Potential role for ligand-gated ion channels after seizure-induced neurogenesis

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Abstract

Epileptic seizures result in an increased generation of new neurons in the dentate gyrus of the adult mammalian hippocampus. The role of these seizure-induced newborn neurons in the process of epileptogenesis remains largely unknown. Recent work, however, suggests an aberrant incorporation of newborn cells into the existing hippocampal network in such a way that they promote hippocampal hyperexcitability. In the present review, we discuss current knowledge about the possible role of seizure-induced newly generated neurons and the putative involvement of ligand-gated ion channels in the process of epileptogenesis.

Introduction

Neurogenesis, defined as a complex process starting with the division of a precursor cell leading to the integration of a new functioning neuron [1], has long been believed to be restricted to the prenatal period. In the 1960s, Altman and Das [2] described for the first time the generation of new neurons in the adult mammalian brain. Studies of the last few decades have provided evidence that neurogenesis persists in adulthood in two selected forebrain regions, namely (i) the SVZ (subventricular zone) which give rise to new interneurons in the olfactory bulb [3], and (ii) the SGZ (subgranular zone) of the hippocampal dentate gyrus [4]. By now, several factors have been found to alter neurogenesis, yet little is known of how newborn neurons blend in the existing neuronal network and what the functional consequences are. In the present paper, we review evidence suggesting that seizures cause newborn neurons to express an altered repertoire of ligand-gated ion channels, thereby affecting hippocampal physiology.

Neurogenesis in the adult hippocampal dentate gyrus

Adult hippocampal neurogenesis has been extensively studied by means of BrdU (5′-bromo-2′-deoxyuridine), a thymidine analogue which integrates into the newly synthesized DNA during the S-phase of mitosis. By making use of this method, Kuhn et al. [4] demonstrated in rats that new neurons are generated from a population of precursor cells being located in the SGZ of the dentate gyrus. Continuous generation of neurons in the adult dentate gyrus has been detected in a similar way in other species such as mice [5], monkeys [6] and, finally, also humans [7]. Newborn hippocampal cells migrate from the SGZ to the granule cell layer, where they differentiate into neurons, form dendritic arbours reaching the molecular layer and project axons into the CA3 region in rats [4,8] and mice [9]. Electrophysiological measurements, after in vivo labelling of the newly generated dentate gyrus cells with a GFP (green fluorescent protein)-expressing retroviral vector, show that newborn cells in the adult mouse hippocampus become functional neurons [10].

In young adult rats, each day, approx. 9000 new cells are generated, which is approx. 0.1% of the granule cell population [11]. The rate of neurogenesis in the adult primate hippocampus is estimated to be lower in comparison with rodents [6,7], i.e. in the macaque monkey, approx. 0.004% of the total granule cell population is renewed per day [6]. Several physiological and pathological factors are known to change the rate of adult SGZ neurogenesis. For instance, stress [12] and aging [4] decrease the rate of neurogenesis, whereas neurogenesis is stimulated by environmental enrichment [13], exercise [5], stroke [14] and seizures. Bengzon et al. [15] reported that even single brief seizures are sufficient to increase neuronal cell proliferation in the adult rat dentate gyrus. Similar increases have been demonstrated in other rodent models of seizures, such as those induced by amygdala kindling [16] and by electroconvulsive shock [17]. Status epilepticus elicited in rats by administration of chemoconvulsants such as pilocarpine, kainic acid or pentylentetrazole also increases neurogenesis [18,19]. A recent study shows that hippocampal neurogenesis is also stimulated after early-life febrile seizures induced by exposing young rat pups to a hyperthermia treatment [20,21]. The demonstration of increased hippocampal neurogenesis after seizures in different animal models has led to the hypothesis that newborn dentate gyrus neurons may contribute to the increased seizure susceptibility characterizing TLE (temporal lobe epilepsy), one of the most frequent seizure disorders.

Key words: adult neurogenesis, hippocampus, hyperexcitability, ligand-gated ion channel, seizure, temporal lobe epilepsy.

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AMPAR, AMPA receptor; GABA, γ-aminobutyric acid; GABAR, GABA type A receptor; HCN, hyperpolarization-activated cyclic nucleotide-gated cation channel; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; SGZ, subgranular zone; TLE, temporal lobe epilepsy.

