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Pathophysiogenesis of Mesial Temporal Lobe Epilepsy: Is Prevention of Damage Antiepileptogenic?

G. Curia[1], C. Lucchi[1], J. Vinet[1], F. Gualtieri[1], C. Marinelli[1], A. Torsello[2], L. Costantino[3] and G. Biagini*[,1,4]

[1]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy;* [2]*Department of Health Sciences, University of Milano-Bicocca, 20900 Monza, Italy;* [3]*Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy;* [4]*Department of Neurosciences, NOCSAE Hospital, 41126 Modena, Italy*

Abstract: Temporal lobe epilepsy (TLE) is frequently associated with hippocampal sclerosis, possibly caused by a primary brain injury that occurred a long time before the appearance of neurological symptoms. This type of epilepsy is characterized by refractoriness to drug treatment, so to require surgical resection of mesial temporal regions involved in seizure onset. Even this last therapeutic approach may fail in giving relief to patients. Although prevention of hippocampal damage and epileptogenesis after a primary event could be a key innovative approach to TLE, the lack of clear data on the pathophysiological mechanisms leading to TLE does not allow any rational therapy. Here we address the current knowledge on mechanisms supposed to be involved in epileptogenesis, as well as on the possible innovative treatments that may lead to a preventive approach. Besides loss of principal neurons and of specific interneurons, network rearrangement caused by axonal sprouting and neurogenesis are well known phenomena that are integrated by changes in receptor and channel functioning and modifications in other cellular components. In particular, a growing body of evidence from the study of animal models suggests that disruption of vascular and astrocytic components of the blood-brain barrier takes place in injured brain regions such as the hippocampus and piriform cortex. These events may be counteracted by drugs able to prevent damage to the vascular component, as in the case of the growth hormone secretagogue ghrelin and its analogues. A thoroughly investigation on these new pharmacological tools may lead to design effective preventive therapies.

Keywords: Antiepileptic drug, astrocyte, blood-brain barrier, ghrelin, growth hormone secretagogue, hippocampus, mesial temporal lobe epilepsy, microglia, neuroinflammation, piriform cortex.

1. INTRODUCTION

Epilepsy is a major neurological disorder with a mean prevalence of, respectively, 0.52% in Europe, 0.68% in the USA and 1.5% in developing countries (reviewed by Strzelczyk *et al.*, [1]). In Europe, the prevalence of epilepsy differs only slightly in pediatric (0.45-0.5%), adult (0.6%), or aged (0.7%) patients. At the beginning of sixties, the prevalence of patients affected by temporal lobe epilepsy (TLE) was 1.7 per 1,000 people, with a corresponding rate of epilepsy in the whole population of 6.2 cases per 1,000 people [2]. However, patients suffering from TLE are those more frequently (41% of all cases) followed by centers dedicated to severe types of epilepsy, so that approximately 73% of patients requiring neurosurgery are affected by TLE. These data suggest that TLE represents a major burden for the health system. Accordingly, a prospective observational study on the burden of epilepsy in Italy showed that candidates to neurosurgery had the highest annual direct cost (€3619/patient), followed by pharmacoresistant subjects (€2190/patient). Using another standardized assessment index, the international dollar purchasing power parities, candidates to neurosurgery are 25% of all cases and account for $5401, whereas patients affected by pharmacoresistance are 20% of all cases and account for $3010. In Italy, it has been estimated that 16% of the total direct costs for antiepileptic drugs is on newly diagnosed epilepsy, 41% on patients with seizure remission, 58% on patients with occasional seizures, and 77% on patients with pharmacoresistant epilepsy [1]. For these reasons, pharmacoresistant TLE represents large part of costs required to the health system in order to assist patients affected by epilepsy.

The most rational therapeutic option for difficult-to-control epilepsy is prevention. Prevention has to be based on knowledge of the causes leading to the targeted disease. In the case of TLE, knowledge on the possible causes is still insufficient. In a remarkable percentage (up to 87%) of cases presenting with TLE and hippocampal sclerosis, usually indicated as mesial TLE [3-9], a primary precipitating injury taking place during infancy is reported in the medical record of the patient [3, 4]. Primary precipitating injuries may consist of febrile seizures (47%), head trauma (10%), birth injuries (19%) or *status epilepticus* (SE; 15%; Fig. **1**) [5], which

*Address correspondence to this author at the Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Laboratorio di Epilettologia Sperimentale, Università di Modena e Reggio Emilia, Via Campi, 287, 41125 Modena, Italy; Tel: +39 059 205-5747; Fax: +39 059 205-5363;
E-mail: gbiagini@unimore.it

