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Review Article

Alterations of GABAergic System which Perturb GABA Mediated Inhibition in Temporal Lobe Epilepsy

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Abstract

Temporal lobe epilepsy (TLE), as a most common type of ungovernable epilepsy is characterized by reduced inhibitory network activity. GABAergic neurotransmission undergoes perturbation and depression in its inhibitory function such as, recapitulation; changes in expression of KCC1 and NKCC2 co-transporters which regulate Cl^- homeostasis, alteration in expression and arrangement of subunits of GABA receptors specially $GABA_A$ (Cl^- channel), sprouting and reorganization of GABAergic neurons and many alterations that we don't account for in this review. All of these changes lead in reduction of GABAergic inhibitory effects, so it can induce epileptic activity in neuronal networks. Understanding the mechanisms of such reorganization and alterations can help us to develop more potent novel therapeutic materials for TLE patients.

Keywords: *GABAergic neurons, KCC1, NKCC2, Temporal lobe epilepsy*

Introduction

Epilepsy is the second frequent neurologic disorder affecting up to fifty million individuals globally and identified by recurrent seizures [1]. More than 30% of the epilepsy patients have uncontrolled seizures despite adequate pharmacological treatment [2]. Various types of epilepsy may be due to developmental [3] or genetic disorders [4]. However, majority of epilepsies appears following various brain injuries such as a mechanical head trauma, brain vascular abnormalities or infection. Occasionally these insults induce neuronal death in the hippocampus, a central part of the temporal cortex [5].

Temporal lobe epilepsy (TLE) with hippocampal sclerosis (HS), constitutes the most common form of refractory epilepsy [6]. In histologic assessments, brains of TLE patients show sclerosis in the Mesial temporal lobe (MTL) due to selective cell loss and astrogliosis in the CA1 area of the hippocampus. Exact molecular mechanism of epileptogenesis in TLE is still remains unknown [7]. This type of epilepsy is associated with reduced inhibitory neurotransmission. The GABA hypothesis of seizure disorders suggests that impairment in GABAergic inhibitory neurotransmission may take a part in synchrony hyperexitible activity of epileptic brain. Effectiveness of anticonvulsant agents which potentiate GABAergic inhibition as well as occurrence of seizure following blockade of $GABA_A$ receptors supports this idea [7]. Thus, in the present review, the importance of GABAergic inhibitory system in TLE is discussed.

Recapitulation of GABAergic neurotransmission, GABA as an excitatory neurotransmitter

Based on several hypotheses GABAergic neurotransmission is impaired in TLE. One interesting hypothesis, according to study by Cohen et al., mentions that perturbed neuronal chloride transport and concentration results in excitatory GABAergic signaling and consequently seizure in TLE [8]. Cohen et al. verified emergence of electrical currents in subicular GABAergic pyramidal neurons of the temporal lobe slices that appeared as spontaneous interictal depolarization. These specimens were obtained from refractory epileptic brains with mesial temporal sclerosis (MTS). In spite of, famous inhibitory effect of GABA in almost all brain regions, excitatory responses by this

neurotransmitter in subicular pyramidal neurons of TLE cases have been reported [9].

Binding of GABA to $GABA_A$ receptors results in entrance of chloride (Cl^-) and sometimes bicarbonate ($HCO3^-$) anions into cells and further synaptic currents induction. $GABA_A$ receptor-mediated conductance, depending on inrtacellular concentrations of Cl^- and $HCO3^-$ either increases or decreases resting membrane potential of a cell. GABAergic signaling can be switched from hyperpolarizing to depolarizing due to pathological changes of Cl^- homeostasis in epileptic tissue [8,10,11]. Two co-transporters are main regulator of Cl^- concentration in neurons [12,13]. Naturally, the Na^+-K^+-$2Cl^-$ co-transporter (NKCC1) depolarizes cell by inflow of Cl^- in response to GABA, while, the K-Cl co-transporter (KCC2) hyperpolarizes the neuron due to outflow of Cl^- to extracellular space. Expression of these transporters is under control by various mechanisms which in turn will affect their function. Developmental alterations are accompanied with up-regulation of KCC2 but down- regulation of NKCC1 [14-17]. As a consequence of these molecular changes, membrane potential of cortical pyramidal cell shifts to hyperpolarizing in response to GABA [12,18,19]. In the adult brain, some pathologic conditions prompt a depolarizing shift in $GABA_A$ mediated currents due to down-regulation of KCC2, and further reduced Cl^-extrusion (Figure 1), [20-23]. In addition, phosphorylation and other post-transcriptional mechanisms adjust both choloride transporters [24-27].

