

Epilepsy: Novel therapeutic targets

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ABSTRACT

Despite of established and effective therapy for epilepsy, 20–25% patients develop therapeutic failure; this encourages finding newer drugs. Novel approaches target receptors which remain unaffected by conventional therapy or inhibit epileptogenesis. AMPA receptor antagonists have shown faster and complete protection compared to diazepam. Protein kinase (PK) plays an important role in the development of epilepsy. PK inhibitors such as K252a, VID-82925, and Herbimycin A have been found effective in inhibition of spread of epileptiform activity and epileptogenesis. Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors classified into three groups. Group 1 mGluRs antagonist and Groups 2 and 3 mGluRs agonists inhibited pentylenetetrazole-induced kindled seizures. Combined use of these agents has also shown favorable results. Mammalian target of rapamycin (mTOR) plays a central role in multiple mechanisms of epileptogenesis. mTOR causes transcription, induction of proapoptotic proteins, and autophagy inhibition. Rapamycin was effective in suppression of recurrent seizures as well as in tuberous sclerosis and acute brain injury model. 5% CO₂ showed potent effects on cortical epileptiform activity and convulsions in animal epilepsy models and in humans with drug-resistant partial epilepsy. It is found to be rapidly acting, safe and cheap, thus it can be a good option in emergency for suppression of seizure. Neurosteroids are considered as fourth generation neuromessengers, they act as positive allosteric modulators of γ -aminobutyric acid (GABAA) receptors. Clinical trial of ganaxolone, an allopregnanolone analogue, has shown a beneficial role in pharmacoresistant epilepsy. However, most of these drugs are tested in early phases of development and the possible use and safety in epilepsy has to be proven in clinical trials.

Key words: AMPA receptor antagonists, mammalian target of rapamycin, metabotropic glutamate receptors, pharmacoresistant epilepsy, protein kinase inhibitors

INTRODUCTION

Epilepsy is the second most common neurological disorder with an incidence rate of 0.3–0.5% worldwide and prevalence of five to ten person per thousand population. Approximately

50 million people are suffering from epilepsy worldwide and among them 90% are in developing countries.^[1] Management of epilepsy requires immediate attention and long-term treatment. About 75–80% patients achieve control with conventional anti-epileptic drugs like phenytoin, carbamazepine, and valproic acid. Most of these drugs have a narrow therapeutic margin and require therapeutic drug monitoring. Moreover, 20–25% patients develop therapeutic failure with these drugs.^[2] This stimulates further research to find newer drugs and new management modalities. The present review discusses newer therapeutic targets [Table 1] which shown promising results and can be used in future for development of new antiepileptic drugs.

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Table 1: Novel therapeutic targets in epilepsy

Target	Mechanism	Drug	Phase of development	Possible use
AMPA receptors	Competitive AMPA receptors antagonism	NS1209	Preclinical stage	Status epilepticus
	Non-competitive AMPA receptors antagonism	GYKI 52466		
Protein kinase	Protein kinase inhibition	Herbimycin A	Approved as antibiotic	Epileptogenesis
mGluRs	Group 1 mGluRs antagonism	K252a	Preclinical stage	Temporal lobe epilepsy Absence seizure
		AIDA((RS)-1-aminoindan-1,5-dicarboxylic acid)	Preclinical stage	
	Group 2 mGluRs agonism	APDC((2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (2R,4R))		
	Group 3 mGluRs agonism	L-AP4(L-(+)-2-amino-4-phosphonobutyric acid)		
mTOR	mTOR inhibition	Rapamycin	Approved as anticancer drug	Status epilepticus Epileptogenesis
CO ₂	Carbonic anhydrase inhibition	5% carbogen	Preclinical stage and Pilot study in Human	Myoclonic epilepsy Partial epilepsy Status epilepticus
Neurosteroids	GABAA receptors modulation	Ganaxolone	Phase II	Infantile spasm Ketamania epilepsy Resistant epilepsy

AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, mGluRs: Metabotropic glutamate receptors, mTOR: Mammalian target of rapamycin

