforms of glutamate decarboxylase (GAD_{65} and GAD_{67}), encoded by two independently regulated genes, are coexpressed in GABAergic neurons, but show differences with regard to their subcellular localisation. GAD_{65} is particularly enriched in nerve endings, and is reversibly membrane-anchored to synaptic vesicles by formation of a protein complex [10-12], whereas GAD_{67} is distributed throughout the cell body. In brain extracts, almost all GAD_{67} is found in the active holoenzyme form, saturated with pyridoxal 5-phosphate (PLP) [13]. In contrast, the majority of GAD_{65} is isolated as apoenzyme, which can be rapidly activated by the addition of PLP [14]. Thus, it has been suggested that GAD_{67} maintains cytoplasmatic basal levels of GABA, whereas GAD_{65} is more important for GABAergic transmission.

GADs are not exclusively expressed in GABAergic neurons, but have been detected in glutamergic hippocampal granule cells as well [15], in which an enhanced GAD_{67} immunoreactivity is observed after seizures [16]. There is no convincing explanation for this observation and the role of GABA in glutamergic cells. GAD may metabolise excess

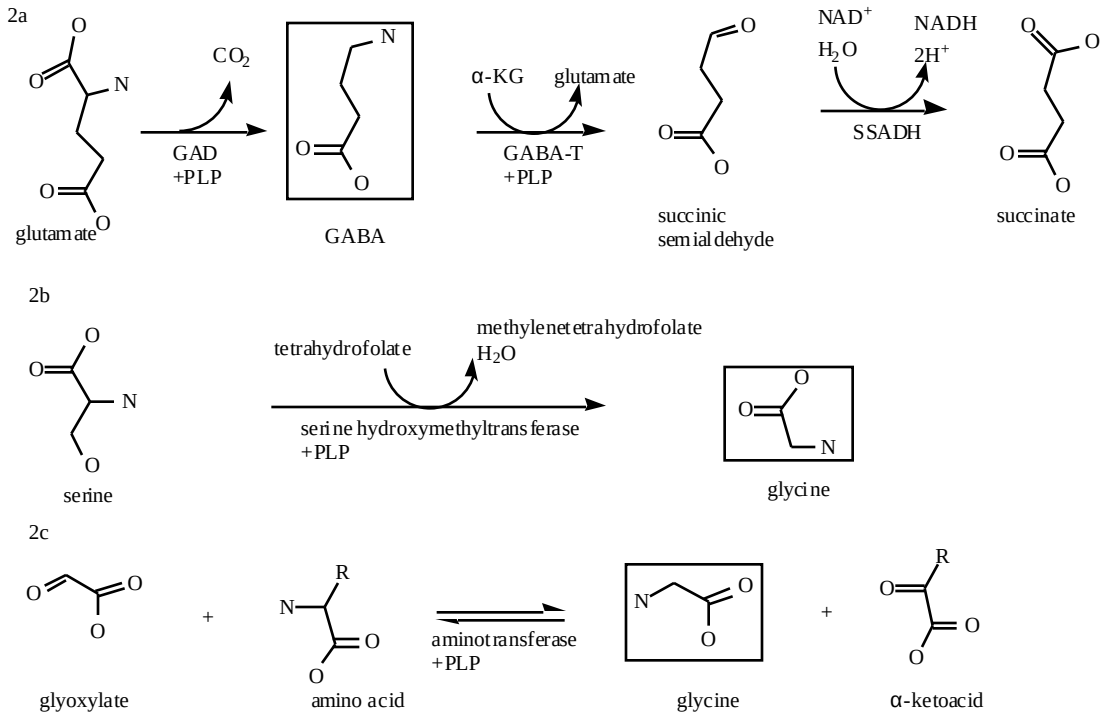


Fig. (2). Pathways of γ-aminobutyric acid (GABA) metabolism (2a) and glycine synthesis (2b, 2c). GAD=glutamate decarboxylase; PLP=pyridoxal phosphate; GABA-T=GABA aminotransferase; α-KG=α-ketoglutarate; SSADH=succinic semialdehyde dehydrogenase.

glutamate, thus regulating neurotransmitter levels in excitatory cells. However, it has also been proposed that excitatory neurons are potentially inhibitory as well [15].

Targeted disruption of the genes for GAD_{65} and GAD_{67} confirmed the distinctive roles of each of the two isoforms in GABA metabolism [17-19]. GAD_{65}-deficient mice display no conspicuous morphological defects and their general behaviour, locomotion, reproduction, and glucose homeostasis appear to be unchanged compared to wild-type controls [17, 19]. Basal GABA-levels and holoenzyme activity are normal, but the reservoir of PLP-inducible apoenzyme is significantly decreased [19]. Both the quantal size and frequency of GABA-mediated miniature inhibitory postsynaptic currents (mIPSCs) are normal in gad_{65}-/- mice, however, during sustained stimulation the release of GABA is markedly impaired [20]. Thus, GAD_{65} seems to participate in the (re)filling and/or secretion of synaptical GABA vesicles.

In contrast to the gad_{65}-/- phenotype, GAD_{67}-deficient mice are born with a cleft palate and die shortly after birth [18, 21]. Furthermore, the GABA content in newborn's cerebral cortex of gad_{67}-/- and gad_{67}+/- mice is reduced to approximately 10% and 65%, respectively, of the respective value for wild-type littermates [18], indicating that GAD_{65} cannot compensate for the loss of GAD_{67}.

These observations in knock-out animals provide evidence that GAD_{67} is essential in the GABA metabolism of the developing brain and controls basal GABA levels, whereas GAD_{65}-generated GABA is insufficient to maintain basic vital functions but provides a dynamic buffer reservoir for vesicular release.

In gad_{65}-/- mice seizures can be easily induced by chemical convulsants [17] or mild stress [19]. Conflicting reports with respect to epileptogenesis in these animals may be due to the different genetic background of the mouse strains used. Anyhow, the findings identifies GAD_{65} as a potential target for anticonvulsants and gad_{65} as a candidate gene for inheritable seizure disorders in humans.

More elaborated knock-out strategies, i.e. techniques which allow an inducible disruption of the gene, may be necessary to reveal the role of GAD_{67} in adult animals, especially its role in epileptogenesis. The observation that GAD_{67} expression is increased in glutamergic hippocampal granule cells *following* seizures should encourage further research on the underlying mechanisms and the development of GAD_{67} specific drugs.

None of the established antiepileptic drugs is known to stimulate GABA anabolism, although gabapentin and valproate (Fig. (**3**)) may exacerbate the activity of GAD. Gabapentin (1-(aminomethyl) cyclohexane-acetic acid) was designed as a cyclic GABA analogue capable of readily crossing the blood-brain-barrier. It proved effective in a wide range of animal seizure models, but contrary to prediction

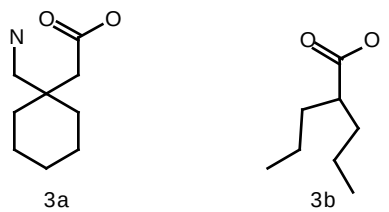


Fig. (3). Gabapentin (**3a**) and valproate (**3b**).

gabapentin does not interact with GABA receptors and, in appropriate doses, does not interfere with the GABA carrier system or GABA-T. Some studies have shown gabapentin to increase GABA levels in the brain of patients with epilepsy and to increase the *in vitro* activity of GAD, but others failed to demonstrate any effects on GABA synthesis (reviewed in [22]). The stimulating effect of valproate (2-propylpentanoic acid) administration on GABA synthesis is more widely accepted, and it was suggested that valproate acts by converting the inactive apoenzyme into the active GAD holoenzyme (see [7]). However, no major specific mechanism(s) of gabapentin and valproate have been identified, leaving GAD as a neglected potential target for antiepileptic drugs. Progress may emerge from a recent study which examined the anticonvulsant activity of the terpenoid bilobalide, a constituent of *Ginkgo biloba* extract. The data indicate a positively modulating effect of bilobalide on GAD activity and GAD_{67} protein levels [23].

An obvious strategy to elevate GABA levels in the brain and hence a potential mechanism of antiepileptic drug action is the blockade of GABA-T, the enzyme initiating GABA catabolism. GABA-T is a mitochondrial protein with a molecular weight of 109 kDa, found in neurons and glial cells. Like GAD, GABA-T requires PLP as cofactor. The enzyme transfers the amino group from GABA to α-ketoglutarate, yielding glutamate and succinic semialdehyde (Fig. (**2**)). Besides its role in GABA catabolism, GABA-T is involved in the transamination of β-alanine, therefore it comes to no surprise that GABA-T is an ubiquitous enzyme not limited to the GABAergic system or even the brain (see [9]). GABA-T can efficiently be blocked by drugs such as γ-acetylenic GABA, aminooxyacetic acid (AOAA), ethanolamine-O-sulfate (EOS), 5-amino-1,3-cyclohexadienecarboxylic acid (gabaculine) and 4-amino-5-hexeonic acid (vigabatrin or γ-vinyl GABA) (Fig. (**4**)). Vigabatrin, one of the first marketed antiepileptic drugs developed through rational design, irreversibly inhibits GABA-T activity. It proved protective in several animal models of induced seizures, and clinical studies have shown vigabatrin to be effective against partial simple and complex seizures. Administration of vigabatrin causes an increase in whole brain GABA, however, the precise manner of its seizure-protective mechanism remains unidentified. As a possible mechanism it is discussed that GABA-T inhibition results in a larger releasable GABA pool, consistent with the finding that vigabatrin preferentially blocks neuronal GABA catabolism (see [4] and references therein). Vigabatrin is generally well tolerated and extraordinarily effective against infantile spasm, however, there is some concern due to several reported side-effects, in particular visual impairment [24, 25], which might be overcome by a second generation of GABA-T inhibitors.

Whereas vigabatrin inactivates GABA-T mainly by a Michael addition, recently a class of time-dependent, mechanism-based inactivators of GABA-T has been introduced. They act via an active enamine intermediate generated by GABA-T, which modifies the PLP coenzyme (depicted in [26]). This class comprises the structurally related compounds 3-amino-4-fluorobutanoic acid [27] and 4-amino-5-fluoropentanoic acid, which are more potent than the conformationally rigid analogues of the latter substance [26].This illustrates that predictions for successful rational drug design are difficult, however, the recent deciphering of the GABA-T crystal structure [28] may facilitate such efforts.