Epileptiform discharges are related to excitatory GABAergic responses resulted from a high intracellular Cl^- following functional changes in co-transporter [12]. Paroxysmal activity down-regulates KCC2 transporter [28]. In patients with TLE, interictal-like activity was produced by depolarizing GABAergic currents in small population of subicular neurons [8]. Thus, failure of GABA mimicking drugs in control of TLE might be related to a perturbed intracellular chloride homeostasis [29]. According to molecular and electrophysiological investigations, GABA-mediated post synaptic potentials were excitatory instead of inhibitory in a remarkable number of subicular pyramidal neurons obtained from TLE patient with HS. While, electrical and anatomical properties of current neurons were similar to neurons in which GABAergic response was inhibitory (hyperpolarizing). In situ data and immunolabeling studies revealed some pyramidal neurons of subiculum were lack of KCC2 mRNA and protein. In contrast, immunohistochemical studies and in situ hybridization indicated that both KCC2 mRNA and protein were expressed by majority of dentate granule cells. Also, KCC2 mRNA was detected in 70% of subicular pyramidal cells [30].

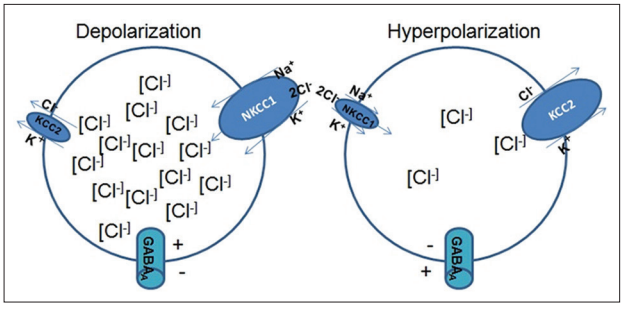

Figure 1. Recapitulation results in excitatory GABAergic neurotransmission.

In a study by Huberfeld et al. combined biocytin staining and immunostaining indicated that most cells that located in the foci of interictal activities showed perturbed expression of KCC2 [31]. Additionally, the biocytin-stained neurons correlated to KCC2-positive neurons in immunohistochemistry which inhibited during interictal activity expressed KCC2 [31]. KCC2 may be detected at low levels in pyramidal cells but is not adequate to maintain basal Cl^- concentration. Furthermore, immunodetected KCC2 may not work normally because of posttranslational modifications [24-26,32]. For example, shift of GABA-mediated hyperpolarization to depolarization following differentiation of the colliculus seems to be more related to posttranslational modifications instead of low level of KCC2 protein [24]. Other molecules that participate in Cl^- homeostasis [12,19,33] may also contribute to this excitatory switch of GABA transmission in human epilepsy. Another research on human epileptic specimens has confirmed the up-regulation of NKCC1 but down-regulation of KCC2 in the subiculum in favor of Cl^- accumulation [34]. parellel to the data, mRNA of NKCC1 in the subicular neurons was up-regulated and KCC2 mRNA down-regulated compared with hippocampal and cortical neurons of epileptic human brain [35]. Recapitulation is the repetition of an evolutionary property or other process during development or growth. Above mentioned changes in expression of transporters is an example of recapitulation.

Brain-derived neurotrophic factor (BDNF) and recapitulation

Shift of neurons to a more immature phenotype thought to be mediated by BDNF pathway seizures activity might result in long-term GABA-mediated excitatory responses due to downregulation of KCC2 and less probably up-regulation of NKCC1 via BDNF signaling through tropomyosin-related kinase B (TrkB). Prolonged epileptic activity increases hippocampal expression of BDNF and its receptor, TrkB [36,37]. Parallel to robust BDNF and TrkB up-regulation in some parts of the epileptic hippocampus, level of KCC2 quickly decreases in the same areas [21]. This seizure activity-dependent down-regulation of KCC2 performs after activation of the TrkB receptor and its downstream cascades for example the phosphoinositide-phospholipase C and Shc-activated MAP kinase pathways [28]. As mentioned above, seizure activity elicits down-regulation of the KCC2 protein or mRNA via BDNF [38]. The mechanism of seizure-induced up-regulation of NKCC1 is not yet clear. There are some similarities between the depolarizing GABAergic transmission (interictal activity) of the epileptic hippocampal cells and unevoked discharges involved in the formation of neuronal networks during development [12,39]. Developmentally, excitatory GABA-mediated currents open high conductance calcium channels and prompt neuronal growth, maturation and repair via surge of calcium, but such excitatory GABAergic responses are responsible for excitotoxic neuronal death following repetitive seizure activities [12,39].

