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Review

Research advances in basic mechanisms of seizures and antiepileptic drug action

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Abstract:
Epilepsy is a common neurological disease but the mechanism of seizure generation has been only partially unraveled. Furthermore, almost 30% of epileptic patients are resistant to pharmacological treatment. Therefore, elucidation of the basic mechanism of seizures and search for new antiepileptics in order to treat the drug-resistant form of epilepsy and to improve the efficacy of current therapies seem justified. The aim of this overview is a brief presentation of some new concepts and research directions in pathogenesis and pharmacotherapy of epilepsy. Development of ideas on the mechanisms of seizures and antiepileptic drugs reflects the progress in our understanding of the central nervous system physiology, particularly of neurotransmission. Hyperactivity of excitatory amino acid systems, insufficient $GABA_A$ receptor-mediated neurotransmission, and disturbances in intrinsic properties of neuronal membranes are still regarded as the most important mechanisms of seizures. New data add to the complexity of GABA-glutamate interaction showing both excitatory and inhibitory role of GABA and glutamatergic neurons in the central nervous system. Moreover, besides synaptic NMDA and $GABA_A$ receptors, also extrasynaptic receptors for the amino acid transmitters have been recently implicated in the pathomechanism of epilepsy. Changes in expression, polymorphisms, lost- or gain-function mutations as well as cellular energetic imbalance can contribute to the disturbed function of the ligand- and voltage-dependent sodium, potassium, chloride and calcium channels, resulting in epileptiform activity. Voltage-dependent sodium and calcium channel blockers, and GABA mimetics are the most clinically useful groups of antiepileptic drugs and the newest research in this field is focused on more selective and subtle regulations of their molecular targets. Of interest is an emerging role of extrasynaptic $GABA_A$ receptors, various kinds of potassium ion channels, hyperpolarization-activated cyclic nucleotide gated (HCN) channels, acid-sensing ion channels, and gap junctions in the regulation of neuronal excitability and seizures. Iono- and metabotropic glutamate receptors used to be viewed as an attractive target for new anticonvulsants, however, opinions are now less enthusiastic, since their competitive and non-competitive antagonists possess undesired side effects. Positive or negative allosteric modulators of glutamate receptors with fewer side-effects can be more promising. The introduction of new compounds acting through novel pharmacological mechanisms gives hope that the proportion of patients with uncontrolled epilepsy will substantially decrease. However, this may be possible if molecular background of the pharmacoresistance in epilepsy is deciphered.

Key words:
seizures, epilepsy, antiepileptic drugs, molecular mechanisms

Introduction

Epilepsy is regarded as a multifactor and symptomatologically highly diversified neurological disorder characterized by recurrent unprovoked seizures. Physiological studies show that seizures reflect transient, abnormal and synchronous hyperactivity of a neuronal population in the brain. Such brain dysfunction can be accompanied by motor, sensory and autonomic disturbances depending on brain region involved in the origin and/or spread of seizures. The relationships between cortical EEG, and extracellular and intracellular recordings from the focus of seizures evoked by local administration of a convulsant to the mammalian cortex was described by Ayala et al. [6]. The simplest form of epileptic activity which can be recorded in cortical or hippocampal neurons is the interictal spike, a synchronized burst of action potentials evoked by recurrent excitation. The depolarization underlying the interictal spike named a paroxysmal depolarization shift is followed by a prolonged potassium current-dependent after-hyperpolarization. The cellular mechanisms of seizure generation involve rhythmic or tonic "runaway" excitation or the synchronized and rhythmic interplay between excitatory and inhibitory neurons and membrane conductances [76]. Seizures are classified into partial (simple or complex) and generalized categories including absence, tonic, clonic, tonic-clonic, myoclonic, and febrile seizures. It has been firmly accepted that seizures can be generated in response to a loss of balance between excitatory and inhibitory influences which results in tonic depolarizations or repetitive, rhythmic burst discharges [76]. Experimentally, epileptiform burst discharges can be evoked in neuronal cultures by removing extracellular magnesium [80], increase in extracellular potassium concentration [118], inhibition of the sodium pump [8] or antagonizing $GABA_A$ receptors [45]. The most common animal models of seizures comprise chemically induced seizures (pentylenetetrazole, kainic acid, pilocarpine), maximal electroshock and electrical or chemical kindling. Neurochemical approach strongly supports the electrophysiological findings that seizures can be generated from excessively enhanced excitatory processes in a given population of neuronal cells in the brain or from hypoactivity of neuronal inhibition. The former mechanism comprises hyperactivity of glutamatergic transmission and functional disturbances of the ligand- or voltage-gated sodium and calcium channels. Deficiency in the inhibitory processes is linked mainly with insufficient $GABA_A$ receptor-mediated neurotransmission and extracellular potassium currents. Changes in expression, polymorphisms, lost- or gain-function mutation of the ligand- and voltage-dependent sodium, potassium, chloride and calcium channels as well as cellular energetic imbalance and inflammatory processes can contribute to neuronal hiperexcitability and seizures [3, 37, 77, 116, 125].