NEWER THERAPEUTIC TARGETS

α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors

AMPA receptors play an important role in pathogenesis of refractory epilepsy. Transition from single self-limiting seizure to repeated prolonged seizure is accompanied by externalization of AMPA and internalization of γ -aminobutyric acid (GABAA) receptors. This process of internalization leads to GABAA receptors agonist ineffective, but AMPA receptors antagonist might be useful due to externalization of AMPA receptors in refractory epilepsy.^[3-5] GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive AMPA receptor antagonist has good penetration in the blood-brain barrier.^[6] Recently, Fritsch *et al.* evaluated effects of GYKI 52466 on early and late kainic acid (KA)-induced status epilepticus.^[7] GYKI 52466 administered in two doses 15 min before seizure induction have stopped seizure activity rapidly as well as its recurrence. All treated animals survived, even in the group that have received 45 mg/kg KA, while 33% mortality observed in untreated animals. When status epilepticus continues, it becomes refractory to conventional treatment. In this study, late administration of diazepam was slow to inhibit and unable to prevent recurrence. In contrast to diazepam, GYKI 52466 was able to terminate seizure and prevented recurrence when administered in the late phase. Pitkänen *et al.* demonstrated that competitive AMPA receptor antagonist NS1209 provided faster and more complete protection as compared to diazepam in status epilepticus.^[8] A major problem with the use of NS1209 is that it required continuous infusion whereas GYKI 52466 was effective in two bolus doses 15 min apart. Due to

noncompetitive antagonism by GYKI 52466, it may have wider anticonvulsant activity as its blocking action cannot be overcome by even high level of glutamate.^[9]

Standard treatment of status epilepticus with diazepam is associated with a decrease in mean arterial pressure (MAP). When MAP falls below 70 mmHg, cerebral blood flow regulation falls and irreversible brain damage occurs. GYKI 52466 at therapeutic dose caused fall in the pulse rate, but no effect observed on MAP. Mice treated with GYKI 52466 appeared to be sedated but responded on stimulation showing moderate sedation. Animals treated with GYKI 52466 gained weight normally after they had recovered from the status epilepticus episode showing good overall tolerability.^[10]

AMPA receptor antagonists may be useful as single drug therapy in status epilepticus with less cardiovascular complications compared to standard treatment with diazepam or phenytoin. AMPA receptor antagonists are promising targets in status epilepticus.

Protein kinase

Protein kinase (PK) is an enzyme protein which primarily phosphorylates receptors, transport molecules, ion channels and affects excitability and functions of neurons and glia.^[11] Experimental models have shown the involvement of protein tyrosine phosphorylation *via* PK in the development of status epilepticus. Phosphorylation of receptors could occur at least through three different intracellular pathways: (1) Src non-receptor tyrosine kinase (2) Eph receptors for the ephrins cell-to-cell adhesion molecules, and (3) Trk receptors for

neurotrophins. These three kinases have been implicated in the epileptogenesis.^[12] Queiroz and Mello showed the preventive role of tyrosin PK inhibitors K252a and herbimycin A in KA induced epileptiform activity and mossy fiber sprouting (MFS).^[13] KA intracerebral injection leads to selective destruction of pyramidal CA3 neurons, which is associated with epileptiform discharges. It also leads to hippocampal neuronal loss, reactive gliosis, MFS, and spontaneous recurrent seizures. Herbimycin A and K252a modified the electrographic epileptiform activity induced by intrahippocampal KA administration but did not alter the cell loss pattern. Only K252a treatment reduced supragranular MFS. Herbimycin A inhibited the spread of the epileptiform activity to the contra lateral hippocampus; this might be due to its strong inhibitory activity on the family of the Src kinases. Src kinases have been described as “a hub for NMDA receptor regulation” and have been involved in NMDA receptor phosphorylation during status epilepticus.^[14] K252a reduced intermittent burst activity by inhibition of BDNF-induced inhibition of GABAA receptor-mediated inhibitory postsynaptic currents in CA1 slices. Both the PK inhibitors failed to prevent limbic cell death. This may be due to animal models with frequent seizures have such widespread cell damage that this cannot be counter-affected by local (intra ventricular) administration of protein tyrosine kinase inhibitors. In future PK inhibitors can be evaluated with the use of more restricted damage animal model to show its effect on cellular death. A study by Zita Gajda *et al.* demonstrated the antiepileptogenic effect of VID-82925, a kinase inhibitor. VID-82925 was found to inhibit the epileptiform activity not only during developmental phase, but also during the long period of stable phase of focus. While levetiracetam pretreatment failed to exert antiepileptogenic effect, the effect of VID-82925 was comparable in intensity with carbenoxolone, a broad-spectrum gap junction channel blocker. It suggests VID-82925 also exerts antiepileptogenic effect by blocking of gap junctional communication.^[15]