A research by Huberfeld et al. revealed that the diuretic bumetanide, re-establishes normal GABAergic inhibitory response in addition to block of interictal activity in the

subiculum through suppressing of NKCC1-mediated Cl^- accumulation. Anti-epileptic effect of bumetanide implies on expression of NKCC1 the subiculum. Dysfunction of KCC2 along with up-regulation of NKCC1 could perturb cellular energy metabolism [31]. According to existing evidence, beside inhibitory effect of bumetanide on interictal activity some other diuretics may also exert antiepileptic effect [16]. However, bumetanide anticonvulsive effect seems to be mediated via affecting on GABAergic signaling and restoring perturbed chloride homeostasis at postsynaptic sites in epileptic tissue [31].

$GABA_A$ receptor dysfunction

Evidences from animal studies introduce $GABA_A$ receptor dysfunction as one possible mechanism that involves in epileptogenesis. Schematic diagram of a normal $GABA_A$ receptor is shown in Figure 2. In addition to animal models of epilepsy, involvement of postsynaptic $GABA_A$ receptors in the pathophysiology of human sclerotic epilepsy has been showed. In agreement with this claim, structural and functional changes of $GABA_A$ receptor in sclerotic hippocampus of TLE patients were recorded [40-44]. Furthermore, auto-radiographic assessments and *in vivo* imaging indicated a reduction of benzodiazepine receptor binding sites which primarily caused by massive neuronal death during the hippocampal sclerosis of TLE cases [45,46] and additional diminish of receptor density in the survived neurons of CA1 [47,48]. Encoding of $GABA_A$ receptor subunits by at least 20 genes in addition to miscellaneous assembly of various subunits together bring heterogeneity of $GABA_A$ receptors [49,50]. Recent animal studies revealed the quantitative and qualitative changes in $GABA_A$ receptors of hippocampus region from TLE models [51-53]. In the normal human hippocampus, $GABA_A$ receptor subtypes express in particular and neuron specific manner, both at the regional and the cellular levels. While, staining in sclerotic areas probably due to extensive dell death was decreased in TLE patients with HS, survived hippocampal cells also underwent specific modification in the $GABA_A$ receptor subtypes, as most important; distribution and frequency of $GABA_A$ receptor subunits in the cell body and apical dendrites were changed compared to the basal dendrites, the α1, α2, and α3subunits were rearranged in the hippocampal CA2 area and in hillus, selective neuronal death of α1- positive cells in various layer of the hippocampus was happened and

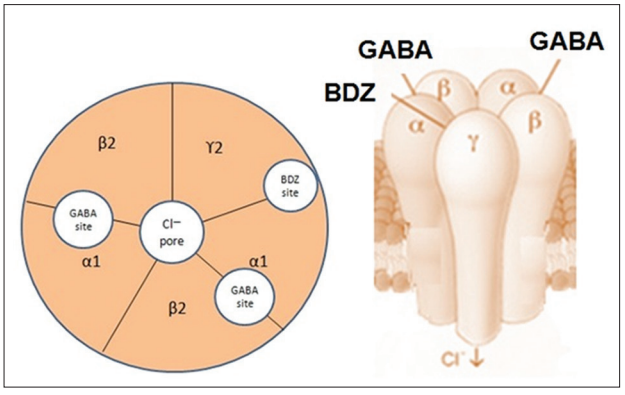

Figure 2. Schematic diagram of $GABA_A$ receptor and its active sites.

dendritic microanatomy in most of α1-positive interneurons was altered [54].

$GABA_A$ receptor subtypes and GABA transporter expression in the normal and TLE patient's hippocampus

$GABA_A$ receptor consists of five subunits which every subunit has at least 3 variants. About 20 genes or more are responsible for encoding these variants in the mammalian CNS (for example α1-6, β1-3, γ1-3) [49]. Differentially assembling of subunits from these families form functionally and pharmacologically distinct receptor subtypes, almost include at least one of each of the α, β and γ subunits [50]. It has been shown that α subunit variants are useful markers for the identification of specific subtypes [55,56], while β and γ subunits are ubiquitous and present in most receptor subtypes. Furthermore, animal studies have showed that depending on the brain regions and neuronal type expression of distinct subtypes are preferred [57].