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However, it remains controversial whether seizures increase hippocampal neurogenesis in epileptic patients. An increase of neuronal precursor cells in the hilus and the SGZ of the dentate gyrus is found in resected hippocampal tissue of TLE patients who were less than 2 years old [22]. On the other hand, Mathern et al. [23] reported a decreased neurogenesis in the dentate gyrus of older patients with seizure onset during childhood [23]. However, a more recent study on surgical hippocampal specimen from adult TLE patients demonstrated large numbers of neuronal progenitor cells in the SGZ [24]. Technical limitations might contribute to the observed conflicting results. Interpretation of the extent of proliferation may also vary according to the marker used to label immature neurons. Indeed, different immature neuronal markers display diversity in the duration of expression [25]. Furthermore, study of the human epileptic hippocampus is only possible after surgical removal of the epileptogenic zone in pharmacoresistant TLE patients who may represent only a subset of the TLE patient population. The individuals studied so far may reflect a diverse population. In those studies, hippocampi obtained during autopsies or tumour resection from patients without epilepsy or other neurological disorders were used as controls. Moreover, seizure-related neurogenesis could be influenced by the age of the patient at the onset of TLE, the individual genetic background as well as the seizure severity. Nevertheless, recent research by Parent et al. [26] points to the importance of increased neurogenesis at some point during the process of epileptogenesis.

Role of seizure-induced newborn neurons in the process of epileptogenesis

Hippocampi of both TLE patients and experimental rodent models of TLE exhibit characteristic morphopathological changes. A frequently observed morphological feature is neuronal cell loss due to seizure-related neuronal death. Neuronal degeneration is predominantly seen in the pyramidal cells of the CA1, CA3 and CA4 regions of the Ammon’s horn, whereas the CA2 pyramidal cells and the granule cells of the dentate gyrus are preserved in most cases. Initially, it was thought that newborn neurons could be beneficial by compensating for the neuronal cell loss. However, it has been suggested that seizure-induced newborn neurons may display a pro-epileptogenic role rather than a repair function. Indeed, it has been already demonstrated that suppression of seizure-induced neurogenesis attenuates later development of spontaneous recurrent seizures [27].

Approx. 20% of the newborn cells induced by experimental seizures survive [20]. The majority of these newborn cells differentiate into neurons in the dentate granule cell layer [21,28] and extend axons to the CA3 region [28]. Seizures accelerate the integration of newborn dentate granule cells, but the biological significance of this phenomenon has not yet been elucidated [29]. Following seizures, mossy fibre axons project abnormally to the supragranular inner molecular layer of the dentate gyrus and the stratum oriens of the CA3 region. But this reorganization of mossy fibres, described as mossy fibre sprouting, does not depend on newborn dentate granule cells [30]. However, adult born dentate granule cells show other abnormalities, including the formation of hilar basal dendrites. The generation of these dendrites, mainly originating from the granule cell somata at the hilar side and extending into the hilus, suggests that they may be postsynaptic targets of the mossy fibre collaterals [31]. Furthermore, Scharffman et al. [32] reported that some newborn granule-like neurons migrate aberrantly to the hilus and the molecular layer after pilocarpine-induced seizures in rats. Such ectopic cells could be relevant to TLE, since they are detected in the hilus and in the dentate molecular layer in surgically removed hippocampi of epileptic patients [26]. The ectopic granule-like neurons develop membrane properties, a firing behaviour, morphological properties and an immunocytochemical profile similar to granule cells of the granule cell layer. However, in contrast with the normal granule cells, ectopic granule cells show, in addition, spontaneous bursts of action potentials which are synchronized with CA3 pyramidal cell discharges [32]. These findings support the ‘neurogenesis hypothesis of epileptogenesis’ which states that seizure-induced newborn hippocampal neurons may incorporate abnormally into the existing hippocampal network in such a way that they promote hippocampal hyperexcitability [28,32,33].

Involvement of ion channels in the process of epileptogenesis

Electrophysiological measurements in several animal models of TLE demonstrate that seizures lead to long-term changes in neuronal excitability. The resulting hyperexcitability neuronal network are likely to be caused by alterations in neuronal excitation and/or inhibition in the hippocampus, from which the seizures originate. Ion channels are known to be key players in the control of neuronal excitability. Hence, a change in the expression and/or properties of ion channels is suggested as a pathophysiological mechanism underlying epileptogenesis.