The GABAergic system

The γ-aminobutyric acid (GABA) since long has been considered to be the principal inhibitory neurotransmitter in the mammalian brain. Enhancers of GABAergic transmission comprise a large group of classical and new generation antiepileptic drugs [30]. It has been firmly established that GABA is produced by intraneuronal decarboxylation of glutamate, and after being released, its intrasynaptic action is quickly terminated by glia and presynaptic neuronal uptake involving the high-affinity GABA transporters. There are four GABA transporters expressed in the human brain that are named GAT-1, GAT-2, GAT-3 and betaine-GABA transporter BGT1. The glial and neuronal GAT-1 received particular attention, as besides the GAT-3, Gat-1 predominantly participates in GABA uptake and is the target for the efficient antiepileptic drug tiagabine [110]. The GABA uptake by GAT-1 was also shown to be compromised in the genetic model of absence epilepsy [27], however, in the light of differential involvement of GABA in the regulation of convulsive and nonconvulsive seizures, significance of this phenomenon remains uncertain. There is a growing interest in amino acid transporters located extrasynaptically such as BGT1, as the inhibitor of both GAT-1 and BGT1 showed anticonvulsant effects in animal models of epilepsy [69, 110]. GABA is intracellularly catabolized by GABA transaminase and the inhibition of this enzyme markedly increases the GABA level in the brain tissue, which correlates with an elevated seizure threshold. The antiepileptic drug vigabatrin is an irreversible GABA transaminase inhibitor, but the alternative mechanism of action of this drug was proposed to involve reverse transport of GABA by GAT-1 and GAT-3 transporters [83]. Be-

sides the impressive progress in knowledge on GABA production and metabolism in the central nervous system, large body of evidence has been recently accumulated on the role of GABA receptors in the mechanism of seizures and in the action of antiepileptic drugs. GABA exerts its action *via* ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors. The pentameric $GABA_A$ receptor complex is composed of two α and two β subunits and one γ or δ subunit which form ligand-gated chloride channel and depending on the subunit composition of the receptor, it mediates tonic or phasic inhibition [83]. The $GABA_A$ receptor configuration also determines the affinity of its agonists, antagonists and allosteric modulators. Activation of $GABA_A$ receptor in the brain generates fast inhibitory postsynaptic potentials (IPSP) and the key role of this receptor complex in prevention and inhibition of seizures (except for the absence nonconvulsive seizures) is commonly accepted [32]. The antagonists of this receptor (e.g., bicuculline, picrotoxin, or pentetrazole) belong to the most potent convulsants, whereas $GABA_A$ receptor agonists or positive modulators (benzodiazepines, barbiturates, some neurosteroids) inhibit seizures in both experimental animals and in humans. Like deficit in GABA synthesis or loss of GABA interneurons, also some changes in expression or mutations of the $GABA_A$ receptor subunits can lead to pathological neuronal discharges. Indeed, impairment of GABAergic transmission is likely to contribute to seizure susceptibility observed in several genetic and acquired animal models of epilepsy [91, 120]. The maintaining of the proper brain excitability level appears to depend on the balance between synaptic excitation and inhibition. Although in the mammalian cortex probably only 10–20% of neurons synthesize GABA and ca. 17% of synapses releases this transmitter, the GABA interneurons *via* $GABA_A$ receptors efficiently control excitatory amino acid transmission in the cortex. The functional balance between inhibitory and excitatory neurotransmission can be attributed to the ability of a single GABA interneuron to inhibit many glutamatergic cells (divergence) and to converge a number of GABA interneurons on the same glutamatergic neuron in local cortical circuits. However, the relationship between GABAergic and glutamatergic neurotransmission can be more complex because the releasable pool of both GABA and glutamate can be co-localized in the same neurons. Moreover, during early stages of CNS development in contrast to adult brain, GABA depolarizes cells, which is likely to be due to a high intracellular chloride level as compared with adult tissue [10]. The ability of GABA to hyperpolarize neurons probably depends on higher expression of the chloride-extruding transporter KCC2 in late stages of brain development and decrease in expression of NKCC1 which transports chloride into cells [34, 102]. Interestingly, NKCC1 expression is elevated in patients with hippocampal sclerosis and cortical dysplasia suggesting its role in the pathogenesis of refractory human epilepsy [113]. The widely accepted view on the role of GABA in mediating predominantly inhibitory processes in the central nervous system has been challenged by some data from experimental studies. In particular, it has been reported that initiation and maintenance of epileptiform synchronization in the hippocampal-entorhinal cortex slices perfused by artificial cerebrospinal fluid devoid of magnesium ions depends on $GABA_A$ receptor activity [4]. The ability of $GABA_A$ receptor-mediated mechanism to synchronize neuronal networks connecting the hippocampus, the entorhinal and perirhinal cortices, or the amygdala, may play a role in generating the recurrent limbic seizures which are characteristic of temporal lobe epilepsy [5]. Taking as an example the status epilepticus-induced reorganization of GABAergic/glutamatergic network in the hippocampus, Ben-Ari has postulated that the increase in the glutamatergic drive and the loss of the GABA-mediated inhibitory drive in the dendrites of the principal cells in the hippocampus accompanied by a persistent increase in intracellular chloride concentration contribute to seizure generation. The author stressed that seizure propagation to the other hippocampus depended on high-frequency oscillation which required active NMDA and $GABA_A$ receptors [10]. The switch $GABA_A$ receptor mode of action from inhibitory to excitatory during seizure activity, that might account for intractability of some epilepsies, was also reported by other investigators [48]. Interestingly, experiments on brain tissue from patients with mesial temporal lobe epilepsy demonstrated that only the subicular pyramidal cells which lacked expression of the potassium-chloride cotransporter KCC2, revealed $GABA_A$ receptor-mediated depolarizing postsynaptic events, further confirming the significance of disturbance in chloride ion homeostasis in the pathomechanism of epilepsy [47]. Disequilibrium of the chloride distribution was observed in some patients with intractable epilepsy [44] and disturbance in chloride ion homeo-