While, three α1, 2, 3 subunits are present in CA1 and CA2 pyramidal cells of human brain, in CA3 only α 2 subunit was expressed. Analyses of samples dissected from HS and non-HS TLE cases revealed differential distribution and expression pattern of $GABA_A$ receptor subunits in the sclerotic hippocampus without changes in non-HS tissue. Remarkable decreases of all $GABA_A$ receptor subunits in extensive cellular death areas, including hippocampal CA1, CA3 and hillus were observed. In contrast, increased $GABA_A$ receptor subunits in the area with partial cell loss such as dentate gyrus were reported. Beside other subunits, presence of the α5 in hippocampus has been confirmed using x-ray and biochemical methods [55-56]. Differential distribution of the α1-α3 subunit variants demonstrates heterogeneity of $GABA_A$ receptor in the human hippocampus. Briefly, while all α -subunits were present in CA1, only α2 was found in CA3 and the expression pattern in CA2 pyramidal neurons was completely different. A2 subunit was detected within the margin of hillus. Also mossy cells strongly were stained for the α1 and the α2 subunits. A wide range of interneurons throughout the hippocampus and dentate gyrus strongly expressed α1 subunit. These interneuons also expressed the β2, 3 and γ2 subunits, and could form functional $GABA_A$ receptors. Due to their morphology and distribution, these interneurons may be GABAergic neurons, as described in human and rodent brain [57-59]. Compared to rodent, α1-positive interneurons are more divergent with higher complex distribution in the human. According to the findings of two separate studies, the rodent brain differs in the several points from those in the human brain [60-61]. The α3 subunit which is negligible in rodent was intensely found in the human CA1 and expressed variably in dentate granule cells. A1 subunit was nearly absent in middle layer of human CA3, since immunoreactivity for α1 in dendrites of rodent CA3 is normal. A1 and α2 subunits profoundly were detected in hilar mossy cells of human brain, but were not found in rodent. In addition human granule cells but not rat sharply were stained [63,64]. While, expression of $GABA_A$ receptor significantly was differed between HS and non-HS specimens, level of receptor in non-HS patients treating with antiepileptic drugs were similar to healthy brain [54]. $GABA_A$ receptor staining remarkably diminished in sclerotic areas of hippocampus and dentate due to most severe

cell death in human epileptic brain [46,47,65]. However, the reorganization of $GABA_A$ receptors along with non-changed or elevated density in areas of partial cell loss could enhance the number of receptors per neuron. For instance, $GABA_A$ receptors containing $\alpha 2$ subunit indicated relative increase. Upregulation of $GABA_A$ receptor including $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, and $\gamma 2$ subunits in the dentate neurons were strongly documented [51-53]. This upregulation as mentioned, indicates activity-dependent manner at GABAergic synapses of the dentate granule cells [51,52,66] and is demonstrated by similar changes in GABAergic cells of human dentate gyrus [67,68].

Despite preservation of $GABA_A$ receptors in TLE brain with HS, density of these receptors significantly decreased in basal dendrites of granule cell [69-71]. This reorganization is a kind of adaptive response to the repetitive excitation in the mossy cells circuits [72,73]. Although, reorganization of mossy fibers sometime led to dysfunction of $GABA_A$ receptor [43,74] or aberrantly innervation in the CA2 area of epileptic cases with HS [75,76]. In addition, CA2 pyramidal cells in HS were free from epileptiform bursts [76] which could be caused by plastic changes in receptor composition specially kind of α variant as well as rate of expression. In spite of, typical neuronal death of HS in the hillus, it has been shown that some neuronal populations such as mossy cells contained the α1 and α2 variants and a group of α1- positive cells survived. In dentate, specific class of GABAergic neurons omitted [77,78], but other subclasses preserved [68,77-79]. This selective vulnerability to seizure-induced insult emphasize on sophisticated specificity of inhibitory GABAergic cells [80]. Dendrites of survived interneurons in the hillus as well as hippocampus displayed some degree of morphological alterations [54,81,78]. Extensive α1- positive cell death in the middle layer of CA2 and CA3 has been occurred. Present findings disclosed stunning complexity in $GABA_A$ receptor composition which likely is required for effective formation and function of inhibitory neuronal networks. Analysis of samples obtained from sclerotic hippocampus of TLE cases revealed similar reorganizations in $GABA_A$ receptors of surviving neurons. Recent discoveries, will be great tools for diagnosis and designing new therapeutic strategies [54].