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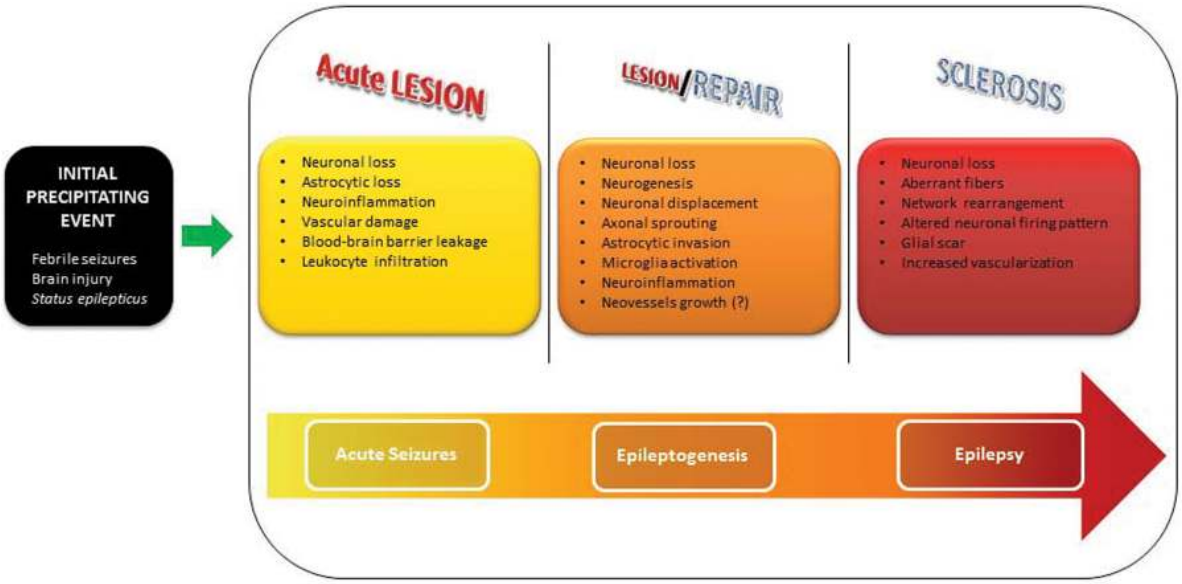


Fig. (1). Synopsis of the main findings of mesial temporal lobe epilepsy (TLE). The different phenomena described in tissue from patients treated with surgical resection of the epileptic foci, and in the brain of animal studied in TLE models, occur at a different extent in the acute period of TLE, during epileptogenesis, or in the chronic period of epilepsy. The failure in tissue recovery by activated repairing mechanisms leads to recurrent spontaneous seizures, frequently resistant to drug treatments.

can be defined as the loss of consciousness for at least 30 minutes or, in less severe cases, cognitive impairment for at least 4 hours [6]. According to a thorough investigation designed by Margerison and Corsellis [7] on 55 brains of patients affected by TLE, the most common histopathological feature is the loss of neurons, also known as "mossy cells", in the hilus of dentate gyrus, a phenomenon defined as "endfolium sclerosis". Additional injuries have been described also in other areas, of which the most frequent is *cornu Ammonis* (CA) sclerosis: a lesion characterized by severe loss of pyramidal neurons (the principal hippocampal cells), and gliosis in the CA1 area, in the prosubiculum (the so-called Sommer's sector) and, less consistently, in CA3b [8]. Hippocampal damage associated with TLE is frequently bilateral but asymmetrical, since one hippocampus is more damaged than the other [9]. Although less frequent, injuries are also found in the entorhinal cortex and amygdala of patients affected by mesial TLE [10], and probably occur also in other brain areas beyond those generally considered as the most vulnerable regions [11-14], and therefore more frequently investigated.

Basic histopathological features of TLE with hippocampal sclerosis are: i) loss of principal cells and interneurons, including changes in neuronal firing patterns related with altered composition or expression of receptors and channels; ii) structural adaptive reactions such as sprouting and neurogenesis with cell dispersion; iii) gliosis, including changes in functioning of glial cells; iv) loss of the integrity of blood-brain barrier; v) neuroinflammation. All these features have been described in surgical or *post mortem* samples obtained from patients suffering from pharmacoresistant TLE [8, 15-19]. Similar alterations have been demonstrated in animal models of TLE, which have been extensively reviewed by others [20-25] and by some of us [26]. Overall, the above mentioned histopathological changes are supposed to occur after the initial precipitating injury and are suspected to contribute to epileptogenesis (Fig. **1**), the process by which a chronic epileptic condition is finally established [27-29]. A crucial point is whether the prevention of structural changes, i.e. lesions, could be of help or not in preventing mesial TLE after a primary precipitating injury potentially able to cause hippocampal sclerosis. Secondarily, it has to be understood whether these phenomena are limited to one or few cerebral regions, in order to decide whether local treatment could be a good strategy to target the key mechanism(s) involved in TLE or, instead, whether multiple cerebral areas are involved, so making a systemic approach mandatory. Most of the studies on TLE focused essentially on changes occurring in the hippocampal formation [30]. However, lines of evidence suggest that other extrahippocampal regions are involved in TLE and could contribute to seizure generation [11, 12, 31-35]. Evaluation of patients affected by TLE without hippocampal sclerosis demonstrated the occurrence of volumetric reductions in regions such as entorhinal and perirhinal cortices, thalamus and amygdala [13, 36-40]. These findings were also supported by histological analyses of human epileptic tissue that showed the presence of selective neuronal loss and synaptic reorganization within extrahippocampal structures, independently of hippocampal sclerosis [10, 41-45]. This aspect of TLE has been overlooked also in animal models, in which the presence of extrahippocampal lesions has not received the necessary attention [46, 47].