The NMDAR [NMDA (N-methyl-D-aspartate) receptor] and the AMPAR [AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor] are ligand-gated ion channels that belong to the family of ionotropic glutamate receptors that are responsible for fast excitatory neurotransmission. In the kindling model of epilepsy, the synaptic transmission in the dentate gyrus is characterized by an increased contribution of NMDARs [34]. Furthermore, analyses of single NMDAR channels and whole-cell NMDA-gated currents in isolated dentate granule cells obtained from kindled animals show specific long-term alterations in the biophysical properties of NMDARs [35]. These reported functional changes may be related to an altered expression or modification in subunit composition of ionotropic receptors. Recent studies provide evidence that experimentally induced seizures can modify the hippocampal expression of NMDAR [36,37] and AMPAR subunits [38,39]. Additionally, human studies show greater NMDAR and AMPAR mRNA levels per neuron in hippocampi from TLE patients compared with
autopsies without cerebral pathology. Specifically, in dentate granule cells, NMDAR2 and AMPA GluR1 (glutamate receptor 1) mRNA levels display the strongest increase relative to the other subunits [40]. These changes in mRNA are paralleled by changes in protein levels [41].

Recent work focused also on the contribution of $I_h$-conducting HCN (hyperpolarization-activated cyclic nucleotide-gated cation) channels to neuronal excitability. Chen et al. [42] reported a depolarization shift of the HCN channel activation and a slowing of the kinetics, thus a change in the $I_h$ current which is associated with long-term hippocampal hyperexcitability in a rat model of febrile seizures. These functional changes are accompanied by an isoform-selective increase of HCN1 expression in surviving dentate gyrus granule cells of human and experimental chronic epileptic hippocampus [43].

Next to changes in excitatory systems, alterations in inhibitory circuits may prove to be equally important in the development of a hyperexcitable network. In this context, the ionotropic GABA$_A$R (GABA ($\gamma$-aminobutyric acid) type A receptor), known for its participation in mediating fast inhibitory neurotransmission, has received much attention. Altered functional properties of GABA$_A$Rs in hippocampal neurons are observed after the development of TLE. Gibbs et al. [44] demonstrated in the pilocarpine model that the whole-cell dentate granule GABA$_A$R maximal current response was increased, whereas the whole-cell CA1 GABA$_A$R maximal current response was decreased. These changes in GABA$_A$R function are associated with alterations in subunit expression and, hence, receptor composition [45–47]. Consistent with findings in several animal models of TLE, Loup et al. [48] found an altered GABA$_A$R subunit expression profile in hippocampal tissue of TLE patients. These changes are most pronounced in surviving dentate granule cells, which display an up-regulation of the $\alpha_1$, $\alpha_2$, $\beta_2/3$, and $\gamma_2$ subunits. It has been suggested that this increased expression of GABA$_A$Rs represents a compensatory response to the hippocampal hyperexcitability and recurrent seizures. The enhanced GABA$_A$R expression is accompanied by a polarized staining pattern with an increased staining on the soma membrane and apical dendrites and a significant reduction in staining in the basal dendrites of the granule cell. This redistribution of GABA$_A$Rs on dentate granule cells is supposed to be compensatory in response to the recurrent excitatory circuits resulting from mossy fibre sprouting [48].

Next to their role in inhibitory neurotransmission, GABA$_A$Rs can also have excitatory effects. A recent study in the pilocarpine model demonstrated a depolarizing shift in dentate granule cell $E_{\text{GABA}}$, resulting in an altered synaptic integration and an increased dentate granule cell excitability [49]. This switch in CI$^-$ homoeostasis is probably due to a down-regulation of the CI$^-$-extruding K$^+$–Cl$^-$ co-transporter (KCC2) synergistically with an up-regulation of the Cl$^-$–importing Na$^+$–K$^+$–2Cl$^-$ co-transporter (NKCC1) [49,50]. The expression of these two main neuronal Cl$^-$ co-transporters is likely to be influenced by brain-derived neurotrophic factor [50].

Taken together, the above-mentioned results describe altered inhibitory and excitatory neuronal hippocampal circuits following seizures. These alterations occur concurrently with an increased hippocampal neurogenesis induced by seizures. Until now, most of the studies reported a net change in excitatory and inhibitory receptor subunit expression, without addressing whether these changes can be attributed to old cells changing their expression profile or to cells that were generated after a seizure. Porter et al. [38] showed a differential alteration of AMPAR subunit expression in dentate granule cells that had been generated before and after seizures. This suggests different contributions of these two cell populations to the process of epileptogenesis. The findings also suggest that seizure-induced neurogenesis may be a source of long-lasting alterations in ligand-gated ion channel expression, and thereby of hippocampal hyperexcitability. Future research should demonstrate whether these neuroplastic changes can be a target for new anti-epileptic therapies.

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