$GABA_A$ RECEPTORS

Most of the fast inhibitory effect of GABA is mediated via the ionotropic $GABA_A$ receptor ($GABA_AR$), a ligand-gated ion channel. The receptor/channel complex transduces GABA signalling by initiating a Cl^- influx into the cell, which hyperpolarises the postsynaptic neuronal membrane. The $GABA_AR$ is a transmembrane pentameric protein, i.e., it is composed of five subunits which form the ion channel. 20 related $GABA_AR$ subunits in mammals, each encoded by

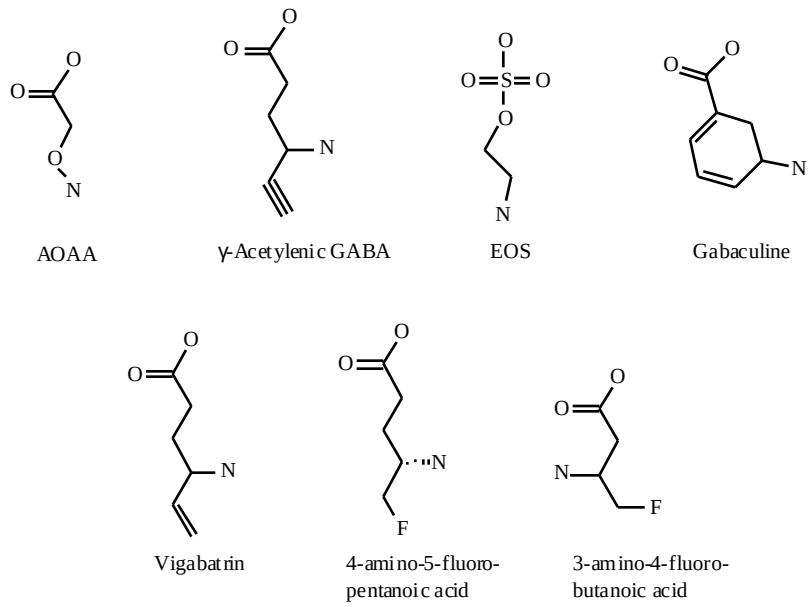


Fig. (4). Inhibitors of GABA-T.

a different gene, have been cloned so far and classified into eight classes of variants, according to their degree of amino acid identity. These yet comprise the α1-6, β1-3, γ1-3, δ, ε, π, ρ1-3, and θ mammalian isoforms (see [29, 30]), the heterogeneity of which is further increased by several alternative splice variants. Thus, from a pentameric subunit arrangement emerges a vast number of putative different $GABA_A$Rs, even under the restriction that some compositions are unlikely to form functional receptors. The subunits display unique patterns of temporal and spatial expression throughout the CNS and only some have been confirmed to coassemble into physiological distinct receptor subtypes. Hence, it is a challenging task to elucidate the composition and stoichiometry of native $GABA_A$Rs and to gain insight into the mechanisms that control subunit expression and assembly on the cellular and subcellular level.

A number of psychoactive drugs exert their beneficial or harmful effects mainly or exclusively via $GABA_A$Rs. These include clinically and/or experimentally important anxiolytics, anesthetics, tranquilizers, and anticonvulsants, like benzodiazepines (BZ), barbiturates, and neurosteroids. However, controversy still surrounds the role of the $GABA_A$R as a potential target of ethanol action, which, however, shares several pharmacological properties with specific $GABA_A$R ligands. Drug actions on $GABA_A$R for BZ and neurosteroids range from agonistic (GABA-enhancing) effects, e.g., anxiolysis, sedation, and hypnosis, to inverse agonistic (GABA-diminishing) effects, e.g., anxiety and vigilance.

During the last decade, target sites for a number of substances have been identified and assigned to specific subunits or receptor subtypes. It became evident that distinct $GABA_A$R subtypes have unique pharmacological profiles based on their particular subunit composition. For instance, an assembly of α1βnγ2 displays high affinity and efficacy for classical BZ agonists, whereas α4βnγ2 and α6βnγ2 subtypes are assemblies without γ2 show a negligible affinity to BZ receptor ligands. Likewise, DMCM (methyl-6,7-dimethoxy-4-ethyl-carboline) exerts an agonistic effecton α2βnγ1 receptors but acts as a weak antagonist on α6β1γ2. A comprehensive summary on specific pharmacological properties of $GABA_A$R subtypes is given in [31].

Several molecular and genetic findings indicate an involvement of $GABA_A$Rs in the generation, outcome, and treatment of epilepsy. Recent studies focused on neural plasticity and unveiled structural changes at the inhibitory postsynaptic membrane in the context of epilepsy. It was found, that the amplitude of $GABA_A$R-mediated mIPSCs was enhanced in dentate gyrus granule cells of rats following electrical stimulation (kindling), an experimental model of temporal-lobe epilepsy [32]. The effect could be attributed to an increase in the number of functional $GABA_A$Rs in the kindled cells as shown by electronmicroscopy and immunocytochemistry [33], providing a molecular basis for the increase in GABAergic inhibition in human temporal lobe epilepsy. However, unchanged mIPSC amplitudes but an lower frequency of mIPSCs were reported in pilocarbine or kainate treated animals [34] -two alternative experimental models of human temporal lobe epilepsy. Combining patch-clamp recording and mRNA amplification techniques, $GABA_A$R plasticity during status epilepticus was recently investigated on the single-cell level, unveiling an increase in the number of $GABA_A$Rs as well as an alteration in their subunit composition [35]. Following treatment with pilocarpine, dentate granule cells from rats displayed profound differences in functional and pharmacological properties of the $GABA_A$Rs compared with cells from untreated control rats. The relative expression of the α4, δ, ε, and β_3 subunits was significantly increased, whereas the relative expression of α1 and β1 were decreased [35]. These changes in subunit expression, but not the increased GABA efficacy, precede the onset of the chronic epileptic state (as defined by recurrent spontaneous seizures) by weeks. Hence, the authors speculate that alteration in $GABA_A$R subunit assembly is involved in the evolution of a chronic seizure state. However, transcription rates of subunits do not necessarily correlate with their translation and assembly and the altered expression may rather be a consequence of drug treatment or kindling, i.e., the induced epileptic state may insufficiently reflect the processes in human temporal lobe epilepsy. Yet, it is unquestionable that plasticity of inhibitory synapses is a feature of epileptic disorders, whether resulting from or contributing to seizures. In turn, these changes directly influence the efficacy and activity of drugs acting on $GABA_A$Rs.

Several approaches to disrupt genes encoding specific $GABA_A$R subunits in mice generated interesting data regarding the significance of $GABA_A$Rs in embryonic and neonatal development and were instrumental in identifying subunits contributing to epileptogenesis. Mice devoid of the γ2 subunit nearly completely lack the BZ binding site, and the physiological properties of their $GABA_A$Rs are drastically different from that of wild-type animals. Prenatal brain development appears unaffected, but after birth the mice show severe growth retardation, sensorimotoric and behavioural dysfunctions, and a drastically reduced life-span [36], which makes them unsuitable tools to investigate effects related to seizure disorders. Gene disruption of the δ subunit revealed a decreased sensitivity to neuroactive steroids and an alteration in behavioural responses to these drugs, but apart from this, no special phenotypical or behavioural quality was observed. Mutant and wild-type mice did not significantly differ in their response to an absence seizure-inducing dose of pentylenetetrazole [37]. The most promising results with respect to epilepsy were obtained with $GABA_A$R β3 knock-out animals [38]. Approximately 90% of the null mutants die within one day after birth, about one half of them suffering from cleft palate, which has been also observed in gad_{67}-/- mice [18, 21]. The surviving mice show several behavioural and physiological abnormalities that vary greatly between individuals. Most striking, however, was the spontaneous development of seizures, ranging from mild to severe, in all β3-/- animals [38, 39]. These studies had been inspired by previous work on pink-eyed dilution (p) mutant mouse strains. They originally lacked the β3, α5, and γ3 subunit genes due to a radiation induced deletion on murine chromosome 7 and die at birth from cleft palate or show neurological abnormalities when rarely surviving. This conspicuous phenotype could be attributed to deletion of β3 [40], stimulating the above described efforts to produce a β3-knock-out mouse. Mice

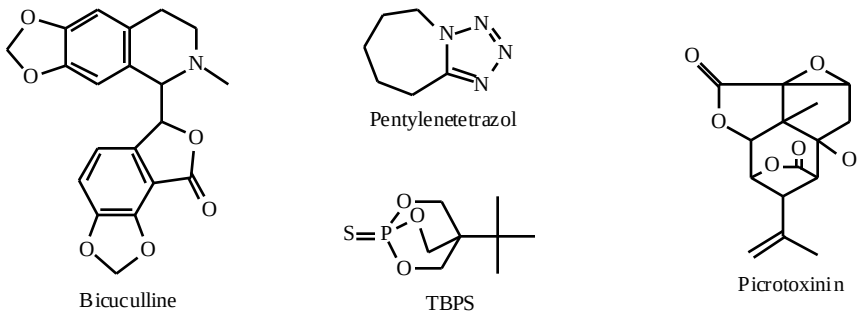


Fig. (5). Important convulsive antagonists of $GABA_A$ receptors.

deficient in β3 are thought to represent an animal model for the human inherited epilepsy-like neurological disorder Angelman syndrome (see [41] and [3] for further details).

A vast amount of indirect pharmacological evidence further strengthens the hypothesis that $GABA_AR$ mediated neurotransmission may play an essential role in convulsive partial epilepsy. First, the two potent convulsants bicuculline and picrotoxinin (Fig. (**5**)) bind preferentially to $GABA_ARs$. Bicucullin, the prototypic $GABA_AR$ antagonist, efficiently and competitively blocks the site-specific binding of GABA to its receptor, whereas picrotoxinin, the active component of picrotoxin, inhibits GABA-evoked chloride influx by blocking the channel pore. The same mechanism underlies the convulsive action of the non-competitive $GABA_AR$ antagonists pentylenetetrazol and *t*-butylbicyclophosphorothionate (TBPS) (Fig. (**5**)), which are both used experimentally to induce seizures in animal models of epilepsy. On the other hand, a number of $GABA_AR$ agonists are successfully used for the treatment of epilepsy. These include the antiepileptic 1,4-BZs clonazepam, clorazepate, diazepam (Fig. (**6**)), lorazepam, midazolam, and nitrazepam, and the 1,5-BZ clobazam (Fig. (**6**)). Binding of BZs occurs to a specific recognition site on the $GABA_AR$ and is thus allosteric to GABA binding. BZs shift the GABA dose-response curve to lower concentrations, hence, they do not evoke a Cl^- influx in the absence of GABA and do not increase the effect obtained at maximal GABA concentrations. The augmentation of GABA-evoked currents by BZs occurs through an increased channel-opening frequency, whereas they have no effect on single-channel conductance or average channel open duration. A recent report demonstrates that BZs exert different actions via distinct receptor types [42]. This study found that some but not all effects of diazepam are impaired in mice carrying a histidine-to-arginine point mutation at position 101 of the α1 subunit gene, which leads to a diazepam-insensitive binding site. Protection by diazepam against pentylenetetrazole-induced seizures was reduced in these transgenic mice compared to wild-type controls, indicating that the anticonvulsant activity of BZ site ligands is partly mediated by α1-containing assemblies.

Barbiturates, including the antiepileptic phenobarbital (Fig. (**6**)), enhance the binding of GABA, thereby increasing GABA-induced Cl^- currents. At high concentrations, they directly activate $GABA_ARs$ through a yet unspecified recognition site, possibly being the cause for the toxicity of barbiturates. In contrast to the action of BZs, barbiturates increase the average open duration without altering the frequency of channel openings. Like BZs barbiturates do not affect single channel conductance.