We have recently reappraised the distribution of lesions in the pilocarpine and kainate models of chemoconvulsive SE. This has been reviewed also by others who focused mainly on neuronal cell loss occurring also in extrahippocampal regions [48, 49]. In their work, Covolan and Mello [49] found a large overlap of injuries caused by pilocarpine or kainate, which mainly differed for the extent and time

course of damage rather than localization. At variance, considering also damage occurring to astrocytes, identified by an antibody against glial fibrillary acidic protein (GFAP), as well as damage to blood vessels, using an antibody against laminin, we were able to disclose some clear cut differential aspects in lesions found in CA3 and entorhinal cortex of pilocarpine or kainate groups of treatment [50]. In particular, kainate damaged mainly the pyramidal cell layer in CA3, in which neurons identified by an antibody against the neuron-specific nuclear protein (NeuN) were almost completely ablated in the 3b sector, and presented a pattern of distribution largely superimposed to the loss of GFAP immunostaining and to the increased laminin immunoreactivity. At variance, in pilocarpine-treated rats a prominent astrocytic lesion occurred in the CA3 stratum lacunosum-moleculare, superimposed to a remarkable increase in laminin immunoreactivity with minimal neuronal losses. Almost absent neuronal damage was also noticed in regions of pilocarpine-treated rats such as the anterior olfactory nucleus, the piriform cortex and the amygdala (Table **1**), areas that are strongly interrelated [51, 52], whereas the pattern of astrocytic loss and vascular lesions were similar in both pilocarpine- and kainate-treated rats (*cf.* [50]). Interestingly, the changes in laminin immunoreactivity were inversely related to loss of GFAP immunostaining in the piriform cortex of both pilocarpine and kainate groups of treatment, suggesting a causal relationship between these two phenomena [50]. Notably, the piriform cortex was the only region in which the interrelationship between loss of GFAP and increase in laminin immunostaining was significant in both groups of chemoconvulsant administration. This phenomenon could be relevant for the development of the remarkable vasogenic edema observed in this limbic region [53, 54], and it further supports the hypothesis that end-feet of astrocytes may be disconnected from the basal lamina of capillaries after SE, causing the derangement of the blood-brain barrier [55, 56]. Investigation on animal models indicated the piriform cortex as one of the most epileptogenic regions in the brain [57, 58]. The piriform cortex was recently outlined as a region relevant for partial seizures also in patients [59], so that the pathophysiological mechanisms involved in damaging this region could be important targets for preventive therapies.

2. NEURONAL LOSS AND PLASTICITY

Many pathophysiological processes observed in the pilocarpine model, such as mossy fiber sprouting, interneuron loss, and granule cell dispersion in the dentate gyrus, have been demonstrated also in human TLE patients and are thought to contribute to epileptogenesis [8, 9].

2.1. Principal Cells

Neuronal loss has been reported in several brain areas of TLE animal models; among others, the hippocampus [60], subiculum [61-63], entorhinal cortex [41, 64-66], amygdala [67, 68], and thalamus [69, 70]. A marked reduction in the number of neurons is observed in the CA1 of pilocarpine-treated animals and in the CA3b pyramidal layer of kainate-treated rats (*cf.* [26, 27, 50]). A remarkable lesion occurs in the CA3 stratum lacunosum-moleculare, involving neuronal cell processes from the entorhinal cortex or from the pyramidal cell layer, and occasionally enlarging to involve the granular cell layer of dentate gyrus [50, 71]. The subiculum is reported to be only partially damaged [50, 62]. In the pilocarpine model of TLE, indeed, about 14% of ventral subicular neurons are lost in the molecular layer, while no loss is observed in the pyramidal cell layer [72, 73].

Patients affected by TLE present variable patterns of neuronal loss. In particular, hippocampal neuronal loss can be classified in 5 different patterns: 1. hippocampi with no significant difference in cell densities compared to age-matched autopsy controls (no mesial temporal sclerosis) (18%); 2. severe cell loss in CA1 and moderate neuronal loss in all other subfields excluding CA2 (19%); 3. extensive neuronal loss in all hippocampal subfields (53%); 4. severe neuronal loss restricted to CA1 (6%); 5. severe neuronal loss restricted to the hilar region (4%) [74]. Neuronal loss has been described also in the subiculum of patients with TLE [61]. In pilocarpine- and kainate-treated animals, cell loss is severe in layer III of medial entorhinal cortex [50, 64-66, 75]. In patients with TLE, a distinct loss of neurons is observed in the anterior portion of medial entorhinal cortex, it is more pronounced in layer III and less marked in layer II [10, 41].

Cell loss occurs in other areas in TLE animal models although with less severity and consistency. Neuronal loss is found in the perirhinal cortex in just 20-40% of TLE rats (depending on the used chemoconvulsant) and, occasionally, in some hypothalamic nuclei, such as the posterior hypothalamic nucleus [50]. Cell loss is detected in 20% of kainate-treated rats in the basolateral and lateral regions of amygdala, and in 60% of kainate-treated rats in piriform cortex and in the anterior olfactory nucleus (Table **1**), while no frank damage is found in these regions in pilocarpine-treated rats treated with diazepam 10 minutes after the onset of SE [50]. Neuronal lesions have been described also in the amygdala, thalamus and neocortex of TLE patients [8, 10-14]. In TLE patients with Ammon's horn sclerosis or focal lesion of the temporal lobe, neuronal density of the lateral amygdaloid nucleus is significantly decreased and the mean volumetric density is reduced to 59% as compared to normal controls [44].