stasis and GABAergic transmission facilitate epileptic discharges [46]. Results from animal models and human studies indicate that changed expression of $GABA_A$ receptor subunits may contribute to the pathomechanism of intractable epilepsy [19, 63]. Laschet et al. [56] found that maintaining the $GABA_A$ receptor in a phosphorylated state was necessary to sustain efficient GABAergic inhibition in the epileptogenic cortex. These authors suggested the existence of a functional link between the regional cerebral glucose hypometabolism in patients with partial epilepsy and dysfunction of GABAergic mechanism due to deficiency in $GABA_A$ receptor phosphorylation. This is an interesting hypothesis, especially if one takes into account that the majority of antiepileptic drugs decrease metabolic processes in the brain tissue. Of great interest is an emerging role of extrasynaptic $GABA_A$ receptors in the regulation of neuronal excitability and seizures. Using the pilocarpine model of temporal lobe epilepsy in mice, Zhang et al. showed that surprisingly, tonic inhibition in dentate granule cells was observed in epileptic mice and that these animals had altered expression of $GABA_A$ receptor subunits on dentate granule cell dendrites and the γ2 subunit was shifted from synaptic to perisynaptic location [140]. It is now well established that there are not only synaptic, but also extrasynaptic high affinity $GABA_A$ receptors differentially distributed in the brain. The latter receptors which are activated by extrasynaptic GABA and some endogenous modulators are responsible for persistent tonic inhibition and are likely to control susceptibility to seizures [127]. Indeed, it has been demonstrated in some models of seizures that the tonic inhibition is maintained in epileptic tissue, in contrast to defective function of synaptic $GABA_A$ receptors and diminished synaptic inhibition [92]. It has been also suggested that the decrease in neocortical phasic GABA inhibition and enhancement of tonic inhibition in the thalamo-cortical circuits are likely to be involved in the pathomechanism of absence epilepsy as demonstrated in both pharmacological and genetic models of this disorder [29]. In addition, selective activation of extrasynaptic $GABA_A$ receptors in the thalamus was sufficient to induce absence seizures in non-epileptic animals [27]. It is believed that the extrasynaptic $GABA_A$ receptors, which are sensitive to ambient concentration of extracellular GABA can be promising targets for antiepileptic, antiparkinsonian, antipsychotic and procognitive drugs [18].

In contrast to the well-recognized role of ionotropic $GABA_A$ receptor in the pathomechanism of seizures, less consistent data have been obtained on engagement of metabotropic $GABA_B$ receptor in the regulation of brain excitability. $GABA_B$ receptors mediate slow inhibition and are localized presynaptically, thus, they can inhibit release of both excitatory and inhibitory neurotransmitters [53]. Agonists of $GABA_B$ receptors show pro- or anticonvulsant effect in pentetrazole-induced seizures in immature animals [71] and inhibit cocaine-induced seizures [41]. Agonists and antagonists of $GABA_B$ promote and suppress generalized absence seizures in genetic animal models of this disorder, respectively. Peripherally administered GABA enhancers in a majority of cases suppress convulsive seizures in both animals and humans, but promote the occurrence of spike and wave discharges in thalamo-cortical circuits that are typical of generalized absence epilepsy [22, 32]. The GABA receptor-mediated hyperpolarization of relay nuclei of the thalamus de-inactivates low-threshold calcium currents and facilitates rhythmic activity in thalamo-cortical circuit [32].

The glutamatergic system

Glutamic acid is the main excitatory amino acid neurotransmitter in the central nervous system. It activates both ionotropic receptors named by their agonists NMDA (N-methyl-D-aspartic acid), AMPA (α-amino-3-hydroxy-5-methyl-isoxazolopropionic acid), and kainate receptors, as well as metabotropic glutamate receptors, which *via* G-proteins affect intracellular processes and ion channel activities [123]. Ionotropic glutamate receptor agonists show potent proconvulsant properties and are often used in experimental models of seizures.

The NMDA receptors are tetramers, composed of NR1 subunit in various alternative splicing forms and NR2A – NR2D subunits. The subunit composition determines ligand affinity, conductance for Ca^{2+} ions and kinetics of NMDA-gated ion channel [89]. The NMDA receptor activation requires simultaneous binding of glutamate and glycine/D-serine and AMPA receptor-mediated initial depolarization for removing magnesium ions which block NMDA receptors at resting membrane potentials. Glutamate binds with