Tyrosine kinase inhibitors may cause some serious side effects like cardiotoxicity, anemia, and thrombocytopenia.^[16] This drawback can be overcome by the development of PK inhibitors that specifically affect epileptogenesis without affecting normal cell growth. PK may be an effective target for the development of a drug which not only antiepileptic but also prevents the course of epileptogenesis.

Metabotropic glutamate receptors

Glutamate receptors are classified into two types: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are divided into three subtypes: kainate, AMPA, and NMDA receptors.^[17] iGluRs are involved in fast synaptic transmission at glutamatergic synapses.^[18] mGluRs were discovered in mid-1990s. mGluRs are G protein coupled receptors of the glutamate receptor family. It plays an important role in glutaminergic transmission.^[19-21] Based on

signal transduction mechanisms, the pharmacological profile and receptor protein mGluRs are classified into three groups: group 1 (mGluR1 and 5), 2 (mGluR2 and 3), and 3 (mGluR4, 6, 7, and 8).^[18,22] Postsynaptically mGluRs have a role of modulating membrane properties by the second messenger while presynaptic mGluRs are involved in control synaptic release. These properties can be used to control glutamatergic signaling in the central nervous system (CNS), without interfering functions of iGluRs.^[23] Group 1 mGluRs through Gq protein are involved in intracellular mobilization, upregulation of these receptors is seen in patients with epilepsy.^[24] Groups 2 and 3 receptors are located presynaptically where they decrease glutamate release; reduction in their expression has been found in patient with temporal lobe epilepsy.^[25] Recent studies have shown that Group 2 mGluRs inhibit cortical excitation of thalamic neurons, cortical layer 5, and intracortically projecting layer 2/3.^[26] In pentylentetrazole-induced kindled seizures (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), a selective mGluR1 antagonist and (*2R,4R*)-4-aminopyrrolidine-2,4-dicarboxylate (*2R,4R*)-APDC, a selective mGluR2/3 agonist have been shown dose-dependent inhibition of both seizure stage and EEG pattern. 1-(+)-2-Amino-4-phosphonobutyric acid (1-AP4), a selective mGluR4/8 agonist, at a dose of 10 and 20 nmol/site injected at the lateral ventricle has also shown dose-dependent inhibition of pentylentetrazole-induced kindled seizures. The combined use of AIDA, APDC, and 1-AP4 has shown significant inhibition of seizure stages and EEG pattern with decreased individual dose of these drugs. There was no significant effect when AIDA was used by an intraperitoneal route, possibly due to prevention of its entry by blood-brain barrier. 1-AP4 at low dose is found to have antiepileptic effect but when used in high doses, it induces convulsion. It could be due to depolarization of NMDA receptors at higher dose. Combined use of these agents also shown favorable results.^[27]

Group 1 mGluRs are involved in cerebellar motor function and blockage of these receptors may lead to motor incoordination and ataxia. This could be major hindrance in the development of group 1 mGluRs antagonist. Group 2 mGluRs may be a promising target due to their selectivity and inhibitory action on cortical and thalamic neuron.^[26] Group 3 mGluRs agonists have shown mixed effects; some studies have demonstrated it as pro-convulsive while in other studies found it to have a protective role in absence seizure and proconvulsant in generalized seizure. This shows involvement of multiple pathways in Group 3 mGluRs action. Further studies required before labeling it as an important target.