Neuronal loss is not the only event characterizing human and animal TLE. Significant changes in neuronal soma size, number of dendrites and spine densities have also been observed in TLE patients compared to control individuals. In particular, the presence of subicular neurons with a reduced arborization and spine density in the proximal part of the apical dendrites suggests a partial deafferentation from CA1 [62]. In addition, amygdaloid neuronal bodies are smaller and neurons have fewer first-order dendrites, whereas the maximum density of spines per dendritic segment is increased [45]. Dendritic alterations, such as spine bifurcation, are also observed suggesting changes in synaptic connectivity.

It is unclear the meaning of principal cell loss in TLE. Some authors suggested that epileptogenesis could be independent of neuronal loss [76]. However, cell loss can be responsible for a different evolution of the disease, as suggested by studies performed in animal models, which showed more frequent and severe seizures in highly damaged rats [77, 78].

Table 1. Neuronal, Glial and Vascular Lesions in Pilocarpine and Kainate Models of TLE

	Pilocarpine		Kainate	
	% Animals	**Lesion**	**% Animals**	**Lesion**
NEURONAL LESIONS				
Hippocampal regions				
Cornu Ammonis 1	0*	-*/+#	0*	-*/++#
Cornu Ammonis 3	0*	-*/++#	100*	+*/++#
Parahippocampal regions				
Ventral subiculum	40*	+*/-#	80*	+*/+#
Dorsal subiculum	0*	-*/-#	0*	-*/+#
Medial entorhinal cortex	100*	+*/-#	100*	+*/++#
Extrahippocampal regions				
Perirhinal cortex	20*	+*/-#	40*	+*/+#
Amygdala	0*	-*/-#	20*	+*/-#
Agranular insular cortex	0*	-*/+#	0*	-*/+#
Olfactory regions				
Piriform cortex	20*	+*/+#	60*	+*/-#
Anterior olfactory nucleus	0*	-*	60*	+*
Thalamic regions				
Paraventricular thalamic nucleus	0*	-*/-#	0*	-*/++#
Reuniens thalamic nucleus	100*	++*/-#	80*	++*/+#
Paratenial thalamic nucleus	20*	+*	40*	+*
Submedius thalamic nucleus	40*	+*	20*	++*
Subparafascicular thalamic nucleus	40*	+*	60*	+*
Central medial thalamic nucleus	0*	-*/-#	100*	++*/++#
Mediodorsal thalamic nucleus	40*	++*/-#	80*	++*/+#
Geniculate body	0*	-*/+#	0*	-*/++#
Rostral regions				
Tenia tecta	0*	-*	40*	+*
Accumbens	20*	+*	20*	+*
Posterior hypothalamic nucleus	20*	+*	20*	++*
Paraventricular hypothalamic nucleus, parvicellular part	40*	+*	60*	+*
Caudal regions				
Zona incerta	0*	-*/+#	0	-*/++#
Substantia nigra, reticular part	40*	++*/-#	40*	++*/-#
Red nucleus, parvicellular part	0*	-*	20*	+*
Raphe	20*	++*	0*	-*
GLIAL LESIONS				
Hippocampal regions				
Cornu Ammonis 3	100*	++*	100*	+*

(Table 1) contd….

	Pilocarpine		Kainate	
	% Animals	**Lesion**	**% Animals**	**Lesion**
Parahippocampal regions				
Medial entorhinal cortex	20*	+*	100*	+*
Extrahippocampal regions				
Perirhinal cortex	40*	+*	40*	+*
Amygdala	60*	+*	40*	+*
Olfactory regions				
Piriform cortex	80*	++++*	60*	+++*
Anterior olfactory nucleus	40*	+*	80*	+*
Thalamic regions				
Paraventricular thalamic nucleus	20*	++*	60*	+*
Reuniens thalamic nucleus	60*	++*	60*	+*
Submedius thalamic nucleus	20*	+++*	0*	-*
Subparafascicular thalamic nucleus	20*	+*	20*	+*
Central medial thalamic nucleus	0*	-*	40*	+*
Mediodorsal thalamic nucleus	20*	++++*	40*	++*
Rostral regions				
Medial septum	0*	-*	40*	+*
Caudal regions				
Substantia nigra, reticular part	40*	++*	0*	-*
Tegmentum	20*	+*	40*	+*
VASCULAR LESIONS				
Hippocampal regions				
Cornu Ammonis 3	100*	+*	100*	+*
Parahippocampal regions				
Ventral subiculum	0*	-*	20*	+*
Medial entorhinal cortex	0*	-*	40*	+*
Extrahippocampal regions				
Perirhinal cortex	60*	+*	60*	+*
Amygdala	40*	+*	60*	+*
Olfactory regions				
Piriform cortex	100*	+*	80*	+*
Anterior olfactory nucleus	40*	+*	80*	+*
Thalamic regions				
Reuniens thalamic nucleus	20*	+*	40*	+*
Anteroventral thalamic nucleus	20*	+*	20*	+*
Submedius thalamic nucleus	20*	+*	40*	+*
Central medial thalamic nucleus	0*	-*	80*	+*
Mediodorsal thalamic nucleus	0*	-*	40*	+*
Geniculate body	0*	-*	60*	+*

(Table 1) contd….