A representative of the neurosteroids is the antiepileptic drug ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) (Fig. (**6**)), a selective high-affinity positive allosteric modulator of the $GABA_AR$ [43]. A anticonvulsive potency of ganaxolone has been demonstrated in animal models. Like

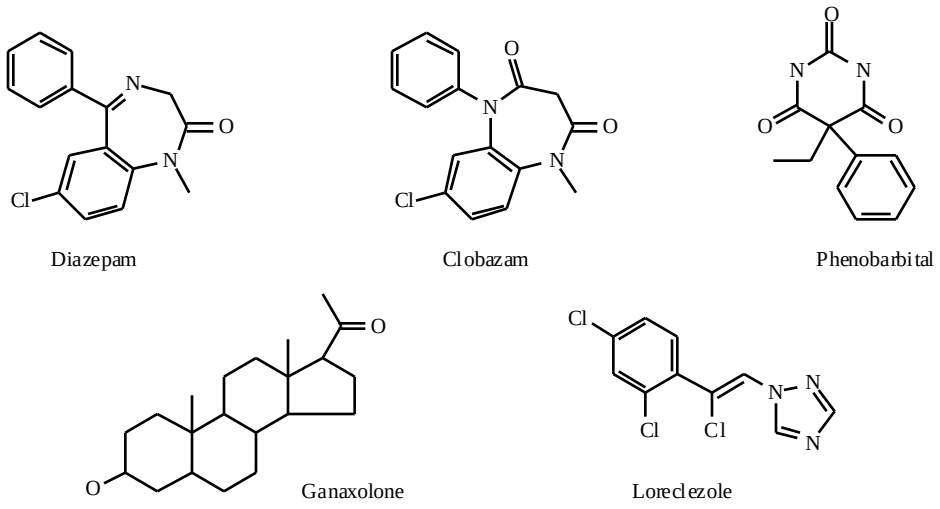


Fig. (6). Prototypical anticonvulsants acting on $GABA_A$ receptors.

other steroid modulators of $GABA_AR$ ganaxolone acts at a site distinct from the barbiturate or BZ recognition site, but has not been proven to posses any subtype specificity. The experimental anticonvulsant loreclezole ((Z)-1-[2-chloro-2-(2,4-dichlorophenyl)ethenyl]-1,2,4-triazole) (Fig. (**6**)) is also reported to specifically interact with $GABA_ARs$. Loreclezole shows a unique subunit dependence displaying highest selectivity for β2/3- rather than β1-containing assemblies [44]. Like BZs, loreclezole acts by increasing the affinity of the receptor for GABA but at a site distinct from the GABA, BZs, barbiturate, and neurosteroid site.

Taken together, there is strong evidence that $GABA_ARs$ can be directly and indirectly involved in the generation of different epileptic disorders. Combining the results from knock out-mice and the selectivity of loreclezole, the β3 subunit must be considered as a potential candidate for $GABA_AR$ mediated antiepileptic drug treatment, and the development of β3 specific ligands should be a major goal of rational drug design. Moreover, we like to emphasise that further research on the distribution, the assembly, the physiological role in health and disease, and the pharmacological properties of different $GABA_AR$ subtypes will elucidate proper targets for highly specific ligands and hence open new directions for an up-to-date drug design.

$GABA_B$ RECEPTORS

Whereas ionotropic $GABA_ARs$ convey fast synaptic inhibition, slow GABA-mediated inhibitory postsynaptic potentials act through the metabotropic $GABA_B$ receptors ($GABA_BR$). $GABA_BRs$ are coupled to guanine nucleotide binding proteins (G proteins) and exert most of their physiological actions via modulation of adenylate cyclase or G protein-gated K^+ and Ca^{2+} channels (see [45] and refs. therein). It has been suggested that pharmacological and functional distinct $GABA_BR$ subtypes are localised pre- and postsynaptically. Whereas activation of presynaptic $GABA_BRs$ is considered to suppress neurotransmitter release by down-regulating high-voltage activated Ca^{2+} channels, postsynaptic $GABA_BRs$ regulate inwardly rectifying K^+ channels (Kir), thereby yielding a prolonged hyperpolarisation of the cell [46].

Although the existence of another GABA receptor molecule aside from the $GABA_AR$ has been proposed years ago based on pharmacological data, it took 16 years until the first $GABA_BR$ cDNA was eventually isolated using a radioligand-binding screening approach [47]. Initially, two splice variants of $GABA_BR$ mRNA had been identified, encoding two closely related proteins (R1a and R1b), which differ in length and composition of the amino terminus but share identical functional domains [46, 47]. $GABA_BR1$ transcripts are expressed in almost all neuronal cell populations throughout the CNS, however, in the cerebellum each splice variant shows spatial restriction to distinct cell types, i.e. R1b can be detected in Purkinje cells, whereas R1a is abundant in the granular cell layer [46, 48]. Yet, it is questionable whether alternative splicing implies a functional diversification of the receptor [46].

The agonist affinity of the recombinant $GABA_BR1s$ was significantly lower than that of native receptors, functional coupling to Kir3 channels proved difficult, and the coupling to other assumed effector systems like adenylate cyclase and Ca^{2+} channels remained elusive in mammalian expression systems. Hence, $GABA_BR1$ could account for some but not all of the functional and pharmacological properties of the native receptor. These discrepancies were solved when several groups simultaneously cloned a new $GABA_BR$ subtype, $GABA_BR2$, and revealed that both subtypes assemble into a heteromeric fully functional $GABA_BR$ complex [49-54]. The two $GABA_BRs$ share a similarity at the protein level of about 35%, they correspond in size, and -like other G protein coupled receptors- contain seven transmembrane domains (7TM). $GABA_BR1$ and $GABA_BR2$ most likely assemble as dimers but may possibly exist as oligomers as well. Furthermore, it cannot be ruled out that $GABA_BR2$ receptors may be functional as monomers and that additional members of this receptor family exist, providing further molecular explanation for different pharmacological and functional $GABA_BR$ subtypes. [49-52]. The heterodimerization of two 7TM receptors represents a new principle of G protein coupled receptor processing and the finding may stimulate further research on the underlying mechanisms and accelerate the search for 'partner molecules' in other receptor systems.

Even without knowledge about its molecular structure and despite the lack of a suitable expression system, $GABA_BR$ were at the centre of intense pharmacological research, in which the involvement of the receptor in some pathophysiological processes became apparent. $GABA_BR$-mediated events are considered to influence pain, anxiety, depression, and cognitive capacity, and the agonist baclofen (β-(4-chlorophenyl)-γ-aminobutyric acid) (Fig. (**7**)), the first marketed $GABA_BR$ drug, is used as a muscle relaxant for the treatment of spasticity in multiple sclerosis and spinal injury.

First evidence for a participation of $GABA_BRs$ in epileptic disorders arose from observations in pharmacologically induced models and genetic rodent strains which provide an model of generalised, non-convulsive epilepsy, designated as absence seizures [55-59]. Absence seizures differ clinically and experimentally from other types of epilepsy. They are characterised by 3/s spike-and-wave patterns in the EEG associated with a behavioural arrest or a transient loss of consciousness, a rapid postictal normalisation, and the absence of abnormalities in the interictal state. A typical pharmacological feature of absence seizures is their *exacerbation* by drugs that increase GABAergic activity in the brain, hence, therapeutic interventions useful in the treatment of convulsive epilepsy forms tend to be harmful in the context of absence seizures and vice versa. $GABA_BRs$ have been implicated in the generation of absence seizures, as receptor antagonists suppress the spontaneous spike-and-wave discharges, whereas agonists aggravate the symptoms [59-61].

Intense studies on different rodent models begin to reveal the underlying cellular basis of absence seizures and a possible mechanism in which $GABA_BRs$ influence the pathogenesis of this epileptic disorder [62-64]. The

thalamocortical burst-firing observed in absence epilepsy is facilitated by the capacity of thalamic relay cells to generate low-threshold spikes, which are triggered by calcium influx at thresholds near the resting membrane potential. Low voltage-activated (T-type) calcium channels play a crucial role, and most genetic mouse models of absence seizures have been reported to bear defects in voltage-sensitive calcium channels [65-67]. T-type Ca^{2+} channels are rapidly inactivated and can only repeatedly be activated in cells with a hyperpolarised membrane potential. It is assumed that postsynaptic $GABA_BR$-activated K^+ currents provide the required hyperpolarisation of thalamic cells, thereby 'de-inactivating' T-type Ca^{2+} channels and priming the generation of low-threshold spikes, leading to a burst of action potentials. Further discussion on the role of calcium channels in the pathogenesis of epilepsy can be found elsewhere in this issue.

In this model, postsynaptic $GABA_BR$ possess a regulative, but not causative function in the pathogenesis of generalised non-convulsive seizures, which is confirmed by the absence of altered density or functionality of $GABA_BR$ in a rat model (GAERS) of absence epilepsy (see [63]. In contrast, an increase in $GABA_BRs$ was shown in the lethargic (lh/lh) mouse, which also serves as an model of absence epilepsy [63]. These discrepancies point to different or additional molecular mechanisms underlying absence seizures, e.g., the control of the release of GABA, L-glutamate and/or other neurotransmitters via modulation of Ca^{2+} influx by presynaptical $GABA_B$ hetero- and autoreceptors.

Despite of these unsettled issues $GABA_BR$ antagonists are valueable in the treatment of non-convulsive absence seizures, and some of the known antagonists may reveal their therapeutic value in the future. The two presently used anti-absence drugs, ethosuximide and valproate, probably do not act through $GABA_BR$. Therefore, substances specific for $GABA_BR$ might serve as an alternative or a helpful completion to these available drugs. Currently, three classes of potent $GABA_BR$ antagonists are distinguishable with respect to their basic structure (Fig. (**7**)). The first compounds introduced were phaclofen and 2-OH-saclofen, both analogues of the receptor agonist baclofen (see [68]), which, however, showed no activity *in vivo*. But recent studies describe an protective effect of halogenated and non-halogenated p-chlorophenyl alcohol amides in several mouse models of absence epilepsy. Substances such as Cl-HEPP ((+/-)-3-hydroxy-3-[4-chloro]phenyl-pentanamide) and Cl-HEPA ((+/-)-2-hydroxy-2-[4-chloro]phenyl-butyramide) display structural similarity to 2-OH-saclofen and are believed to exert their anticonvulsive effects via $GABA_BR$ [69, 70].

The 3-aminopropyl-phosphinic acid derivative CGP35348 was the first antagonist to penetrate the blood-brain barrier, and subsequently used as lead design for the synthesis of potent orally active compounds, e.g., CGP36742 and CGP46381 (see [68]). These drugs and homologues thereof were mainly used to elucidate function and distribution of $GABA_BR$, and revealed its role in epileptic disorders. Meanwhile, dozens of potent $GABA_BR$ antagonists derived from this design series.

A third class of antagonists has been described, belonging to a series of 2,5-disubstituted 1,4-morpholines, of which Sch 50911 ((+)-(S)-5,5-dimethyl-morpholinyl-2-acetic acid) antagonises $GABA_BR$-mediated function and

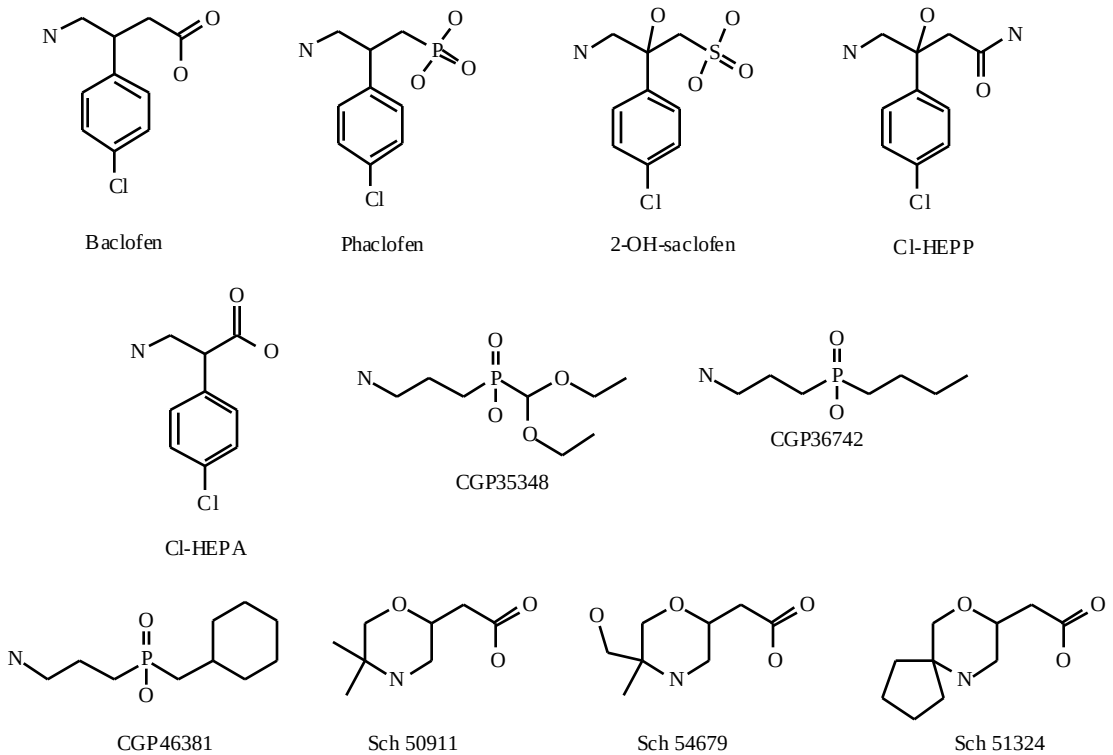


Fig. (7). $GABA_BR$ agonist (baclofen) and members from three classes of antagonists.

suppresses absence seizures. Sch 54679 (5-(S,R)-hydroxymethyl-5-methylmorpholynyl-2-(R,S)-acetic acid) and Sch 51342 (2-(R,S)-5-[spirocyclopentyl]-morpholynyl-acetic acid), two derivatives of Sch 50911 differing in their 5-alkyl substituents on the morpholino-ring, likewise possess antagonistic actions at $GABA_B$ auto- and heteroreceptors, and may soon be tested for their efficacy in epilepsy [71, 72].