In addition to receptor subunits, expression of GABA transporter also shows changes in TLE patients. Mathern et al. showed that in the non-HS cases which total number of neurons was similar to control autopsies, the number of immunoreative astrocytic GABA transporter-3 (GAT-3) in the hilus and Ammon's horn was increased. In contrast, HS patients with decreased neuron densities demonstrated decreased GABA transporter, GAT-3, on remaining granule cells and pyramids. In other words, hippocampal GABA transporter quantities differ in TLE patients compared with autopsies. This reduced number of GABA transporter in the TLE patients with HS results in diminished GABA release and may mediate subsequent loss of inhibition [67].

Axonal spouting and synaptic reorganization

Probable role of axonal sprouting and synaptic reorganization in acquired epilepsy, particularly TLE, has acquired considerable attention for over few decades. Origin of interest in this issue comes from the observation of Timm staining of the dentate gyrus from both cases with TLE and MTS and also related animal models [82-83]. Several studies using electrophysiological field potential recordings with the paired-pulse technique and qualitative light-microscopic observations, have proposed that synaptic reorganization occurs in the GABAergic system and that this reorganization leads to increased GABA-mediated inhibition in animal models of TLE [84].

Two kinds of synaptic reorganization within GABAergic networks have been suggested. Based on first hypothesis, principal cell axon collaterals (usually the mossy fibers of the granule cells) enhance their connectivity to GABAergic interneurons [84]. In the second hypothesis, the GABAergic interneurons sprout axon collaterals and increase their synaptic connections may back to the principal cells [85]. Both of these hypothetical forms of synaptic reorganization would be seen as compensatory responses and expected to potentiate the efficacy of local GABAergic circuits during acquired epileptogenesis [86].

Selective loss of GABAergic interneurons and disinhibition

A well-supported hypothesis concerning cellular mechanisms that likely contribute to acquired epileptogenesis is a selective death of particular types of GABAergic interneurons [87]. Numerous studies have shown that the somatostatin-immunoreactive GABAergic interneurons are particularly vulnerable, and most of them are lost in human temporal lobe epilepsy. Paired whole-cell recordings, indicates that the remaining somatostatin-immunoreactive interneurons formed more synaptic connections to granule cells (i.e., the number of granule cells that received connections was increased 2 to 3 folds). This finding, which was confirmed by several laboratories, proposes immediate death of interneurons and a failure of those to undergo axon projectiong and formation of new inhibitory connections. Although the paired recordings provide direct evidence that axon sprouting of inhibitory interneurons occurs and that GABAergic input is augmented to the principal cells (at least for some types of interneurons), the details of the alterations of the reorganized GABAergic interneuron circuit are quite complicated. Axons sprouting of principal cells and the genesis of new recurrent excitatory circuits probably occur during the latent periods seen in many TLE cases and animal models and other forms of acquired epilepsy. In a pilocarpine model of status epilepticus (SE), unevoked recurrent seizures appeared 45 days after status epilepticus. Mossy fiber sprouting, usually appears after 45 days in Timm staining; however, the functional effects of synaptic reorganization are best seen in many months after status epilepticus. Many animal studies have shown that acquired epilepsy generally undergo sustained progression in seizure frequency and severity [88-90]. Many animals seem to have periods in which seizure frequency is not increased, however, and a lack of progression has been reported in some animals [91]. Profound reorganization of inhibitory GABAergic circuits whereby compromises the enhanced recurrent excitation, could be explanation for these progression free periods.

One may argue that increased connectivity among interneurons, whereby interneurons inhibit other interneurons,