	Pilocarpine		Kainate	
	% Animals	**Lesion**	**% Animals**	**Lesion**
Rostral regions				
Accumbens	60*	+*	20*	+*
Stria terminalis	40*	+*	40*	+*
Medial preoptic nucleus	0*	-*	20*	+*
Posterior hypothalamic nucleus	20*	+*	60*	+*
Globus pallidus	0*	-*	40*	+*
Lateral septum	0*	-*	20*	+*
Caudal regions				
Zona incerta	0*	-*	20*	+*
Substantia nigra, reticular part	40*	+*	40*	+*
Raphe	0*	-*	40*	+*
Tegmentum	40*	+*	80*	+*

Quantification of lesions in pilocarpine- and kainate-treated rats. Animals presenting lesion in investigated areas are illustrated by percentages. The lesion extent is presented by a scale ranging from no lesion (-) to maximal lesion (++++). Data are from ref [49] (#) and from ref [50] (*). Discrepancies between the two studies may be due to different methods. Gualtieri *et al.*, [50] administered diazepam (20 mg/kg) 10 minutes after SE onset, while Covolan and Mello [49] administered thionembutal (25 mg/kg) 90 minutes after SE onset. Gualtieri *et al.* used NeuN as neuronal marker and quantified neuronal lesion extension measuring area of disappeared NeuN immunoreactivity. Covolan and Mello used silver impregnation, which selectively stains injured neurons, and quantified neuronal lesion as percentage area in each investigated structure containing silver stain. Finally, Gualtieri *et al.* harvested tissues 4 days after SE, while Covolan and Mello collected tissue 48 h after SE.

2.2. Interneurons

Patients affected by TLE present variable patterns and extensions of neuronal loss, but the hilus of dentate gyrus seems to be particularly susceptible [7], so to be spared in a minority (11%) of patients [79]. Many hilar neurons are γ-aminobutyric acid (GABA)ergic, and number of GABAergic interneurons are reduced in the dentate gyrus of patients with TLE [80-83] and rodent models [84-90]. The loss of GABAergic interneurons could reduce inhibition of dentate gyrus, thereby lowering seizure threshold. Consistent with this hypothesis, frequencies of miniature inhibitory postsynaptic currents in granule cells are low in epileptic rats [91-93], see Leroy *et al.* [94]. Injured neurons, mainly interneurons, can be found also in the hippocampus (CA1 and CA3 stratum pyramidalis and radiatum), amygdala and piriform cortex [95].

Interneurons can be distinguished in different subclasses based on their protein expression, and alterations in TLE are not homogeneous among them. Parvalbumin-positive interneurons can be found in the CA1, in the subiculum, and in the granule cell layer of the hippocampal formation in control tissue. In pilocarpine-treated rats a substantial reduction of parvalbumin-positive interneurons is evident in the subicular pyramidal cell layer, while a smaller loss is detected in the subicular molecular layer [63, 72]. Loss of parvalbumin-positive cells is also described in CA1, dentate hilus, deep layers of entorhinal, perirhinal and insular cortices in pilocarpine-treated rats [63, 73, 87, 88, 96-98].

In humans affected by TLE, parvalbumin cell loss is reported for neocortex and hippocampus [15, 99], however different patterns can be observed [100]. In human epileptic CA1 region with mild cell loss, a slight loss of parvalbumin-positive cells is observed, mostly in the stratum oriens. In human epileptic CA1 with patchy cell loss, the decrease in number of parvalbumin-positive cells is more pronounced. In the strongly sclerotic human tissue, the number of parvalbumin-positive cells is dramatically decreased, only a few dendrites are usually present in the region, and axon terminals can hardly be found. In human tissue from TLE patients with strong hippocampal sclerosis, parvalbumin-positive cells can hardly be found in CA1, whereas in the subiculum number and distribution of parvalbumin-positive neurons seem to be unchanged [100].

Several investigators report variable loss of other types of interneurons. Neuropeptide Y-positive cells are decreased in perirhinal and insular cortices of pilocarpine-treated rats [97, 98]. Cholecystokinin-positive cells are reduced in superficial layers of perirhinal cortex [98]. Calbindin-positive interneurons are also decreased in pilocarpine-treated mice [73]. Loss of calretinin-positive cells is significant in the molecular layer, while it is less consistent in pyramidal cell and polymorphic layer in the subiculum of TLE animals [72]. Glutamic acid decarboxylase-positive interneurons are reduced in the subiculum (all layers) and in CA1 stratum oriens, but not in pyramidal cell layer and stratum lacunosum-moleculare [72].