$GABA_BR$ antagonists induce convulsions at high doses [61], enhance the susceptibility to audiogenic seizures [61], and aggravate generalised convulsive seizures when administered for long time [73]. It has been proposed that different populations of $GABA_BRs$ are involved in this proconvulsive effect, located either on different nerve endings or in distinct brain structures [61].

It is evident that $GABA_BR$ antagonists possess adverse effects in generalised absence and convulsive seizures, due to the pathophysiological characteristics of the two epilepsy forms. Likewise, such opposite effects have been reported with respect to $GABA_BR$ *agonists*, recommending them as possible therapeutics in convulsive generalised or partial seizures.

GABA TRANSPORTER

GABA-mediated inhibitory synaptic transmission is terminated by removal of GABA from the synaptic cleft within a few milliseconds after its release. Although diffusion cannot be neglected, the majority of neurotransmitter molecules is rapidly recycled by a cellular uptake system of high-affinity sodium- and chloride-dependent transporter proteins, located in the plasma membrane of glial cells and (pre)synaptic nerve endings. Four different GABA transporters (GATs) have been identified and cloned from several mammalian sources. The nomenclature is somewhat confusing, since the transporters were termed GAT-1, GAT-2, GAT-3 and GAT-4 in mice [74], whereas there corresponding counterparts in rat are designated as GAT-1, BGT-1 (betaine/GABA transporter) [75], GAT-2 and GAT-3, respectively [76-78]. Human and other mammalian cDNA homologues to GAT-1, BGT-1 and GAT-3 of the rat have been cloned [79-81]. If not otherwise stated, we use the latter GAT nomenclature in this review.

Together with the creatine and the taurine transporter the four GATs constitute a subfamily of the Na^+/Cl^- neurotransmitter transporters (see [82]). The predicted protein structure of all Na^+/Cl^- transporters contains a motif of twelve hydrophobic transmembrane helices/domains (TM) connected by short internal and external loops, and a single large extracellular loop with putative glycosylation sites. The amino- and carboxy-termini face the cytoplasma [83, 84]. The structural organisation of twelve TM domains has been evolutionary maintained in several other neurotransmitter transporter families, however, its functional significance is not well understood. Yet, extensive study on GAT-1 has revealed the basic mechanism of GABA uptake. The neurotransmitter is cotransported with sodium and chloride in an electrogenic fashion. This process is driven by the electrochemical gradient of sodium ions, which is maintained by a Na^+/K^+-ATPase. Uptake of each GABA molecule is accompanied by cotransport of two Na^+ and one Cl^- [85]. These ions bind first, inducing a conformational switch of the transporter protein to a high affinity state for GABA [86]. Following the transport, Na^+, GABA and Cl^- are successively released, and GAT returns to its original conformation (see [82]). Being dependent on the ion gradients GATs also operate in the reverse direction, i.e. they can cause efflux of GABA, thus giving rise to a non-vesicular neurotransmitter release.

Since GABA is a zwitterion and ions serve as cosubstrates, charged amino acids in the membrane domains could be necessary for GAT function. However, of five charged residues within the transmembrane domains of GAT-1, only arginine-69 in TM 1 proved to be essential for activity [87], possibly playing a role in the binding of Cl^-. Additional approaches to essential functional domains focused on aromatic amino acids, which could interact with positively charged substrates by virtue of their π electrons [88]. Tryptophane-68 has subsequently been shown to participate in the binding and dissociation of Na^+, further strengthening the significant role of the first hydrophobic domain of GAT-1 in the transport mechanism [86]. More recently, a tyrosine residue in position 140 has been shown to be critical for GABA binding and transport by GAT-1 [89], even replacement by other aromatic amino acids resulted in an impaired capacity to bind GABA. It has been proposed that three short external loops may form a pocket into which the initial substrate binding occurs. These loops were cross-substituted among various GATs, resulting in transporters mimicking the GABA affinities of the respective 'donor' sequence [90]. Still, the precise binding domains for GABA, sodium and chloride have yet to be defined, and the conformational changes occurring during substrate uptake remain an enigma.

The process of GABA transport by GAT-1 may not necessarily represent the general mechanism in other GATs, exemplified by the absolute dependence on external Cl^- in GAT-1, whereas GABA transport by GAT-2 and GAT-3 is only moderately reduced in chloride-free medium [77]. Furthermore, functional characterisation of the canine BGT-1 revealed that GABA is cotransported with three Na^+ not two as in GAT-1, and that GABA might bind prior to the ions [91].

The different roles of the individual GATs are not clear, but they are distinctly localised. As GAT-1 and GAT-3 are brain-specifically expressed and display the highest affinity to GABA, they are considered to represent the principal actors in the termination of GABAergic inhibition and in the homeostasis of GABA levels in the central nervous system. In the brain, two major subtypes of GABA transport systems, neuronal and glial, can be differentiated with regard to their biochemical, physiological and pharmacological features. The neuronal uptake system can be inhibited by L-(2,4)-diaminobutyric acid (L-DABA) or cis-3-aminocyclohexanecarboxylate (ACHC) (Fig. (**8**)), but is insensitive to β-alanine. GAT-1 exhibits a corresponding pharmacological profile and it is therefore believed to represent the neuronal GABA transporter. The second transport system, thought to be located in glial cells, is inhibited by β-alanine (Fig. (**8**)) [92]. The high sensitivity of

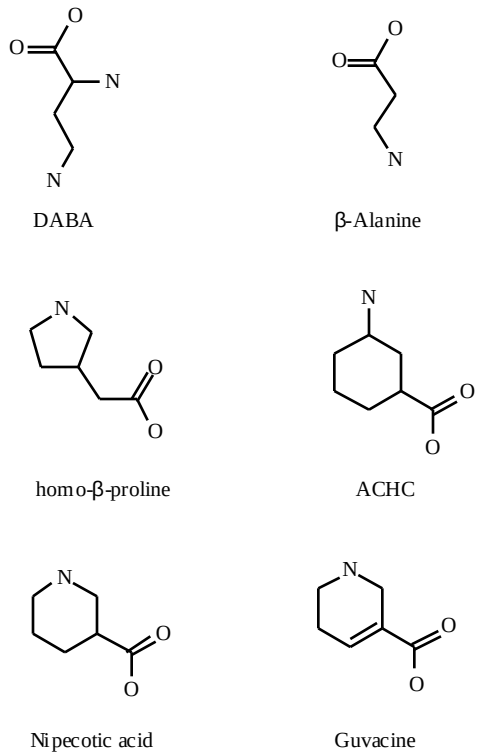


Fig. (8). Amino acid substrate inhibitors of the GABA transporter.

recombinant GAT-3 to β-alanine and virtually no inhibition by ACHC or L-DABA suggests GAT-3 to represent the glial transporter [77]. However, GAT-3 lacks the sensitivity to several inhibitory drugs reported for the pharmacological defined glial-type transporter system [77], and more detailed *in vitro* studies demonstrated that GAT-3 mRNA may be predominantly expressed in neurons rather than in glial cells [78]. Likewise, some results indicate that GAT-1 may also be present in glial cells [93, 94], suggesting variable roles for GAT-1 and GAT-3 in different brain areas. Furthermore, additional transporters may be coexpressed in neurons and/or glial cells, blurring the distinct pharmacological profile of recombinant GAT-1 and GAT-3. GAT-2 may be one candidate, since it combines pharmacological features of both the neuronal, i.e., sensitivity for L-DABA, and the glial, i.e., inhibition by β-alanine, uptake system [77]. However, it cannot be ruled out that further GABA transporters with pharmacological profiles consistent with those found in glial or neuronal cell cultures remain to be identified.

Whereas expression of GAT-1 and GAT-3 is restricted to the brain and the retina, GAT-2 mRNA is also present in peripheral tissues such as liver and kidney [77]. Its distribution in the brain changes during postnatal development and is restricted to few areas in adults. Therefore, GAT-2 is assumed to be involved in the maturation of the central nervous system [82, 95], whereas its role in the adult brain and the peripheral tissues is unknown.

BGT-1 is ubiquitously expressed in most mammalian tissues including the central nervous system. Its ability to effectively transport betaine clearly distinguishes BGT-1 from the three other GATs. Transport of GABA and betaine can be inhibited both by β-alanine and by L-DABA [91]. Due to its wide distribution and its specific substrate properties, BGT-1 is considered to act mainly as an osmolyte transporter, thus controlling the maintenance of a cellular osmotic equilibrium. The significance of GABA transport by BGT-1, if any, however remains elusive.

Though all GATs exhibit high sequence homology and share structural and functional properties, they clearly differ in their substrate affinities. The main feature separating GAT-1 from the other three GABA transporters is the substrate specificity for GABA. Whereas BGT-1 is an effective betaine transporter, GAT-2 and GAT-3 have appreciable affinities for taurine, and all three are able to transport β-alanine, although BGT-1 to a much lesser extent (see [82]). All recombinant GATs display pharmacological diversity, hence, it is possible to design drugs that discriminate between the transporters and exert their effects exclusively on distinct uptake systems. Since blockade of GABA uptake leads to a prolonged presence of GABA in the synaptic cleft and therefore to augmentation of GABAergic inhibition, GATs are an obvious target for therapeutic interventions against partial convulsive seizures. GAT-1 and GAT-3 must be seen as key players in synaptic GABA uptake, and pose themselves as preferential drug targets.

The inhibitory action of conformationally restrained GABA analogs (Fig. (**8**)) such as nipecotic acid (3-piperidinecarboxylic acid), 4-OH-nipecotic acid (4-hydroxy-3-piperidinecarboxylic acid), guvacine (1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) and homo-β-proline (3-pyrrolidineacetic acid) on GABA uptake in cell culture has been known for some time. Unfortunately, determining the *in vivo* efficacy of these compounds has been hampered by their poor penetration of the blood-brain barrier. To overcome this problem, several synthesis programs were commenced to provide lipophilic derivatives of these cyclic amino-acids which combine the high affinity to GATs with the property to readily cross the blood-brain barrier. These efforts resulted in compounds derived from nipecotic acid or guvacine, with either 4,4-disubstituted 3-butenyl or diarylether moieties attached to the nitrogen atom of the cyclic amino acid (Fig. (**9**)) [96]. Examples are SKF 89976A, SKF 100330A [97], tiagabine ((R)-N-[4,4-di(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid) [98], NC-711 [99] and CI-966 [100], which have a suitable lipophilicity to enter the central nervous system after oral administration, are potent inhibtors of GABA uptake, and proved protective against seizures in some animal models. In addition, SKF 89976A, tiagabine, NC-711, and CI-966 are highly selective for GAT-1 [101].