a higher affinity to NMDA receptors than to AMPA receptors and the NMDA-gated channel remains open even for a couple of seconds after agonist binding have taken place. Also the conductance of NMDA receptors for calcium ions is higher than that of other cation-conducting ionotropic receptors. Depending on the concentration of calcium ions entering the cells determining frequency of synaptic stimulation, one can observe activation of protein kinases (when the intracellular calcium level is low) or phosphatases (when calcium level is high). These phenomena may be responsible for processes of synaptic potentiation or depression *via* the regulation of AMPA receptor phosphorylation. The NMDA receptor activity is regulated by a number of agents, such as polyamines, zinc ions, nitric oxide or steroid hormones. Experimental data provided a strong evidence for the key role of NMDA receptors in neuronal plasticity, but also in seizures and neuronal damage. In experimental animals NMDA receptor antagonists, e.g., dizocilpine or ketamine, inhibit seizures evoked by pentetrazole, pilocarpine, maximal electroshock or sensory stimulation. These NMDA antagonists appear to delay development of kindling but have a weaker effect on the fully developed kindling [61]. Unfortunately, both competitive and non-competitive NMDA receptor antagonists showed in experimental and clinical studies serious undesired effects, such as motor coordination and memory impairment and disorientation. Moreover, these unwanted symptoms are aggravated in animal models of epilepsy. Therefore, some allosteric modulators of NMDA receptors, especially those which interact with glycine and polyamine binding sites seemed more promising as potential antiepileptic drugs [86]. Antagonists of glycine binding sites show affinity for NR1/NR2A subunits, whereas ligands of polyamine site bind to NR1A/NR2B subunit complexes [42]. The anticonvulsant effect of the partial agonist of the glycine-binding site, D-cycloserine, probably involves desensitization of the NMDA receptor. Moreover, D-cycloserine potentiates anticonvulsant effects of some antiepileptic drugs and in low doses has a positive influence on memory processes [134, 135]. The new generation antiepileptic drug, harcoseride is an antagonist of the glycine binding site on NMDA receptor complex. Also felbamate, besides blocking voltage-dependent sodium channels and enhancing GABAergic transmission, showed antagonistic activity at glycine binding site in the NMDA receptor. It is noteworthy that also concomitant administration of glycine and a polyamine site antagonists had beneficial effects in experimental seizure models. It should also be mentioned here that through the activation of GABAergic interneurons, NMDA receptors can suppress circuit level neural activity, especially the limbic circuits [38]. Epileptic seizures are associated with a drop of brain tissue pH, which should lead to proton inhibition of NMDA receptor activity. On the other hand, inhibitors of acid-sensing ion channels decrease seizure-like bursting in hippocampal slices suggesting a new target for antiepileptic therapy [137].

In contrast to the well-recognized involvement of synaptic NMDA receptors in the regulation of brain excitability, less is known about functional role of extrasynaptic NMDA receptors in pathomechanism of seizures. These extrasynaptic receptors, which can be activated by excitatory amino acid synaptic spillover, have different subunit composition and couple with distinct signaling pathways than the synaptic sites [59]. It has been postulated that extrasynaptic NMDA receptor activation may lead to neuronal damage [59, 95], and in this way may contribute to development of seizures.

The AMPA receptor which is responsible for generation of fast excitatory postsynaptic potentials (EPSP) in the central nervous system forms channels permeable to sodium, potassium and, with some limitation, calcium ions. In the presence of an agonist, the AMPA receptor desensitizes and the desensitization is inhibited by positive allosteric modulators of this receptor. Negative modulators of AMPA receptors, e.g., 2,3-benzodiazepines were also synthesized. The AMPA receptor is composed of four homologous proteins GluR1–4 (or GluRA–D) in various configurations. Each of these proteins can exist in two alternative-splice variants, named "flip" and "flop". The presence of GluR2 subunit in AMPA receptor complex determines low permeability to calcium ions. Those AMPA receptors that do not possess GluR2 show a two-fold higher permeability to calcium ions than to sodium ones. The AMPA receptor is phosphorylated by several protein kinases, and this process is often initiated by NMDA receptor-dependent intracellular flow of calcium ions. Phosphorylation of AMPA receptor further enhances ion flow and can lead to pathological hyperactivity of glutamatergic transmission and epileptic discharges. Contrariwise, calcineurin-induced dephosphorylation of AMPA receptor decreases its activity.

AMPA and kainate receptor antagonists showed anticonvulsant effects in genetic and chemical models

of seizures. Their adverse effects on memory and motor coordination in experimental animals are less profound than those evoked by the NMDA receptor antagonists [105, 107]. Moreover, the AMPA and kainate receptor antagonists enhance efficacy of classical antiepileptic drugs and their interactions with phenobarbital and valproate appear to be especially positive. Among antiepileptic drugs, barbiturates and topiramate showed ability to block the AMPA receptors [67, 108]. Also another potential antiepileptic drug, talampanel, is a noncompetitive AMPA receptor antagonist with broad spectrum of anticonvulsant activity. Kainate receptors are several times less abundant in the brain tissue than AMPA receptors, but their widespread distribution suggests that they are present in the majority of neuronal cells. Cloning studies showed, that kainate receptors could be composed of GluR5, GluR6, GluR7 and KA1 and KA2 subunits. The GluR5 and GluR6 can form homomeric channels, whereas other subunits are a part of heteromeric combinations with the GluR5 and GluR6 proteins. Long-lasting depolarization of neurons by postsynaptically localized kainate receptors leads to intracellular flow of calcium ions *via* the voltage-dependent calcium channels, and this effect may be of significance in synaptic plasticity, but also in seizurogenic and neurotoxic effects of kainate [14, 126]. The well-established convulsant and neurotoxic effects of kainate can be also associated with presynaptic inhibition of GABA release. This assumption is supported by observation that kainate diminishes GABA-dependent inhibitory synaptic potentials in hippocampal CA1 region. Another interesting hypothesis is that presynaptic kainate receptors are involved in enhancement of glutamate release, as the activation of these receptors increases the concentration of calcium ions in hippocampal synaptosomes. The above-mentioned facts suggest that some selective modulators of kainate receptors can be considered as potential antiepileptic drugs.