Interestingly, interneurons were shown to be progressively reduced during epileptogenesis, and this phenomenon was hypothesized to be associated with the appearance of spontaneous recurrent seizures [88, 101]. Interneuron loss after intensive kindling was associated with the development of recurrent spontaneous seizures [89]. Thus, loss of interneurons appears to be a phenomenon involved in epileptogenesis, but mechanisms responsible for cell loss are still not defined. We [19] recently investigated this phenomenon by using the biomarker of hypoxia exposure pimonidazole (Fig. **2**), also known as the "hypoxyprobe" [102, 103]. Pimonidazole, administered in rats presenting spontaneous recurring seizures, was revealed with a specific antibody and, in double immunofluorescence confocal microscopy experiments, was found in interneurons expressing neuropeptide Y in neocortex (Fig. **2**) and in other brain regions [19]. These findings suggest that a subtle, cell-specific damage may occur in the brain exposed to recurrent seizures, affecting a population of interneurons critically involved in the control of seizure activity. Importantly, repeated hypoxic seizure-related insults could explain the progressive course of some epileptic disorders like TLE with hippocampal sclerosis, in which neuropsychological and morphometric neuroimaging studies have demonstrated chronic temporal and extra-temporal damage [104, 105]. In the long term, this process could contribute to aggravate the course of epilepsy.

2.3. Adaptive Changes

Intracellular injection of depolarizing current pulses induces two patterns of firing in principal cells in normal tissue: the first consists of regular repetitive firing, while the second one is characterized by an initial burst of action potentials followed by regular firing. In the subiculum and entorhinal cortex, the incidence of regular firing neurons is higher compared to intrinsically bursting neurons and with similar proportions in pilocarpine-treated and in control tissue [63, 96]. However, as observed for insular cortex, adaptation rate of regular firing neurons is lower in pilocarpine-treated slices, and single-shock stimulation induces bursting responses with higher incidence compared to non-epileptic tissue [97], suggesting that different brain regions are variably hyperexcitable in TLE animal models.

Subicular neurons recorded intracellularly from pilocarpine-treated rats generate bursts or doublets of action potential in response to stimuli delivered in the CA1 stratum radiatum; in contrast, this stimulation protocol disclose a single action potential, followed by hyperpolarization, in control rats [63]. Similar finding is observed in layer V neurons of lateral entorhinal cortex. They responded to single-shock stimulation with a postsynaptic depolarization followed by a biphasic hyperpolarization in control tissue, and with action potential bursting (a depolarizing envelope overridden by action potential bursting) in pilocarpine-treated slices [96].

Dysfunction of $GABA_A$ receptor-mediated inhibition is characterized by positive shift of the reversal potential of inhibitory postsynaptic potentials coupled with a decreased peak conductance. These alterations are caused by a reduction in the expression of potassium-chloride transporter member 5 along with a decrease number of parvalbumin-positive interneurons in subiculum, perirhinal and insular cortices [63, 97, 98].

2.4. Sprouting

Mossy fiber sprouting has been described in dentate gyrus, subiculum and entorhinal cortex of epileptic rats [63, 88, 106, 107], and in the dentate gyrus and CA3 of TLE patients [107-109]. Sprouting of mossy fiber in the inner molecular layer of the dentate gyrus occurs in the kainate [110], kindling [111] and pilocarpine [112] models, and in patients with TLE [107]. Sprouted mossy fibers establish synaptic connections with newly generated hilar basal dendrites in kindling model of TLE [113], with granule cells and in-

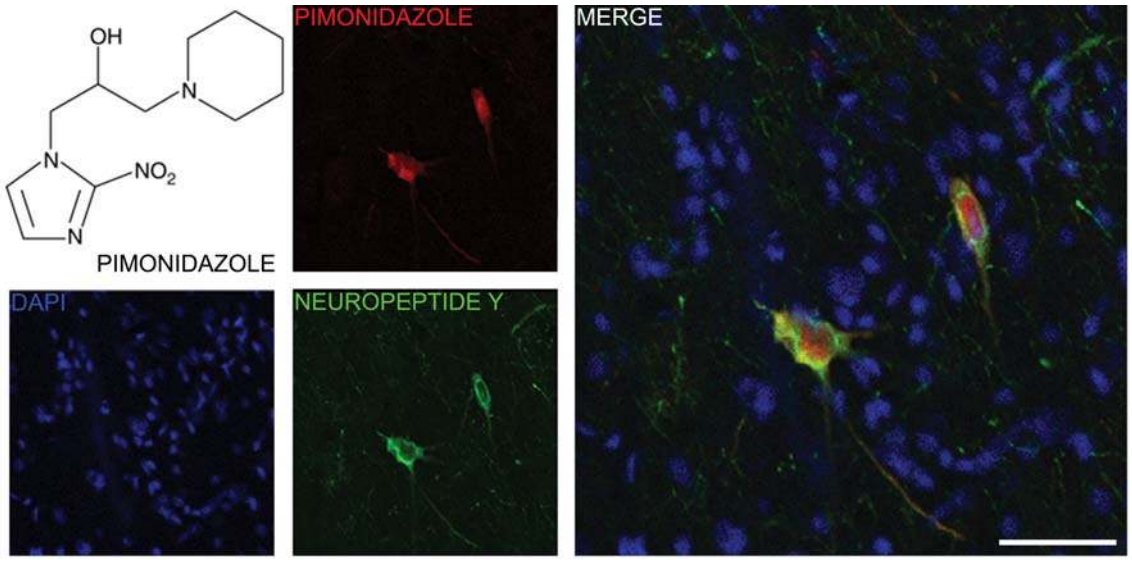


Fig. (2). Interneurons expressing neuropeptide Y bind the hypoxia marker pimonidazole in epileptic pilocarpine-treated rats. Hypoxia was probed with pimonidazole and revealed by an anti-pimonidazole antibody in rats that developed spontaneous recurrent seizures, after *status epilepticus* (methods as in [19]). Photomicrographs illustrate the co-localization of neuropeptide Y and the hypoxia marker pimonidazole in neocortex. Cell nuclei are stained in blue using 4',6-diamidino-2-phenylindole (DAPI). Scale bar, 37.5 µm.

terneurons of dentate gyrus [114, 115], but also with CA1, CA3 and subicular neurons [116-119]. Sprouted mossy fibers can establish synaptic connections with glutamate receptor 1-, calbindin-, calretinin- and parvalbumin-immunopositive neurons [118, 119].