The inhibitory action of these substances on GABA uptake is much more potent than that of their parent cyclic amino acids, and their potency is strongly stereo-selective and dependent on structural features. It has been assumed that these inhibitors bind to the GABA recognition site via the cyclic amino acid moiety and to a separate domain via the attached side chain, whereas the parental compounds nipecotic acid and guvacine only recognise the GABA site [98]. In accordance, the lipophilic derivatives prevent

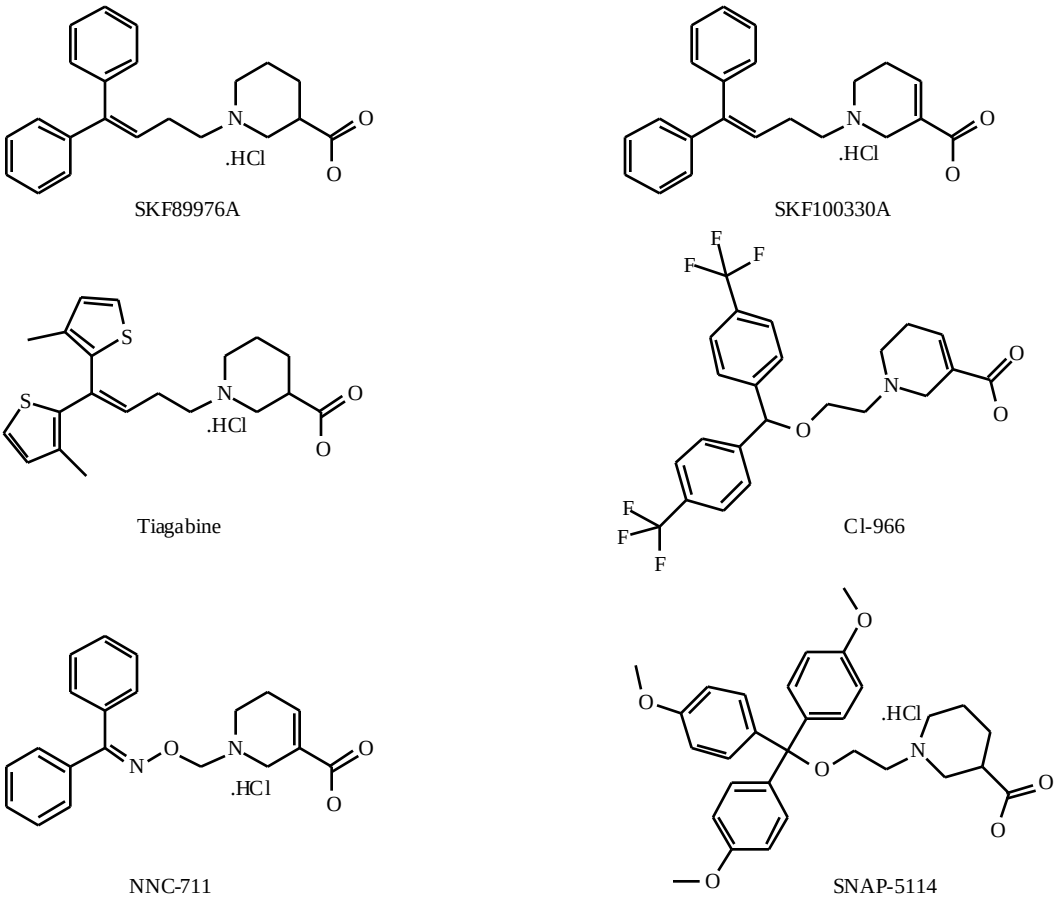


Fig. (9). Lipophilic derivatives of nipecotic acid or guvacine.

transport of GABA without being substrates for GAT themselves.

As the first of these drugs, the nipecotic acid analogue tiagabine [96, 98] successfully completed clinical trials and was introduced into clinical practice. Tiagabine represents a prime example for an anticonvulsant developed by rational strategy [6], and is mechanistically the best described antiepileptic drug in clinical use. It exclusively acts on GABAergic synapses by preventing GABA uptake by GAT-1, thus augmenting GABA-mediated inhibitory neurotransmission. No other mechanism has been invoked to explain its anti-seizure activity, i.e., tiagabine is unlikely to act as transmitter on GABA receptors, and lacks affinity to any other neurotransmitter system. The drug is active in a number of animal seizure models, and clinical data show that tiagabine is well tolerated and efficacious. Currently, tiagabine is licensed in several countries (including the European Union, Australia, and the USA) as add-on therapy for partial seizures in adolescents and adults, but (pre)clinical studies and data from animal models of epilepsy suggest a potential broader spectrum of use [102-105].

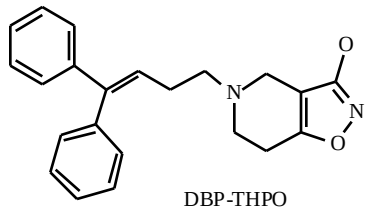
The promising results with the lipophilic derivatives of nipecotic acid and guvacine stimulated comparable approaches with different lead substances, e.g., the bicyclic isoxazole GABA analogues THPO (4,5,6,7-tetrahydroisoxazolo(4,5-c)pyridin-3-ol) and THAO (5,6,7,8-tetrahydro-4H-isoxazolo(4,5-c)azepin-3-ol) act as anticonvulsant agents by inhibiting GABA uptake. THPO is not a substrate for GAT, indicating a different mechanism of inhibition compared to nipecotic acid [106]. THPO, THAO, and their lipophilic 4,4-diphenyl-3-butenyl (DPB) derivatives (Fig. (**10**)) were characterised on astrocytes, cortical neurones, and on the four recombinant GATs [107]. In general, DPB substitution led to more potent inhibitors of GABA uptake. None of the substances had any effects on recombinant GAT-3, although THPO and THAO inhibited GABA uptake into glial cells with a slight preference compared to neurones. Since DPB-THPO displays highest affinity for GAT-1 but weak affinity for GAT-2 and BGT-1, it is to question, whether it has any advantages over the established derivatives based on guvacine and nipecotic acid. However, substitution of THPO and THAO with other lipophilic side chains might enhance the selectivity for BGT-1 or GAT-2 (see below).

Recently, phosphinic acid analogues of nipecotic acid and SKF89976A were introduced as novel GABA uptake inhibitors, but their potential to act specifically on distinct transporters has yet to be explored [108].

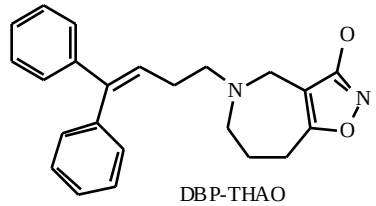
Whereas most GABA analogues inhibit substrate transport by GAT-1, only a few compounds selective for BGT-1, GAT-2, and GAT-3 have been identified so far. The bicycloheptane derivative (-)-2-phenyl-2-[(dimethyl-amino)ethoxy]-(1R)-1,7,7-trimethylbicyclo [2.2.1]heptane (EGYT-3886), which has an unusual structure compared to

other known GAT inhibitors, shows moderate and non-selective potency to all four cloned GATs, and was chosen as design lead for a series of triarylnipecotic acid derivatives [109]. From this series, 1-(2-(tris(4-methoxyphenyl)methoxy)ethyl-3-piperidinecarboxylic acid (SNAP-5114) (Fig. (**9**)) has been shown to inhibit GABA uptake by human GAT-3 [80, 109], indicating that subtype selectivity can be achieved by different side chains.

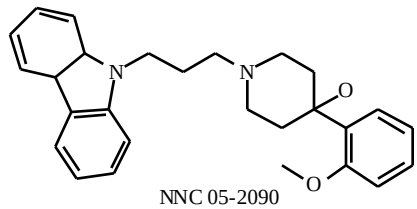
Recently, 1-(3-(9H-carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol (NNC 05-2090) (Fig. (**10**)), was identified to exhibit preference for murine GAT-2 [110]. Although mGAT-2/BGT-1 specific compounds may not be useful as antiepileptic drugs, due to the assumed physiological role of this transporter (see above), they may prove to be of great value as ligands to elucidate the distribution and transport mechanism of BGT-1.



DBP-THPO



DBP-THAO



NNC 05-2090

Fig. (10). Novel lipophilic inhibitors of GABA uptake.

In conclusion, just ten years after cloning of the first neurotransmitter transporter has begun to reveal the molecular basis of GABA uptake, the design of novel and the improvement of established GABA transporter inhibitors is progressing [111, 112], and drugs with high selectivity for distinct GAT subtypes may soon emerge.

GAT inhibitors exert their effects only under conditions when GABA is released physiologically. At a first glance, they would be expected to have less side effects than GABA-concentration independent receptor agonists or drugs which interfere with GABA metabolic enzymes. However, extra- and intracellular GABA concentrations modulate the expression and function of several components in the GABAergic system, i.e., GAD_{65} activity is regulated by a feedback mechanism and inhibited by even a small increase in the neuronal GABA concentration [113], and transport by GAT-1 is up-regulated by high extracellular GABA concentrations [114]. In addition, the pooling and prolonged presence of GABA in the synaptic cleft activates postsynaptic $GABA_BRs$ [115]. With regard to the predicted role of $GABA_BRs$ in generalised absence epilepsy (see the $GABA_BR$ section) inhibition of GABA uptake may aggravate absence seizures, thus GAT inhibitors are contraindicative in such epileptic disorders.

THE INHIBITORY GLYCINERGIC SYSTEM

Next to GABA, glycine is the most abundant inhibitory amino acid neurotransmitter in the CNS. Whereas GABA functions predominantly in the cortex and the cerebellum, glycinergic synapses constitute the major inhibitory system in the spinal chord and brain stem [116], inhibiting spinal motor neurons and hence controlling the tonus of skeletal muscles. Glycine receptors are also found in higher brain regions such as the olfactory bulb, cerebellum, midbrain and cerebral cortex, indicating that glycine mediates a vast number of motoric and sensoric functions [116], and maybe acting in concert with GABA, which is colocalised in some cell types [117-119]. In addition, glycine plays a major role in excitatory glutamergic synapses as a high-affinity coagonist of NMDA receptors (see elsewhere in this issue).

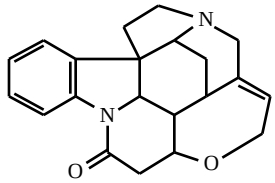
Glycine can be synthesised via two alternative pathways (Fig. (**2**)), one of them is the transamination of glyoxylate. However, this pathway seems to be of minor importance in the CNS, where most glycine is derived from serine through the action of the mitochondrial PLP-dependent enzyme hydroxymethyltransferase, which catalyses the transfer of the serine β-carbon atom on tetrahydrofolate. Catabolism of glycine proceeds through reversal of these synthesis pathways, however, different routes are also involved in the breakdown of the transmitter pool [2].

In contrast to the highly specialised GABA, glycine is also an essential building block of proteins and a metabolite in a number of biochemical pathways beside its role as a neurotransmitter. Thus, glycine is present in all tissues, and drugs which operate somewhere along the metabolic pathway of glycine will undoubtedly cause severe side-effects.