The family of metabotropic glutamate receptors comprises 8 subtypes of receptors, mGluR1 – mGluR8, which were divided into 3 groups on the basis of differences in amino acid sequence, pharmacological characteristic and intracellular signaling pathways. The activation of mGluR belonging to Group I (mGluR1 and mGluR5) stimulates phosphatidylinositol (IP3) synthesis and release of intracellular calcium, whereas the stimulation Group II (mGluR2 and mGluR3) and group III (mGluR4, mGlu6-8) mGluRs inhibits adenylate cyclase [25]. The group I mGluRs are localized postsynaptically on both glutamatergic hippocampal and cortical cells and GABAergic interneurons. Their activation leads to phosphorylation and inactivation of several types of potassium channels, which leads to depolarization of neuronal cells. The group III mGluRs are presynaptic inhibitory autoreceptors localized on glutamatergic cell endings and presynaptic inhibitory heteroreceptors on some GABAergic neuronal cells.

Pharmacological studies show that agonists of group I mGluR induce seizures in experimental animals and prolong duration of interictal and ictal discharges in hippocampoal slices, whereas antagonists of these receptors suppress seizures. In contrast, the stimulation of group II and III mGluRs prevents seizures. Furthermore, the group II mGluR agonist LY354740 significantly enhanced anticonvulsant effect of diazepam while the mGluR5 antagonist MPEP showed a positive interaction with some conventional antiepileptic drugs in the amygdala-kindling model of complex partial seizures [16, 52]. Metabotropic glutamate receptors can be also involved in the regulation of non-convulsive seizures because ligands of these receptors suppress seizure activity by the selective reduction of hyperactive glutamatergic synaptic communication within the cortex and thalamus [2, 85]. Therefore, group I mGluR antagonists and group II and III mGluR agonists can be regarded as potential antiepileptic and neuroprotective drugs, provided that they lack serious undesired effects [72, 81].

The process of glutamate release and uptake in brain tissue engages several membrane transporters. There are 5 glutamic acid transporters (GLAST, GLT-1, EAAC1, EA4 and EAAT5) which are localized in membranes of astrocytes and neurons. The synthesis and activity of glutamate transporters is regulated by the concentration of glutamic acid, as the main signal, whereas inactivation of these proteins results in seizures and neurotoxicity [122, 123, 130].

A decrease in hyperactivity of glutamatergic transmission in order to inhibit seizures can be achieved *via*: 1) inhibition of glutamic acid synthesis using azaserine or glutaminase inhibitors, e.g., 6-diazo-5-oxy-1-norleucine. Among antiepileptic drugs, gabapentin attenuates glutamic acid synthesis through the inhibition of BCAA aminotransferase, whereas vigabatrin decreases both glutamic acid and D-aspartic acid levels in brain tissue. Also valproate reduces the concentration of the excitatory amino acid, aspartate in nerve terminals [82];

2) decrease in synaptic release of glutamic acid through modulation of its presynaptic receptors or calcium-dependent neurotransmiter release. Lamotrigine is an antiepileptic drug which, besides blocking voltage-dependent sodium channels, inhibits in micromolar concentrations glutamic acid and D-aspartic acid release;
3) enhancement of glutamate re-uptake;
4) attenuation of postsynaptic glutamate effects using antagonists of ionotropic and group I metabotropic glutamate receptors, which induce desensitization or decrease density of glutamate receptors. Among antiepileptic drugs which in their mechanism of action show antagonistic component at glutamate receptors, one can mention felbamate, topiramate and phenobarbital.

Some data suggest that when analyzing glutamate role in seizure phenomena, one should take into account the complex and only partly recognized interactions between glutamatergic neurons, glia and extracellular matrix [60, 62]. In fact, an interesting hypothesis was formulated suggesting that epileptic discharges resulted from pathological high-frequency calcium waves produced by astrocyte syncytia, which led to synchronization of neuronal discharges. These processes are accompanied by an augmented release of excitatory neurotransmitters which further facilitate generation of calcium waves in astrocytes [23, 58]. Adenosine is an endogenous anticonvulsive and neuroprotective agent of glial origin which is regarded as a promising target for antiepileptic therapy [15, 33, 60]. Gap junctions responsible for electrotonic coupling between neurons and astrocytes and synchronization of epileptoidal discharges are also considered as a potential target for future antiepileptic drugs. The role of the extracellular matrix in the mechanism of seizures has been only recently taken into account [66, 138]. Correspondingly, metalloproteinase-9 inhibition was shown to attenuate experimental epileptogenesis [133], but these results have to be supported by further studies.