Sprouting of axon collaterals of CA1 pyramidal cells occurs in kainate and pilocarpine models, and in patients with TLE [117, 120]. In addition to CA1 itself, CA1 fibers extend to CA3 stratum radiatum and to subiculum [117, 121]. CA3 sprouted fibers project to CA1, subiculum, presubiculum and parasubiculum, and medial and lateral entorhinal cortex [122]. Sprouted axons from neurons in the entorhinal cortex project to the subiculum, amygdala, and perirhinal cortex in mouse pilocarpine model of TLE [60]. Basolateral amygdala neurons sprouted axons project to the ventral subiculum and to the perirhinal cortex. These axons and terminal boutons became extremely large or even aggregated in the mouse pilocarpine model [60].

A number of studies addressed the role of mossy fiber sprouting in TLE (*cf.* [107]). In view of the particular time course of mossy fiber sprouting, it looked attractive to hypothesize that epileptogenesis could correspond to the period required for the growth of functionally active sprouted axons. However, several reports confuted this view [123-126].

2.5. Neurogenesis

Neurogenesis is a developmental process that involves the proliferation, migration and differentiation of neuroblasts and the synaptic integrations of newborn neurons with previously established circuitries (*cf.* [127-129]). Neurogenesis in adult brain largely occurs in two areas, the subventricular zone of the anterior lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus, but many agree that adult neurogenesis could occur at other sites, such as the striatum [130-133].

In this section we will focus on neurogenesis in the dentate gyrus, since this is the primary neurogenesis site in the temporal lobe, key structure for mesial TLE. The newly born granule cells in the dentate gyrus resemble other granule cells: they send axonal projections to their normal target zone, the mossy fiber pathway [134], and develop electrophysiological properties similarly to other granule cells [135]. Newly born neurons from the subgranular zone are functionally integrated into the existing hippocampal circuitry [136] and may provide network plasticity necessary for certain forms of hippocampus-dependent learning and memory. Progenitors can differentiate into glia and GABAergic interneurons [133]; however, these fates appear to be relatively rare compared to the proportion of cells that become granule cells.

Neurogenesis in the adult dentate gyrus is regulated by a variety of factors: aging, stress, exercise, growth factors, neurotransmitters, hormones. Seizure activity also influences dentate granule cell neurogenesis. Dramatic increases of neurogenesis occurs in human hippocampus [137-139] and in animals after acute seizures induced by kindling [140-142] or by electroconvulsive shock [143, 144], but also after SE initiated by systemic administration of pilocarpine [145] or unilateral intracerebroventricular injection of kainate [146, 147]. In chronic epilepsy, however, decreased dentate gyrus neurogenesis is evident in human TLE patients and in animal models of chronic TLE [138, 148-150]. The substantially declined dentate gyrus neurogenesis seems to be associated with spontaneous recurrent seizures, learning and memory impairments, and depression present in most of TLE patients [151-154]. In the chronic epileptic rat model of TLE, however, it has been hypothesized that the severely diminished dentate gyrus neurogenesis is not associated with either decreased production of new cells or reduced survival of newly born cells in the subgranular zone and in the granular cell layer. Rather, it may be linked to a dramatic decline in the neuronal fate choice of newly generated cells. Indeed, in the dentate gyrus of control tissue, the majority of the adult-born neurons become granule cell, which are primary cell types in the region, and use glutamate as a neurotransmitter [155], while in epileptic tissue newly born cells differentiate primarily into glial cells [156].

In addition to proliferation, seizures may alter also migration of the newly born cells, giving rise to ectopic granule cells [157, 158]. However, there is a second hypothesis to explain the presence of ectopic granule cells in the hilus. In normal development, granule cells are primarily born in the hilus; as the dentate gyrus matures the primary site of proliferation shifts from the hilus to the subgranular zone [159]. There is evidence that not all adult-born granule cells follow such a normal pathway, therefore some of them are located in the hilus. These hilar ectopic granule cells are rare in normal adult Sprague-Dawley rats, but their number is greatly increased in animal models of TLE [116, 145, 160, 161]. The ectopically placed granule cells integrate abnormally into the CA3 network [116], and establish afferent connectivity with mossy fiber terminals [162], exhibit spontaneous bursts of action potentials [116], and may therefore contribute to network hyperexcitability.