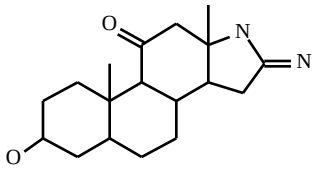
Once released from synaptic vesicles, glycine exerts its inhibitory action via the postsynaptically located ionotropic glycine receptor (GlycR). The GlycR functions in a similar way as the $GABA_AR$. Binding of glycine opens an intrinsic Cl^- channel, resulting in chloride influx and thus hyperpolarisation of the membrane potential. GlycR and $GABA_AR$ belong to the same superfamily of ligand-gated receptor channels and share a number of structural features, such as the heteromeric assembly of five subunits, which forms the functional receptor with the channel pore and the outer-membrane ligand binding-site (see [116, 120]. In contrast to the highly variable $GABA_AR$, so far only two classes of GlycR subunits have been identified: the 48-kDa α subunit, of which there are four isoforms (α1-4), and one 58-kDa β subunit [121, 122]. The α subunits carry the binding site for glycine. A 93-kDa protein, belonging to a group of similar proteins, is copurified with the receptor and

designated gephyrin [123]. It is implicated in synaptic anchoring and cluster formation of GlycR and possibly $GABA_AR$ but does not contribute to the transmembrane ion channel [124]. Most native GlycRs in the adult brain are considered to be assemblies of α1 and β, with a proposed stoichiometry of 3α:2β, whereas fetal and neonatal receptors primarily posses the α2 isoform [116].

Studies on the functional role of GlycR have been hampered by the fact that only a few high-affinity ligands have been identified. The alkaloid strychnine (Fig. (**11**)) is the most prominent compound and the prototypical antagonist. It acts on GlycR, efficiently blocks glycine-mediated inhibition, and has been indispensible in mapping the distribitution of GlycR in the CNS as the radiolabeled derivative. Strychnine and glycine recognise overlapping though conformationally distinct binding sites on the α subunits, however, the antagonist displays a high affinity for α1 but virtually none for α2 [125].



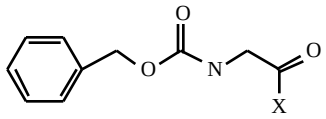
Strychnine



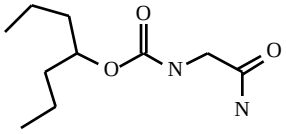
RU 5135

Fig. (11). Antagonists of Glycine receptors

Strychnine, like other GlycR antagonists such as the synthetic high-affinity but less specific steroid ligand RU5135 (Fig. (**11**)) is a potent convulsant in accordance with the proposed role of GlycR in sensorimotor function. Likewise, GlycR agonists are considered to exert anticonvulsant activity [125]. However, the range of agonistic compounds is limited to few substances. Beside glycine, some related amino acids such as β-alanine or taurine show agonistic activity at GlycR, but lack specificity and high affinity. The low number of potential agonists may be the consequence of steric restrictions at the recognition site, since, due to its size, glycine might interact via a tight binding pocket. Consequently, substitution of larger moieties, e.g. pyrazoles or aminohydroxyisoxazoles, to the glycine molecule results in a loss of GlycR agonistic action [126]. More recently, a number of glycinamide derivatives (Fig. (**12**)) have been shown to exert anticonvulsive action in animal tests [127, 128]. Of these compounds, *N*-valproyl glycinamide (TV 1901) is currently in phase II clinical trial [5]. However, these substances were not examined for their efficacy on GlycR or related receptor classes. It remains to be determined whether the anticonvulsant activity is mediated by an agonistic mechanism on GlycR. A wide spectrum of ligands recognises both GlycR and $GABA_AR$, e.g., the above mentioned RU5135, and tests in animal models do not necessarily discriminate between drug action on either the glycinergic or the GABAergic system. Thus, no anticonvulsive drug has been proven to selectively act via the GlycR.



X=OH Z-glycine
$X=NH_2$ Z-glycinamide



TV 1901 (N-valproyl glycinamide)

Fig. (12). Examples for anticonvulsive glycine/glycinamide derivatives.

In analogy to GABA uptake, glycine is removed from the synaptic cleft by means of a high-affinity, Na^+/Cl^- dependent carrier system. However, in contrast to the GAT-system, glycine uptake does not affect the time course of IPSPs [129], and thus is more likely to ensure the rapid resupply of the releasable pool than to terminate GlycR-mediated inhibition. Five glycine transporters have been identified in the mammalian CNS, which derive from two genes, glyT1 and glyT2 [130-133]. The transporter proteins vary in their tissue and cellular distribution, with GlyT1b being predominantly expressed in astroglial cells, whereas GlyT2a is found in glycinergic neurons [134]. Both subtypes differ in their mechanistic properties, as GlyT1b carries 2 Na^+ and 1 Cl^- per glycine molecule, whereas transport by GlyT2a requires a third sodium ion. Moreover, transporter-mediated non-vesicular release of glycine is differentially regulated in both carriers [135].

So far, no data are available covering the anticonvulsive properties of GlyT inhibitors. However, since uptake of glycine is not assumed to influence the time course of glycinergic inhibition, it is questionable if blocking the uptake carrier molecules will augment the effect of glycine on GlycR. In addition, inhibition of the transporters may lead to a glycine spillover to nearby glutamergic excitatory synapses and cause effects contrary to those desired in antiepileptic treatment.

FUTURE PERSPECTIVES AND CONCLUSIONS

It has been known for a long time that prolongation of inhibitory neurotransmitter action can suppress convulsive seizures and hence mitigate the symptoms of a vast range of epileptic disorders. During the last decade, the molecular basis of GABAergic and glycinergic inhibition in the CNS was deciphered by cloning the key proteins. This

information has been instrumental in attempts to explain the efficacy of established antiepileptic drugs and to design novel compounds with anticonvulsive potential. Prominent examples are the marketed drugs vigabatrin and tiagabine, which have been developed to some extent by rational strategy. Suitable expression systems for nearly all relevant recombinant enzymes, transporters and receptor subunits can now be employed, facilitating high-throughput screening of newly designed drugs or structural derivatives of known ligands. Furthermore, transgenic and knock-out strategies will equip us with animals, which are more applicable and predictive in preclinical drug evaluation than the presently used rodent models.

Beside progressing from known lead substances, future trends in the search for antiepileptic drugs with respect to the GABAergic system are likely to include the following:

1. Design of $GABA_AR$ ligands with higher specificity to distinct receptor subtypes then the presently known ligands. This trend is surely not restricted to the class of BZ receptor ligands, but may involve, e.g., the loreclezole and the neurosteroid binding site.

2. The possibility to reconstitute functional $GABA_BRs$ in cell culture systems should help to gain insight into the pharmacological properties of the native membrane protein and to design novel drugs that specifically target $GABA_BRs$.

3. A still recent line which opens up a number of chemical approaches is the screening for subtype-specific GAT inhibitors.

4. The development of drugs which interfere with the cellular machinery involved in filling and exocytosis of synaptic vesicles. A vesicular GABA and glycine transporter has recently been cloned [136, 137], however, pharmacological data are still missing.

5. Substances which increase GABA synthesis by stimulating GAD activity, however, such compounds are presently not in sight.

Whereas virtually every component of the GABAergic system represents a proper target for antiepileptic drug intervention, there is still a lack of evidence whether augmentation of glycinergic inhibition is appropriate in the treatment of epilepsy. Since glycine is mainly involved in sensorimotoric functions, agonists of GlycRs may, of course, exert anti*convulsive* effects, however, an anti-*seizure* potency of such substances is questionable. Still, drugs which enhance glycinergic inhibition may relieve some motoric symptoms of convulsive seizures.

Epilepsy is a disease with multiple etiologies, and excitatory as well as inhibitory neurons contribute to the symptoms. Most established antiepileptic drugs act by several cellular mechanisms, and this may be an explanation for their efficacy. Hence, it has been presumed that such drugs may be advantageous over selective substances [6]. Although broadly acting drugs are certainly more beneficial than compounds restricted to a specified target, the latter should exhibit less undesired side-effects. Moreover, specifically acting drugs are imperative in efforts toward an elucidation of the underlying molecular basis of different epileptic forms. In the future, an assortment of substances might enable us to prescribe for epileptics adjusted cocktails of specific drugs, thus achieving optimal therapeutic results by simultaneous actions at several distinct targets.

For this goal, it will be essential that physicians, pharmacologists, molecular biologists, and medicinal chemists collaborate in antiepileptic drug research in the third millenium. Since epilepsy is a multicausal and multifactorial disease, it requires a multidisciplinary task force to defeat it.

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LIST OF ABBREVIATIONS

BGT	=	Betaine/GABA transporter
BZ	=	Benzodiazepine
CNS	=	Central nervous system
GABA	=	γ-Aminobutyric acid
$GABA_AR$	=	$GABA_A$ receptor
$GABA_BR$	=	$GABA_B$ receptor
GABA-T	=	GABA aminotransferase
GAD/gad	=	Glutamate decarboxylase (protein/gene)
GAT	=	GABA transporter
GlycR	=	Glycine receptor
GlyT/glyT	=	Glycine transporter (protein/gene)
Kir	=	Inwardly rectifying K^+ channels
mIPSCs	=	Miniature inhibitory postsynaptic currents
PLP	=	Pyridoxalphosphate
PTZ	=	Pentylenetetrazole
TM	=	Transmembrane domains

REFERENCES

[1] Puranam, R.S.; McNamara, J.O. *Curr Opin Neurobiol* **1999**, *9*, 281.

[2] Feldman, R.; Meyer, J.; Quenzer, L. *Principles of Neuropsychopharmacology*, Sinauer Associates, Inc., **1997**.

[3] Olsen, R.W.; Avoli, M. *Epilepsia* **1997**, *38*, 399.

[4] Rho, J.M.; Sankar, R. *Epilepsia* **1999**, *40*, 1471.

[5] Bialer, M.; Johannessen, S.I.; Kupferberg, H.J.; Levy, R.H.; Loiseau, P.; Perucca, E. *Epilepsy Res.* **1999**, *34*, 1.

[6] Löscher, W. *Eur. J. Pharmacol.* **1998**, *342*, 1.

[7] Löscher, W. *Prog. Neurobiol.* **1999**, *58*, 31.

[8] Emilien, G.; Maloteaux, J.M. *Int. J. Clin. Pharmacol. Ther.* **1998**, *36*, 181.

[9] Waagepetersen, H.S.; Sonnewald, U.; Schousboe, A. *J. Neurochem.* **1999**, *73*, 1335.

[10] Christgau, S.; Schierbeck, H.; Aanstoot, H.J.; Aagaard, L.; Begley, K.; Kofod, H.; Hejnaes, K.; Baekkeskov, S. *J. Biol. Chem.* **1991**, *266*, 21257.

[11] Christgau, S.; Aanstoot, H.J.; Schierbeck, H.; Begley, K.; Tullin, S.; Hejnaes, K.; Baekkeskov, S. *J. Cell. Biol.* **1992**, *118*, 309.

[12] Hsu, C.-C.; Davis, K.M.; Jin, H.; Foos, T.; Floor, E.; Chen, W.; Tyburski, J.B.; Yang, C.-Y.; Schloss, J.V.; Wu, J.-Y. *J. Biol. Chem.* **2000**, M001403200.

[13] Kaufman, D.L.; Houser, C.R.; Tobin, A.J. *J. Neurochem.* **1991**, *56*, 720.

[14] Martin, D.L.; Rimvall, K. *J. Neurochem.* **1993**, *60*, 395.

[15] Cao, Y.; Wilcox, K.S.; Martin, C.E.; Rachinsky, T.L.; Eberwine, J.; Dichter, M.A. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9844.