The voltage-gated sodium channels

Voltage-gated sodium channels are integral membrane glycoproteins composed of an α-subunit that is associated with β-subunits [24, 109]. The α-subunit contains four multispanning homologous domains that form the central pore of the channel. The loop between segments 5 and 6 of each domain forms the ion selectivity filter [24, 139]. The α-subunits NaV1.1, NaV1.2, NaV1.3, and NaV1.6 are predominantly expressed in the central nervous system. The β-subunits consist of one N-terminal domain, one transmembrane domain, and one C-terminal domain. The β-subunits can regulate the expression of VGSC, increasing sodium channel density and neuronal excitability [90]. The voltage-dependent sodium channels play the key role in generating both normal action potentials and seizures [54]. Epileptic seizures are associated with modest sustained depolarization below the inactivation threshold for action potential generating sodium channels. The depolarization enables synchronous, high-frequency neuronal firing, the electrocorticographic correlate of which comprises ictal epileptic field potentials. Antagonists of voltage-dependent sodium channels decrease the maximal amplitude of sodium current and prolong the time of the channel inactivation. In this manner they decrease availability of sodium channels during high-frequency epileptic discharges [108]. Inactivation refers to depolarization-induced conformational changes from an open-channel to its non-conducting state. Usually, the isoforms Nav1.1–Nav1.4, Nav1.6, and Nav1.7 have fast inactivation kinetics, whereas Nav1.5, Nav1.8, and Nav1.9 have slower inactivation [109]. Most of the traditional antiepileptic drugs which are sodium channel antagonists stabilize mainly the fast inactivation of the voltage-gated sodium channels, whereas lacosamide acts on the process of slow inactivation. Thus, phenytoin carbamazepine, lamotrigine, felbamate, topiramate, oxcarbazepine, zonisamide, rufinamide, eslicarbazepine acetate, and probably gabapentin, and sodium valproate bind to the fast-inactivated state of the voltage-gated sodium channel and produce a transient, voltage- and frequency-dependent reduction in channel conductance [28, 77]. A new generation antiepileptic drug, lacosamide binds to the slow-inactivated state of the voltage-gated sodium channel and evokes a long-lasting, voltage- and frequency-dependent decrease in channel conductance [9, 119].

The voltage-gated calcium channels

Voltage gated calcium channels are heteromeric complexes with the pore-forming α1 subunit and acces-

sory subunits. Their molecular structure and mechanism of inactivation are similar to the voltage-gated sodium channels. The neuronal voltage-gated calcium channels are divided into L-, P/Q-, N- and T-type channels. The L-type calcium channels are predominantly localized postsynaptically and belong to the slowly inactivating category. They mediate sustained calcium ion influx in postsynaptic neurons, which further augments neuronal depolarization. L-type calcium channel antagonists show antiepileptogenic properties but they can aggravate absence epilepsy [55, 124]. N-type and P/Q-type channels are expressed presynaptically and are involved in neurotransmitter exocytosis. The T-type channels open with slight depolarization, and are quickly inactivated. The low-threshold Ca^{2+} current regulates oscillatory activity of thalamic neurons (pacemaker) and probably participates in generating generalized absence seizures characterized by 3 Hz spike-wave discharges in the EEG recordings. Indeed, polymorphisms or mutations in the CACNA1H gene coding for the voltage gated T-type Ca^{2+} channel can be associated with pathogenesis of some generalized epilepsies, e.g., the childhood absence epilepsy [1]. Voltage-gated calcium channels can be divided into low-voltage-activated channels and high-voltage-activated channels. The low-voltage-activated (T-type) calcium channel blockers, such as ethosuximide, zonisamide, and probably sodium valproate prevent synchronized depolarization of neurons, particularly in the thalamocortical circuits. The high-voltage-activated calcium channel blockers, such as lamotrigine (N-type, P/Q-type) felbamate, gabapentin ($\alpha_2\beta$-1 subunit), topiramate, levetiracetam (N-type), pregabalin ($\alpha_2\beta$-1 subunit), like phenobarbital, prevent neurotransmitter release (N- and P/Q-type) and post-synaptic depolarization (L-type) [74, 77].

The voltage-dependent potassium channel

Potassium channels form the largest and the most diverse family of ion channels involved in the regulation of a variety of physiological functions. The subunits of potassium channels are encoded in humans by 78 genes, however, their alternative splicing and heteromerization add to the complexity of the functional protein complexes. Taking into account the structure and mode of activation, potassium channels can be classified in four big subfamilies, i.e., inwardly rectifying K^+ channels (K_{ir}), two-pore K^+ channels (K_{2P}), calcium-activated K^+ channels (K_{Ca}) and voltage-gated channels (Kv) [131, 136]. Presently, 12 subfamilies of voltage-gated potassium channels Kv1–Kv12 with various homology and ability to form tetramers and encoded by 40 genes in humans have been identified [50]. The subfamily of voltage-dependent potassium channels KCNQ (Kv7) comprises: KCNQ1/KCNE1 responsible for action potential repolarization in the cardiac muscle, KCNQ2/3 underlying M-current, which controls resting membrane potentials and frequency of action potentials in neurons, KCNQ4 expressed in the inner ear and the KCNQ5 which forms complexes with the KCNQ3 [111]. Functional significance of the various voltage-dependent potassium channels is supported by genetic studies. The mutation of KCNQ1/KCNE1 genes results in the long QT syndrome (LQTS), mutation of KCNQ2/3 is associated with the benign familial neonatal convulsions (BFNC), whereas mutation of KCNQ4 is linked with the congenital deafness [70]. The KCNQ2 and KCNQ3 genes encode proteins which are important regulators of neuronal membrane excitability and, generally, the activation of Kv7 channels inhibits depolarization of neuronal cells [21, 78, 79]. The KCNQ (Kv7) channels regulate the response of neurons to excitation and form a potent inhibitory mechanism in the brain. In 2000, it was discovered that KCNQ potassium channels were a molecular target for retigabine [78]. Thus, retigabine was the first drug that was recognized as a selective activator of neuronal KCNQ2-5 channels, but devoid of affinity for KCNQ1 channels in the cardiac muscle. The KCNQ2/3 potassium channels are key players in the mechanism of inhibition of neuronal excitability so, one can postulate that retigabine influences one of the most fundamental mechanisms of epilepsy [106]. It has been pointed out that even a slight reduction in KCNQ2/KCNQ3 function can be epileptogenic and mutations in either KCNQ2 or KCNQ3 cause BFNC epilepsy [12]. The defective functioning of the voltage dependent potassium channel leads to the loss of control over excitability. In contrast, heteromerization of KCNQ2 and KCNQ3 strongly potentiates the M-current resulting in anti-epileptogenic effects [36]. Modulation of M-currents has a great impact on neuronal excitability because these currents are the only ones which are maintained during initiation of action potential and have ability to inhibit epileptic dis-