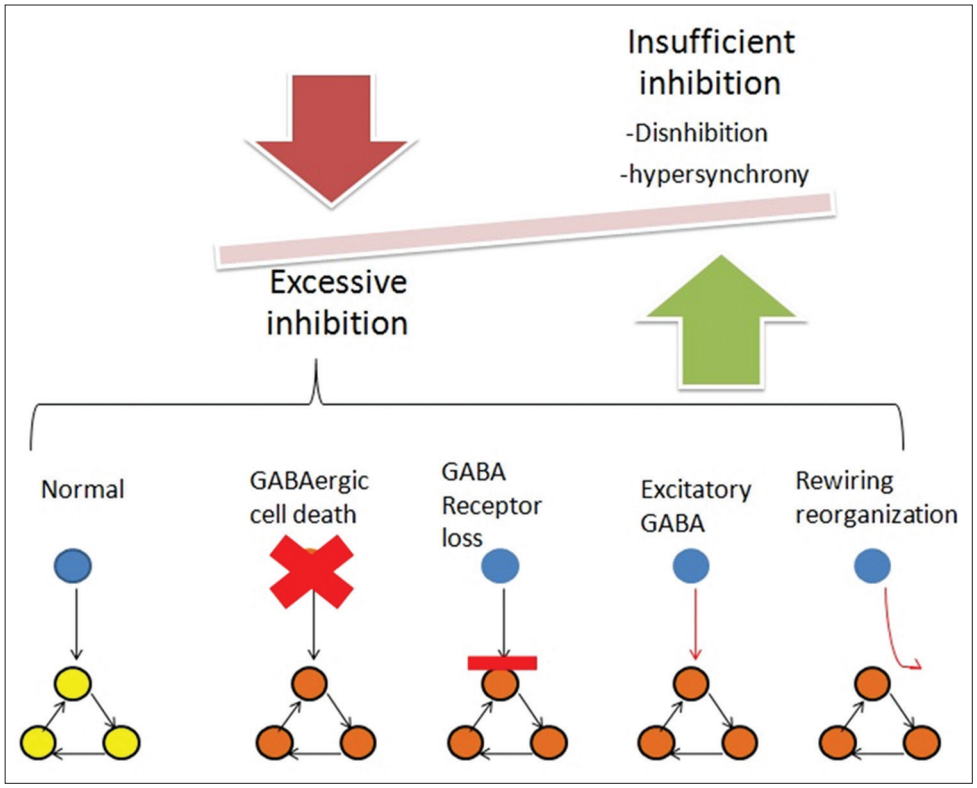

Figure 3. Summary of GABAergic system perturbation in epileptic condition.

can lead to a decrease of GABAergic inhibition. Additional studies combining anatomic and electrophysiological mapping at the single-cell level, with quantitative analyses of axonal distributions and postsynaptic currents, should ultimately reveal the exact mechanisms of reorganization occurs among the GABAergic inhibitory interneuron system and clarify the negative or positive role it may play in epileptogenesis [92].

Conclusion

Depending on the neurotransmitter, the type of receptor, and the prevailing ionic gradients the receiving neuron is either excited or inhibited. The present paper provides some of the evidences to date that inhibitory interneurons undergo several changes lead to acting as excitatory neurons (Figure 3) such as; 1. disturbed homeostasis of Cl^- in favor to the intracellular accumulation of Cl^- caused by upregulation of NKCC2 and down-regulation of KCC1 and therefore GABAergic excitatory function in spite of inhibition. Due to an unknown reason expression of NKCC2 cotransporter was enhanced in TLE patients, while expression of KCC1 shows contrary changes. These alterations lead to accumulation of Cl^- inside the cell, consequently driving force for Cl^- gets reversed. Whenever GABA acts on its receptor (Cl^- channel) Cl^- leaves the cell outwardly and raising membrane potential, it means cell get depolarized. So, GABA plays as an excitatory neurotransmitter 2. another main alteration; rearrangement and dysfunction of GABA receptors, include $GABA_A$ and $GABA_B$. In addition to ionic concentration, alteration of GABA-A and B receptors subtypes composition had been reported. 3. Synaptic reorganization, the last effect may be relatively small and seen only in some interneurons, and it may provide no more than partial compensation for the epilepsy-induced GABAergic interneurons loss. In addition, the observation that the mean amplitude of the unitary inhibitory presynaptic currents (IPSCs) was unchanged has other interpretations than the possible conclusion that the number of GABAergic synaptic connections from the somatostatin-immunoreactive interneurons in the reorganized hippocampus is the same as in normal brain. Several studies have suggested that the properties of GABAA receptors are altered during epileptogenesis, which might be expected also to modify the mean amplitude of unitary evoked IPSCs [74]; thus, changes in the amplitude of IPSCs could be related to both changes in connectivity and receptor subunits.

The conservative interpretations of the data serve to bold the complexity of the issue. Taken together such these changes cause dysfunction of GABA neurotransmission. In this review we only provide some of hypotheses of this issue and discount other probably mechanisms. We need more studies to detect all involved mechanisms of GABAergic signaling impairment in acquired epileptogenesis to address new effective therapeutic drugs or strategies for intractable and surgically non resectable TLE patients.

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