[16] Schwarzer, C.; Sperk, G. *Neuroscience* **1995**, *69*, 705.

[17] Asada, H.; Kawamura, Y.; Maruyama, K.; Kume, H.; Ding, R.; Ji, F.Y.; Kanbara, N.; Kuzume, H.; Sanbo, M.; Yagi, T.; Obata, K. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 891.

[18] Asada, H.; Kawamura, Y.; Maruyama, K.; Kume, H.; Ding, R.G.; Kanbara, N.; Kuzume, H.; Sanbo, M.; Yagi, T.; Obata, K. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6496.

[19] Kash, S.F.; Johnson, R.S.; Tecott, L.H.; Noebels, J.L.; Mayfield, R.D.; Hanahan, D.; Baekkeskov, S. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14060.

[20] Tian, N.; Petersen, C.; Kash, S.; Baekkeskov, S.; Copenhagen, D.; Nicoll, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12911.

[21] Condie, B.G.; Bain, G.; Gottlieb, D.I.; Capecchi, M.R. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11451.

[22] Kelly, K.M. *Neuropsychobiology* **1998**, *38*, 139.

[23] Sasaki, K.; Hatta, S.; Haga, M.; Ohshika, H. *Eur. J. Pharmacol.* **1999**, *367*, 165.

[24] Arndt, C.F.; Derambure, P.; Defoort Dhellemmes, S.; Hache, J.C. *Neurology* **1999**, *52*, 1201.

[25] Leppik, I.E. *Epilepsia* **1998**, *39 Suppl 5*, 2.

[26] Qiu, J.; Silverman, R.B. *J. Med. Chem.* **2000**, *43*, 706.

[27] Silverman, R.B.; Roscher, C.L. *Bioorg. Med. Chem.* **1996**, *4*, 1521.

[28] Storici, P.; Capitani, G.; De Biase, D.; Moser, M.; John, R.A.; Jansonius, J.N.; Schirmer, T. *Biochemistry* **1999**, *38*, 8628.

[29] Barnard, E.A.; Skolnick, P.; Olsen, R.W.; Mohler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A.N.; Langer, S.Z. *Pharmacol. Rev.* **1998**, *50*, 291.

[30] Bonnert, T.P.; McKernan, R.M.; Farrar, S.; le Bourdelles, B.; Heavens, R.P.; Smith, D.W.; Hewson, L.; Rigby, M.R.; Sirinathsinghji, D.J.; Brown, N.; Wafford, K.A.; Whiting, P.J. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9891.

[31] Hevers, W.; Lüddens, H. *Mol. Neurobiol.* **1998**, *18*, 35.

[32] Otis, T.S.; De Koninck, Y.; Mody, I. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7698.

[33] Nusser, Z.; Hajos, N.; Somogyi, P.; Mody, I. *Nature* **1998**, *395*, 172.

[34] Hirsch, J.C.; Agassandian, C.; Merchan Perez, A.; Ben Ari, Y.; DeFelipe, J.; Esclapez, M.; Bernard, C. *Nat. Neurosci.* **1999**, *2*, 499.

[35] Brooks Kayal, A.R.; Shumate, M.D.; Jin, H.; Rikhter, T.Y.; Coulter, D.A. *Nat. Med.* **1998**, *4*, 1166.

[36] Gunther, U.; Benson, J.; Benke, D.; Fritschy, J.M.; Reyes, G.; Knoflach, F.; Crestani, F.; Aguzzi, A.; Arigoni, M.; Lang, Y.; *et al. Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7749.

[37] Mihalek, R.M.; Banerjee, P.K.; Korpi, E.R.; Quinlan, J.J.; Firestone, L.L.; Mi, Z.P.; Lagenaur, C.; Tretter, V.; Sieghart, W.; Anagnostaras, S.G.; Sage, J.R.; Fanselow, M.S.; Guidotti, A.; Spigelman, I.; Li, Z.; DeLorey, T.M.; Olsen, R.W.; Homanics, G.E. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12905.

[38] Homanics, G.E.; DeLorey, T.M.; Firestone, L.L.; Quinlan, J.J.; Handforth, A.; Harrison, N.L.; Krasowski, M.D.; Rick, C.E.; Korpi, E.R.; Makela, R.; Brilliant, M.H.; Hagiwara, N.; Ferguson, C.; Snyder, K.; Olsen, R.W. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4143.

[39] DeLorey, T.M.; Handforth, A.; Anagnostaras, S.G.; Homanics, G.E.; Minassian, B.A.; Asatourian, A.; Fanselow, M.S.; Delgado Escueta, A.; Ellison, G.D.; Olsen, R.W. *J. Neurosci.***1998**, *18*, 8505.

[40] Culiat, C.T.; Stubbs, L.J.; Montgomery, C.S.; Russell, L.B.; Rinchik, E.M. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2815.

[41] DeLorey, T.M.; Olsen, R.W. *Epilepsy Res.* **1999**, *36*, 123.

[42] Rudolph, U.; Crestani, F.; Benke, D.; Brunig, I.; Benson, J.A.; Fritschy, J.M.; Martin, J.R.; Bluethmann, H.; Mohler, H. *Nature* **1999**, *401*, 796.

[43] Carter, R.B.; Wood, P.L.; Wieland, S.; Hawkinson, J.E.; Belelli, D.; Lambert, J.J.; White, H.S.; Wolf, H.H.; Mirsadeghi, S.; Tahir, S.H.; Bolger, M.B.; Lan, N.C.; Gee, K.W. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 1284.

[44] Wafford, K.A.; Bain, C.J.; Quirk, K.; McKernan, R.M.; Wingrove, P.B.; Whiting, P.J.; Kemp, J.A. *Neuron* **1994**, *12*, 775.

[45] Bettler, B.; Kaupmann, K.; Bowery, N. *Curr. Opin. Neurobiol.* **1998**, *8*, 345.

[46] Kaupmann, K.; Schuler, V.; Mosbacher, J.; Bischoff, S.; Bittiger, H.; Heid, J.; Froestl, W.; Leonhard, S.; Pfaff, T.; Karschin, A.; Bettler, B. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14991.

[47] Kaupmann, K.; Huggel, K.; Heid, J.; Flor, P.J.; Bischoff, S.; Mickel, S.J.; McMaster, G.; Angst, C.; Bittiger, H.; Froestl, W.; Bettler, B. *Nature* **1997**, *386*, 239.

[48] Bischoff, S.; Leonhard, S.; Reymann, N.; Schuler, V.; Shigemoto, R.; Kaupmann, K.; Bettler, B. *J. Comp. Neurol.* **1999**, *412*, 1.

[49] Kaupmann, K.; Malitschek, B.; Schuler, V.; Heid, J.; Froestl, W.; Beck, P.; Mosbacher, J.; Bischoff, S.; Kulik, A.; Shigemoto, R.; Karschin, A.; Bettler, B. *Nature* **1998**, *396*, 683.

[50] Jones, K.A.; Borowsky, B.; Tamm, J.A.; Craig, D.A.; Durkin, M.M.; Dai, M.; Yao, W.J.; Johnson, M.; Gunwaldsen, C.; Huang, L.Y.; Tang, C.; Shen, Q.; Salon, J.A.; Morse, K.; Laz, T.; Smith, K.E.; Nagarathnam, D.; Noble, S.A.; Branchek, T.A.; Gerald, C. *Nature* **1998**, *396*, 674.

[51] White, J.H.; Wise, A.; Main, M.J.; Green, A.; Fraser, N.J.; Disney, G.H.; Barnes, A.A.; Emson, P.; Foord, S.M.; Marshall, F.H. *Nature* **1998**, *396*, 679.

[52] Kuner, R.; Kohr, G.; Grunewald, S.; Eisenhardt, G.; Bach, A.; Kornau, H.C. *Science* **1999**, *283*, 74.

[53] Ng, G.Y.; Clark, J.; Coulombe, N.; Ethier, N.; Hebert, T.E.; Sullivan, R.; Kargman, S.; Chateauneuf, A.; Tsukamoto, N.; McDonald, T.; Whiting, P.; Mezey, E.; Johnson, M.P.; Liu, Q.; Kolakowski, L.F., Jr.; Evans, J.F.; Bonner, T.I.; O'Neill, G.P. *J. Biol. Chem.* **1999**, *274*, 7607.

[54] Ng, G.Y.; McDonald, T.; Bonnert, T.; Rigby, M.; Heavens, R.; Whiting, P.; Chateauneuf, A.; Coulombe, N.; Kargman, S.; Caskey, T.; Evans, J.; O'Neill G, P.; Liu, Q. *Genomics* **1999**, *56*, 288.

[55] Marescaux, C.; Vergnes, M.; Liu, Z.; Depaulis, A.; Bernasconi, R. *Epilepsy Res. Suppl.* **1992**, *9*, 131.

[56] Snead, O.C.d. *Eur. J. Pharmacol.* **1992**, *213*, 343.

[57] Bernasconi, R.; Lauber, J.; Marescaux, C.; Vergnes, M.; Martin, P.; Rubio, V.; Leonhardt, T.; Reymann, N.; Bittiger, H. *J. Neural. Transm. Suppl.* **1992**, *35*, 155.

[58] Liu, Z.; Vergnes, M.; Depaulis, A.; Marescaux, C. *Neuroscience* **1992**, *48*, 87.

[59] Hosford, D.A.; Clark, S.; Cao, Z.; Wilson, W.A., Jr.; Lin, F.H.; Morrisett, R.A.; Huin, A. *Science* **1992**, *257*, 398.

[60] Marescaux, C.; Vergnes, M.; Bernasconi, R. *J. Neural. Transm. Suppl.* **1992**, *35*, 179.

[61] Vergnes, M.; Boehrer, A.; Simler, S.; Bernasconi, R.; Marescaux, C. *Eur. J. Pharmacol.* **1997**, *332*, 245.

[62] Snead, O.C.d. *J. Neural. Transm. Suppl.* **1992**, *35*, 7.

[63] Hosford, D.A. *Curr. Opin. Neurol.* **1995**, *8*, 121.

[64] Futatsugi, Y.; Riviello, J.J., Jr. *Brain Dev.* **1998**, *20*, 75.

[65] Fletcher, C.F.; Lutz, C.M.; O'Sullivan, T.N.; Shaughnessy, J.D., Jr.; Hawkes, R.; Frankel, W.N.; Copeland, N.G.; Jenkins, N.A. *Cell* **1996**, *87*, 607.

[66] Burgess, D.L.; Jones, J.M.; Meisler, M.H.; Noebels, J.L. *Cell* **1997**, *88*, 385.

[67] Letts, V.A.; Felix, R.; Biddlecome, G.H.; Arikkath, J.; Mahaffey, C.L.; Valenzuela, A.; Bartlett, F.S., 2nd; Mori, Y.; Campbell, K.P.; Frankel, W.N. *Nat. Genet.* **1998**, *19*, 340.

[68] Bittiger, H.; Froestl, W.; Mickel, S.; Olpe, H.R. *Trends Pharmacol. Sci.* **1993**, *14*, 391.

[69] Meza Toledo, S.E.; Martinez Munoz, D.; Carvajal Sandoval, G. *Arzneimittelforschung* **1998**, *48*, 1051.

[70] Meza Toledo, S.E.; Juarez Carvajal, E.; Carvajal Sandoval, G. *Arzneimittelforschung* **1998**, *48*, 797.

[71] Ong, J.; Marino, V.; Parker, D.A.; Kerr, D.I.; Blythin, D.J. *Eur. J. Pharmacol.* **1998**, *362*, 35.

[72] Ong, J.; Marino, V.; Parker, D.A.; Kerr, D.I.; Blythin, D.J. *Eur. J. Pharmacol.* **1999**, *369*, 33.