Mammalian target of rapamycin

Mammalian target of rapamycin (mTOR) is a conserved serine/threonine PK. mTOR activity is upregulated or downregulated in the tuberous sclerosis complex, cortical dysplasia, brain tumors, neurodegenerative disorders, and traumatic brain

injury.^[28] mTOR plays the central role in multiple mechanisms of epileptogenesis^[29] [Figure 1]. A variety of extracellular and intracellular signals like nutrients, energy status, growth factors, and stress regulate the upstream pathway which in turn regulates mTOR activity.^[30,31] mTOR responds to this pathway by modulating downstream pathways by increasing or decreasing growth, proliferation, metabolism, and survival. These effects are produced by direct translation of proteins.^[32] Therefore, in anabolic states it increases cellular growth and catabolic states decreases it. It has been demonstrated that the mTOR pathway upregulated during 1–24 hours (acute phase) after seizure onset and 3 days–5 weeks (second phase) after status epilepticus (SE). Acute phase of mTOR activation can be induced directly by initial SE-evoked synaptic activity; while the second activation may be a consequence of ensuing spontaneous action potential discharges due to enhancement of synaptic efficacy after the initial episode of SE. mTOR causes transcription, induction of proapoptotic proteins such as BAX and leads to activation of intrinsic cell pathway.^[33] Another mechanism proposed is the role of mTOR in autophagy inhibition.^[34] Autophagic stress-induced neuronal death in *in vivo* models of neuron death and neurodegeneration, including the kainate-induced seizure model has been found.^[35] Zeng *et al.* demonstrated the role of rapamycin in neurogenesis and MFS, which are major mediators in epileptogenesis. Rapamycin was effective in the suppression of recurrent seizure when given before and after seizure induction by kainate.^[36] Wong demonstrated the antiepileptogenic role of rapamycin in tuberous sclerosis and acute brain injury model.^[29]

Safety is a major concern in usefulness of mTOR inhibitors. Rapamycin approved by U.S. Food and Drug Administration (FDA) as an immunosuppressant drug, and there are known risks associated with it. Rapamycin increases risk of infection, adversely affects growth and development on the long term use in children.^[29] The question needs to be answered in the use of rapamycin is therapeutic window or up to how much time after brain injury rapamycin treatment can be initiated to exert antiepileptic effect. Other factor needs to be considered is the mTOR pathway that may also be associated with recovery of brain after injury and inhibition of these may adversely affect recovery process. The future use of mTOR inhibitors depends on careful titration of dose which inhibits pathological process but allows the normal physiological process involving the mTOR pathway.

However, it is a good signal of shifting epilepsy treatment from seizure suppression to prevention of epilepsy.

Role of CO₂ in epilepsy

Suppression of the electroencephalography (EEG) spike-wave pattern in a patient of petit mal epilepsy by carbogen (10% CO₂) was demonstrated in early 1990s. Number of studies