charges. M channels are not involved in repolarization of individual action potentials but exert a dampening effect on repetitive discharges and excitability of neurons [21]. Retigabine binds to activation gate region interacting with a specific amino acid fragment and stabilizing the neuronal KCNQ (Kv7) channels in an open conformation. Specifically, it has been proposed that retigabine binds to a hydrophobic pocket formed upon channel opening between the cytoplasmic parts of S5 and S6 segments of Kv7.2 involving Trp236 and the channel's gate, which explains the large hyperpolarizing shift in voltage-dependent activation [136]. Such characteristic amino acid pocket to which retigabine binds in neuronal cells is not present in the cardiac muscle cells. Thus, retigabine activates KCNQ2/KCNQ3 potassium channel shifting their voltage-dependence to more negative values [132]. By opening the Kv7 channels, retigabine prevents sodium depolarizing currents and occurrence of epileptic discharges. Retigabine action seems to be not restricted to hyperpolarization of the resting potential of neuronal membranes and enhancement of neuronal resistance against generation of epileptiform activity. It has been reported that through the activation of presynaptic M-currents, retigabine can also inhibit the depolarization-evoked excitatory amino acid release from cerebrocortical synaptosomes [65, 132]. The broad antiepileptic activity of retigabine was proved in several experimental models of chemical, electrical and genetic models of seizures and was confirmed in clinical trials [13]. One can expect that retigabine can be a prototype drug for a new generation of antiepileptic drugs. Indeed, some benzanilide- and adamantane-derived compounds with agonistic activity at KCNQ2/KCNQ3 channels and possessing antiepileptic properties have recently been presented [39, 104, 132]. Several other newly identified compounds displayed isoform-specific prolongation of Kv7 potassium channel opening and induced significant reduction in activation that differed from that of retigabine [40]. KCNQ2 and KCNQ3 genes encode Kv7.2 and Kv7.3 potassium channel subunits, respectively. Kv7.2 and Kv7.3 form heteromeric voltage-gated potassium channels, which are responsible for the generation of M-current which regulates repeated neuronal discharges [128]. Mutations of KCNQ2 and KCNQ3 genes that reduce the current-M amplitude cause the rare hereditary benign familial neonatal seizures [26]. It has been found that the mouse model of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions shows spontaneous generalized tonic-clonic seizures neuronal plasticity, but, like in human BFNC, without neuronal loss and hippocampal mossy fiber sprouting [115]. The Szt-1 mice with a spontaneous mutation involving KCNQ2 (Kv7.2) have a reduced baseline seizure threshold and lower sensitivity to an M-current enhancer [88]. On the other hand, in two animal models of temporal lobe epilepsy (amygdalar kindling and electrical stimulation of the hippocampus) the KCNQ2 immunoreactivity was increased in the basolateral amygdala. It was concluded that the KCNQ2 subunit upregulation could be an adaptive response to counteract the limbic seizures [94]. The Kv7.2/Kv7.3 channels are targets for pharmacotherapy of epilepsy, stroke, migraine, dementia, bipolar depression, anxiety and neuropathic pain. The non-inactivating K^+ M-current is known to be slowly activated during neuron depolarization towards the threshold for action potential hyperpolarizing the cell and decreasing neuronal excitability. Numerous second messengers converging pathways and intracellular calcium control the M-current, and the activation of various Gq/G11-coupled receptors predominantly inhibits this current [21]. The M-current appears to play a key role in controlling neuronal excitability because it is the only sustained current which counteracts depolarization near the threshold of action potential [20, 73]. Therefore, the M-current is thought to act as an efficient brake for repetitive action potential firing. M-current inhibition by muscarinic receptor agonists enhances neuronal excitability, as shown in the cells derived from both peripheral and central nervous system. This is in line with proconvulsant effects of cholinergic receptor agonists, e.g., systemic administration of pilocarpine in high doses produces recurrent limbic seizures in rodents widely accepted as a model of temporal lobe epilepsy [121]. The blockade of M-current in the hippocampus-entorhinal cortex slices of neonatal and adult rats produced epileptiform activity with a developmental pattern resembling BNFC [93]. This is in agreement with the study that showed that M-current inhibition in the Mg^{2+}-free seizure model in hippocampal slices of immature rats readily transited the interictal bursting to sustained depolarization [98]. The significance of the M-current in seizure prevention was supported by introduction of a new antiepileptic drug to the clinic, that is retigabine which acts mainly, if not exclusively, through a positive modulation of Kv7.2–7.5 channels. Muscarinic receptor stimulation with linopiridine in-

creased neuronal firing in slices from human epileptogenic cortex, whereas retigabine had opposite effects [43]. It is of note that the activation of K^+ M current can play also an important role in the mechanism of action of neuropeptides with anticonvulsant activity, such as dynorphin and somatostatin [68, 75, 112]. In low concentrations, the endogenous κ opioid receptor agonists dynorphin A and dynorphin B augmented M-current in CA3 neurons of the rat hippocampus, but in high concentration, dynorphin A suppressed the M-current. Dynorphin was found to selectively augment the M-current in the hippocampal CA1 neurons by an opiate receptor mechanism [68]. Qiu et al. [99] reported that somatostatin receptor subtype 4 coupling to M-channels was critical to the inhibition of epileptiform activity. They found that the somatostatin receptor 4 knockout mice showed shorter latencies and increased seizure severity when compared with wild-type mice in both kainate and pentetrazole seizure models [9].