[73] Getova, D.; Froestl, W.; Bowery, N.G. *Brain Res.* **1998**, *809*, 182.

[74] Liu, Q.R.; Lopez Corcuera, B.; Mandiyan, S.; Nelson, H.; Nelson, N. *J. Biol. Chem.* **1993**, *268*, 2106.

[75] Yamauchi, A.; Uchida, S.; Kwon, H.M.; Preston, A.S.; Robey, R.B.; Garcia Perez, A.; Burg, M.B.; Handler, J.S. *J. Biol. Chem.* **1992**, *267*, 649.

[76] Guastella, J.; Nelson, N.; Nelson, H.; Czyzyk, L.; Keynan, S.; Miedel, M.C.; Davidson, N.; Lester, H.A.; Kanner, B.I. *Science* **1990**, *249*, 1303.

[77] Borden, L.A.; Smith, K.E.; Hartig, P.R.; Branchek, T.A.; Weinshank, R.L. *J. Biol. Chem.* **1992**, *267*, 21098.

[78] Clark, J.A.; Deutch, A.Y.; Gallipoli, P.Z.; Amara, S.G. *Neuron* **1992**, *9*, 337.

[79] Nelson, H.; Mandiyan, S.; Nelson, N. *FEBS Lett.* **1990**, *269*, 181.

[80] Borden, L.A.; Dhar, T.G.; Smith, K.E.; Branchek, T.A.; Gluchowski, C.; Weinshank, R.L. *Receptors Channels* **1994**, *2*, 207.

[81] Borden, L.A.; Smith, K.E.; Gustafson, E.L.; Branchek, T.A.; Weinshank, R.L. *J. Neurochem.* **1995**, *64*, 977.

[82] Nelson, N. *J. Neurochem.* **1998**, *71*, 1785.

[83] Bennett, E.R.; Kanner, B.I. *J. Biol. Chem.* **1997**, *272*, 1203.

[84] Clark, J.A. *J. Biol. Chem.* **1997**, *272*, 14695.

[85] Kavanaugh, M.P.; Arriza, J.L.; North, R.A.; Amara, S.G. *J. Biol. Chem.* **1992**, *267*, 22007.

[86] Mager, S.; Kleinberger Doron, N.; Keshet, G.I.; Davidson, N.; Kanner, B.I.; Lester, H.A. *J. Neurosci.***1996**, *16*, 5405.

[87] Pantanowitz, S.; Bendahan, A.; Kanner, B.I. *J. Biol. Chem.* **1993**, *268*, 3222.

[88] Kleinberger Doron, N.; Kanner, B.I. *J. Biol. Chem.* **1994**, *269*, 3063.

[89] Bismuth, Y.; Kavanaugh, M.P.; Kanner, B.I. *J. Biol. Chem.* **1997**, *272*, 16096.

[90] Tamura, S.; Nelson, H.; Tamura, A.; Nelson, N. *J. Biol. Chem.* **1995**, *270*, 28712.

[91] Matskevitch, I.; Wagner, C.A.; Stegen, C.; Broer, S.; Noll, B.; Risler, T.; Kwon, H.M.; Handler, J.S.; Waldegger, S.; Busch, A.E.; Lang, F. *J. Biol. Chem.* **1999**, *274*, 16709.

[92] Kanner, B.I.; Bendahan, A. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 2550.

[93] Minelli, A.; Brecha, N.C.; Karschin, C.; DeBiasi, S.; Conti, F. *J. Neurosci.***1995**, *15*, 7734.

[94] Conti, F.; Melone, M.; De Biasi, S.; Minelli, A.; Brecha, N.C.; Ducati, A. *J. Comp. Neurol.* **1998**, *396*, 51.

[95] Jursky, F.; Nelson, N. *J. Neurosci. Res.* **1999**, *55*, 394.

[96] Andersen, K.E.; Braestrup, C.; Gronwald, F.C.; Jorgensen, A.S.; Nielsen, E.B.; Sonnewald, U.; Sorensen, P.O.; Suzdak, P.D.; Knutsen, L.J. *J. Med. Chem.* **1993**, *36*, 1716.

[97] Ali, F.E.; Bondinell, W.E.; Dandridge, P.A.; Frazee, J.S.; Garvey, E.; Girard, G.R.; Kaiser, C.; Ku, T.W.; Lafferty, J.J.; Moonsammy, G.I.; *et al*. *J. Med. Chem.* **1985**, *28*, 653.

[98] Braestrup, C.; Nielsen, E.B.; Sonnewald, U.; Knutsen, L.J.; Andersen, K.E.; Jansen, J.A.; Frederiksen, K.; Andersen, P.H.; Mortensen, A.; Suzdak, P.D. *J. Neurochem.* **1990**, *54*, 639.

[99] Suzdak, P.D.; Frederiksen, K.; Andersen, K.E.; Sorensen, P.O.; Knutsen, L.J.; Nielsen, E.B. *Eur. J. Pharmacol.* **1992**, *224*, 189.

[100] Bjorge, S.; Black, A.; Bockbrader, H.; Chang, T.; Gregor, V.; Lobbestael, S.; Nugiel, D.; Pavia, M.; Radulovic, L.; Woolf, T. *Drug Dev. Res.* **1990**, 189.

[101] Borden, L.A.; Murali Dhar, T.G.; Smith, K.E.; Weinshank, R.L.; Branchek, T.A.; Gluchowski, C. *Eur. J. Pharmacol.* **1994**, *269*, 219.

[102] Adkins, J.C.; Noble, S. *Drugs* **1998**, *55*, 437.

[103] Leach, J.P.; Brodie, M.J. *Lancet* **1998**, *351*, 203.

[104] Uthman, B.M.; Rowan, A.J.; Ahmann, P.A.; Leppik, I.E.; Schachter, S.C.; Sommerville, K.W.; Shu, V. *Arch. Neurol.* **1998**, *55*, 56.

[105] Morimoto, K.; Sato, H.; Yamamoto, Y.; Watanabe, T.; Suwaki, H. *Epilepsia* **1997**, *38*, 966.

[106] Juhasz, G.; Kekesi, K.A.; Nyitrai, G.; Dobolyi, A.; Krogsgaard Larsen, P.; Schousboe, A. *Eur. J. Pharmacol.* **1997**, *331*, 139.

[107] Bolvig, T.; Larsson, O.M.; Pickering, D.S.; Nelson, N.; Falch, E.; Krogsgaard Larsen, P.; Schousboe, A. *Eur. J. Pharmacol.* **1999**, *375*, 367.

[108] Kehler, J.; Stensbol, T.B.; Krogsgaard Larsen, P. *Bioorg. Med. Chem. Lett* **1999**, *9*, 811.

[109] Dhar, T.G.; Borden, L.A.; Tyagarajan, S.; Smith, K.E.; Branchek, T.A.; Weinshank, R.L.; Gluchowski, C. *J. Med. Chem.* **1994**, *37*, 2334.

[110] Thomsen, C.; Sorensen, P.O.; Egebjerg, J. *Br. J. Pharmacol.* **1997**, *120*, 983.

[111] Andersen, K.E.; Sorensen, J.L.; Huusfeldt, P.O.; Knutsen, L.J.; Lau, J.; Lundt, B.F.; Petersen, H.; Suzdak, P.D.; Swedberg, M.D. *J. Med. Chem.* **1999**, *42*, 4281.

[112] Knutsen, L.J.S.; Andersen, K.E.; Lau, J.; Lundt, B.F.; Henry, R.F.; Morton, H.E.; Naerum, L.; Petersen, H.; Stephensen, H.; Suzdak, P.D.; Swedberg, M.D.B.; Thomsen, C.; Sorensen, P.O. *J. Med. Chem.* **1999**, *42*, 3447.

[113] Porter, T.G.; Martin, D.L. *J. Neurochem.* **1984**, *43*, 1464.

[114] Bernstein, E.M.; Quick, M.W. *J. Biol. Chem.* **1999**, *274*, 889.

[115] Scanziani, M. *Neuron* **2000**, *25*, 673.

[116] Betz, H. *Trends Neurosci.* **1991**, *14*, 458.

[117] Chen, S.; Hillman, D.E. *J. Neurocytol.* **1993**, *22*, 81.

[118] Triller, A.; Sur, C.; Korn, H. *J. Comp. Neurol.* **1993**, *338*, 83.

[119] Maxwell, D.J.; Todd, A.J.; Kerr, R. *Brain Res.* **1995**, *690*, 127.

[120] Bechade, C.; Sur, C.; Triller, A. *Bioessays* **1994**, *16*, 735.

[121] Grenningloh, G.; Pribilla, I.; Prior, P.; Multhaup, G.; Beyreuther, K.; Taleb, O.; Betz, H. *Neuron* **1990**, *4*, 963.

[122] Grenningloh, G.; Schmieden, V.; Schofield, P.R.; Seeburg, P.H.; Siddique, T.; Mohandas, T.K.; Becker, C.M.; Betz, H. *EMBO J.* **1990**, *9*, 771.

[123] Prior, P.; Schmitt, B.; Grenningloh, G.; Pribilla, I.; Multhaup, G.; Beyreuther, K.; Maulet, Y.; Werner, P.; Langosch, D.; Kirsch, J.; *et al*. *Neuron* **1992**, *8*, 1161.

[124] Kuhse, J.; Betz, H.; Kirsch, J. *Curr. Opin. Neurobiol.* **1995**, *5*, 318.

[125] Becker, C. In *Handbook of Experimental Pharmacology, Selective Neurotoxicity*, H. Herken and F. Hucho, Ed. Springer Verlag: Berlin Heidelberg, **1992**; 102, 539.

[126] Drummond, J.; Johnson, G.; Nickell, D.G.; Ortwine, D.F.; Bruns, R.F.; Welbaum, B. *J. Med. Chem.* **1989**, *32*, 2116.

[127] Sussan, S.; Dagan, A.; Blotnik, S.; Bialer, M. *Epilepsy Res.* **1999**, *34*, 207.

[128] Sussan, S.; Dagan, A.; Bialer, M. *Epilepsy Res.* **1999**, *33*, 11.

[129] Titmus, M.J.; Korn, H.; Faber, D.S. *J. Neurophysiol.* **1996**, *75*, 1738.

[130] Kim, K.M.; Kingsmore, S.F.; Han, H.; Yang Feng, T.L.; Godinot, N.; Seldin, M.F.; Caron, M.G.; Giros, B. *Mol. Pharmacol.* **1994**, *45*, 608.

[131] Adams, R.H.; Sato, K.; Shimada, S.; Tohyama, M.; Puschel, A.W.; Betz, H. *J. Neurosci.***1995**, *15*, 2524.

[132] Borowsky, B.; Hoffman, B.J. *J. Biol. Chem.* **1998**, *273*, 29077.

[133] Ponce, J.; Poyatos, I.; Aragon, C.; Gimenez, C.; Zafra, F. *Neurosci. Lett.* **1998**, *242*, 25.

[134] Jursky, F.; Tamura, S.; Tamura, A.; Mandiyan, S.; Nelson, H.; Nelson, N. *J. Exp. Biol.* **1994**, *196*, 283.

[135] Roux, M.J.; Supplisson, S. *Neuron* **2000**, *25*, 373.

[136] McIntire, S.L.; Reimer, R.J.; Schuske, K.; Edwards, R.H.; Jorgensen, E.M. *Nature* **1997**, *389*, 870.

[137] Sagne, C.; El Mestikawy, S.; Isambert, M.F.; Hamon, M.; Henry, J.P.; Giros, B.; Gasnier, B. *FEBS Lett.* **1997**, *417*, 177.