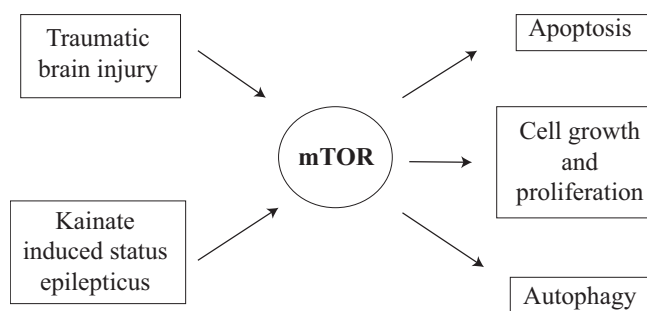


Figure 1: Role of a mammalian target of the rapamycin pathway in epileptogenesis

performed on various concentrations (10–30%) of CO₂ have demonstrated the antiepileptic effect. Recently, the effect of standard medical carbogen with low level of CO₂ (5%) has been investigated for antiepileptic activity in the rat model of myoclonic epilepsy, non-human primate epilepsy model and in humans with partial epilepsy. In this study 5% CO₂ showed potent effects on cortical epileptiform activity and convulsions in animal epilepsy models and in humans with drug-resistant partial epilepsy.^[37] Epilepsy terminated within 1 min after administration of CO₂ and prolonged administration was not needed. The elevated level of pCO₂ causes acidosis which has a direct suppressant effect on brain excitability. Acetazolamide, a carbonic anhydrase inhibitor, has shown antiepileptic activity in previous studies.^[38,39] Changes in brain pH through systemic carbonic anhydrase inhibition and retention of CO₂ may account for its anticonvulsant properties. It is also proposed that direct effects on the interneuron carbonic anhydrase inhibitor may be an alternative mechanism. pH also plays an important role in the antiepileptic effect of CO₂. *In vivo* and *In vitro* studies demonstrated that excessive neuronal activity during seizure leads to acidosis, which helps in termination of seizure.^[40,41] A slight change in ambient pressure of CO₂ precipitates seizures shows that seizure is highly sensitive to pCO₂.^[42] Safety is major deciding factor in treatment with CO₂; however, little discomfort was reported in patients breathing 5% CO₂ for a couple of minutes. High percentage of CO₂^[43] for the prolonged period^[44] may lead to symptoms such as anxiety. It has a very small effect on non-epileptic cortical activity as compared to antiepileptic activity.

On the basis of these findings CO₂ due to its rapid action, safety and low cost may be a good option to treat prolonged seizures in the setting of the emergency room, intensive care unit, or ambulance especially in developing countries.

Neurosteroids

Neurosteroids are considered as fourth generation neuromessengers which are synthesized within the neurons. Neurosteroids are synthesized directly by brain from cholesterol. Pregnenolone is synthesized from cholesterol, which is then converted to allopregnanolone and

allotetrahydrodeoxycorticosterone. Gonadal sex steroids and dehydroepiandrosterone (DHEA) are not considered as neurosteroids, as they are not synthesized in the brain. Only PREG(S) sulfur ester of DHEA, pregnanolone, allopregnanolone, and allotetrahydrocorticosterone are considered as 'true' neurosteroids. Neurosteroids have ability to modulate neurotransmission and act as positive allosteric modulators of GABAA receptors.^[45] Studies have shown that during development of seizure, the level of neurosteroids fluctuates (expression of GABAA receptors increases when neurosteroids administered chronically and withdrawn). Enzymes 5-a-reductase (5aR) and 3-a-hydroxysteroid dehydrogenase (3aHSD) are found in brain that may be involved in their synthesis and metabolism. Epilepsy-like disorder (EL) mice is a genetic model for idiopathic complex partial seizures in humans.^[46] During the development of seizure in EL mice, interictal period levels of neurosteroids, enzymes and GABAA receptor alpha4, gamma2, and delta subunits change. In third week of EL mice life, the level of neurosteroids increases which later at 5th to 10th week decreases and there is a sharp withdrawal of neurosteroids just before mice exhibit frequent seizures. Along with the decrease in the level of neurosteroids, there are upregulation of receptor alpha4, gamma2, and delta subunits.^[47]

A recent clinical trial of ganaxolone, an allopregnanolone analogue, has shown the beneficial role in pharmacoresistant epilepsy.^[48] No serious adverse reactions have been noted with ganaxolone treatment in this trial. In future neurosteroids which have shown to act on GABAA receptors may be a target for new drug development.

Future prospects

In summary, various therapeutic targets described in this review may have possible advantages to the available antiepileptic drugs (Some of these targets have shown to be effective in resistant cases, which may be an important option in therapeutic failure cases). Apart from the development of drug suppressing seizure, a new concept of prevention of epileptogenesis has arrived. Drugs acting through mTOR and PK pathways may be new rays in this direction. However, most of these drugs are tested in animals only and the possible use and safety in epilepsy have to be proven in clinical trials.

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