Pharmacological and molecular data indicate that, besides KCNQ2/KCNQ3, also other potassium channels, e.g., KATP, Kv1.1 and GIRK2 regulate neuronal excitability and can be involved in pathomechanism of seizures [132]. Specifically, the Kv1channels are involved in regulating the action potential firing patterns and neuronal excitability [103]. Mutations Kcna1 and Kcna2 are associated with epilepsy and ataxia in humans, whereas mice devoid of Kv1.1 or Kv1.2 genes showed high susceptibility to seizures [17, 117]. Developmental seizure susceptibility of Kv1.1 potassium channel knockout mice has been described by Rho et al. [100] as a clinically relevant model of early-onset epilepsy. The α-dendrotoxin--induced inhibition of these channels results in seizures, whereas compounds that prevent Kv1.1 inactivation decreased pentetrazole- and maximal electric shock-evoked seizures in mice [7, 64].

Another subtype of the potassium channel has an additional link to CNS diseases. This is *KCNH2* (HERG1; human ether-à-go-go-related gene) which was initially discovered in the hippocampus [129], and it was later demonstrated that this gene was expressed in many regions of the central nervous system. Further studies reported that hippocampal expression of the ERG family of potassium channels was distributed preferentially in hippocampal astrocytes that may regulate neuronal excitability [35]. Later, the mutations in that gene, causing the loss of its function, were found to be responsible for the LQTS type 2 and for many years this has been a major focus of clinical and research interest. However, only recently a clear link was identified between KCNH2 mutations and epilepsy in humans [51, 87]. It was observed that LQT2-causing perturbations in the *KCNH2*-encoded potassium channel might result in susceptibility to recurrent seizure activity. Additionally, ERG potassium channels blockade in astrocytes changed potassium concentrations extraneuronally, and such changes were shown to be epileptogenic [49]. This has clinical implications as post-traumatic changes in hippocampal glia mimic the effects of acute blockade by external antagonists, including loss of K conductance with subsequent failure of glial potassium homeostasis, which in turn promotes abnormal neuronal excitation [31]. Further research should focus on this interesting mechanism as it might prove to be an important component of the complex pathophysiology of drug refractoriness in epilepsy. In addition, ion channels co-expressed in the brain and the heart, such as potassium channels are logical candidates as risk factors for sudden unexplained death in epilepsy (SUDEP) because defects in intrinsic membrane excitability could underlie both epilepsy and cardiac arrhythmias that cause death [84].

Hyperpolarization-activated cyclic nucleotide gated (HCN) channels structurally resemble voltage-gated potassium channels. Altered expression levels of the HCN1 subunit have been shown in the hippocampus of patients with temporal lobe epilepsy as well as in animal models of this disorder [101]. On the other hand, the HCN2 subunit is abundant in the thalamus, and silencing of the gene coding for this protein leads to non-convulsive seizures in mice. These observations suggest that HCN could play a role in both limbic and absence epilepsy and can be a potential target for antiepileptic therapy [96, 114]. Lamotrigine and gabapentin were shown to activate the hippocampal HCN channels, but it is premature to draw any conclusion, to what extent this effect might contribute to antiepileptic properties of these drugs [97].

Concluding remarks

There is general consensus that seizures result from excessively enhanced excitatory processes in a given population of neuronal cells in the brain or from defi-

cient neuronal inhibition. Traditionally, the above mechanism was thought to comprise hyperactivity of glutamatergic transmission and insufficient $GABA_A$ receptor-mediated neurotransmission. New data add to the complexity of GABA-glutamate interaction showing both excitatory and inhibitory role of GABA and glutamatergic neurons in the central nervous system. Moreover, besides synaptic NMDA and $GABA_A$ receptors, also extrasynaptic receptors for the amino acid transmitters have been recently implicated in the pathomechanism of seizures. Changes in gene expression, polymorphisms, lost- or gain-function mutation as well as cellular energetic imbalance can contribute to disturbed function in the ligand- and voltage-dependent sodium, potassium, chloride and calcium channels, resulting in seizure activity.

The principal targets for antiepileptic drugs comprise voltage-dependent sodium, potassium and calcium channels, $GABA_A$ receptors, GABA-metabolizing enzymes and GABA transporters. Excitatory amino acid receptors and some synaptic proteins can also be involved in the mechanism of antiepileptic drug action [11, 57, 77]. Looking for future directions of therapy of epilepsy, one may stress significance of molecularly characterized new drug targets, drugs acting in direct vicinity of seizure focus (focal method of drug delivery), and gene and cell therapy. A significant progress has been made in elucidating the role of amino acid receptor subunits, various kinds of potassium ion channels, HCN channels, acid-sensing ion channels, and gap junctions in the regulation of neuronal excitability. There is a great hope that molecular genetics and proteomics strategy will help in creating new, effective anticonvulsants. However, it seems that the real breakthrough in our understanding of the mechanism of epilepsy and antiepileptic drug action will depend on integration of molecular and functional studies and on proper transformation and presentation of data, which should facilitate the substantive discussion between specialists of various fields of experimental and clinical epileptology.

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