Ectopic granule cells are not detected in 100% of tissue from patients with intractable TLE. This may be due to the fact that ectopic cells are not always present, are sometimes underestimated or missed, or do not exist in patients with severe hippocampal damage, where all kinds of hilar neurons are lost. Patients without severe sclerosis may contain more ectopic granule cells, but more studies are necessary for conclusive statements [128]. In addition to ectopic hilar cells, also granule cell layer dispersion is observed in animal and human TLE [80, 108, 139, 163]. Dispersed granule cells, which lead to an irregular widening of the granule cell layer, are detected in 50% of TLE patients with hippocampal sclerosis [164]. Dispersion of granule cell layer has been initially hypothesized to be due to an excessive neurogenesis; however, recent evidences show that it may be due to displacement of mature neurons [165].

2.6. Functional Significance

It is generally accepted that TLE is related to an impaired balance between excitation and inhibition where excitation is in excess and inhibition is reduced compared to control tissue [100]. This unbalance may be stronger in some regions. For example, in the mouse model of TLE, significant loss of the total neurons (both principal and interneurons) in layers II-III of entorhinal cortex, and drastic loss of interneurons in

layer IV-VI of entorhinal cortex, may result in a marked unbalance between excitatory and inhibitory components [166]. In addition, an excess excitation is provided by the sprouted CA1 pyramidal cell axons [117, 167, 168], as well as by the sprouting of afferents from the dentate gyrus, CA3, and/or the CA2 region [169-172], and/or from extrahippocampal pathways [168]. Certain populations of dendritic inhibitory cells survive in epilepsy, and participate in the intense synaptic reorganization [172], while others are lost [82, 173]. Perisomatic inhibitory input is preserved in the non-sclerotic epileptic CA1 region, where pyramidal cells survive. The preserved perisomatic inhibition together with an excess of excitation and an impaired dendritic inhibition might lead to an abnormal synchrony in output regions of the hippocampus, while still contain large enough numbers of projecting neurons [100].

Network reorganization in pilocarpine-treated animals occurs presumably as consequence of neuronal loss and SE-induced sprouting [117]. SE also appears to induce formation of ectopic cells that are recruited into the nascent network [174]. Studies in cultures show that kainate-induced injury precedes the increase in neurogenesis [175]. However, an argument against the role of seizure-induced injury in stimulating proliferation is the fact that proliferative progenitor response can occur independently of cell death [176]. In addition, data report that newly born neurons do not necessarily survive for long periods of time [140, 177].

3. GLIAL CELLS

Several lines of evidence indicate that also astrocytes degenerate in rats exposed to SE [71, 178, 179]. We have recently confirmed that damage to astrocytes can be detected in a variety of brain regions [50]. In addition, astrocytes are activated by seizures and neuronal damage and, in response to these events, produce gliosis leading to scar formation. Also microglia are activated in response to seizure and participate in the modulation of neuroinflammation. These different populations of glial cells are now recognized to have a growing role in the pathophysiology of mesial TLE.

3.1. Astrocytes

Since the discovery that astrocytes are able to release transmitters (gliotransmitters) such as adenosine, adenosine triphosphate, D-serine, GABA and glutamate [180-182], they are now considered as major players in modulating neuronal transmission [183-186], including synaptic plasticity and postsynaptic currents [182, 187-189]. Thus, they play a major role in neuronal synchronization in undamaged tissue: glutamate released by astrocytes in response to calcium oscillations in the hippocampus contributes to neuronal firing synchronization [188]. Astrocytes thus can potentially participate in generating seizures in the presence of ictogenic stimuli. Indeed, the frequency of astrocytic calcium oscillations is increased during epileptiform activity [189], and calcium elevation in astrocytes correlates with the initiation and maintenance of ictal-like activity, which is further enhanced by stimulation of astrocytic calcium signaling [190]. This neuron-astrocyte excitatory loop could be responsible in focal seizure promotion [191, 192]. Recently, the cannabinoid receptor 1, which is expressed in astrocytes, has been shown also to modulate epileptiform activity [193].

Alterations in glutamate and GABA metabolism/transmission by astrocytes could have major consequences on the ability of neurons to synchronize and generate epileptic activity. Decreased astrocytic expression of glutamine synthetase in epileptic brain could lead to extracellular glutamate accumulation by impairing glutamate uptake function [194, 195], theory supported by observation of ictogenesis in knockout animals for the glutamate GTL-1/EAAT2 transporter [196]. Moreover, a dysfunction in astrocytic GABA transporter GAT-1 of the thalamus is associated with absence seizures [197]. Astrocytes can also release neurosteroids, such as dehydroepiandrosterone and pregnanolone and their sulfated forms, which are potent modulators of glutamatergic transmission [198-201]. Pregnanolone sulfate and other neurosteroids can also modulate GABAergic transmission [202]. Thus neurosteroid synthesis and release by astrocytes may initiate and sustain ictal-like synchronization.

Moreover, astrocytes in epileptic brain undergo activation especially in presence of brain damage, perhaps as recovery mechanism. These reactive astrocytes release trophic factors [203], cytokines [204], as well as neurosteroids [205, 206], which may alter their modulatory properties towards neuronal transmission. For example, increase of reactive astrocytes in CA1 leads to impairment of inhibitory synaptic currents, which is paralleled by a relative increase of synaptic excitability consequent to impairment of the astrocytic glutamate-glutamine cycle [207]. Moreover, it was observed in epileptic mice that the non-overlapping domains organization of astrocytes is partially lost during gliosis, leading to morphological changes in neurons: hypertrophy of apical dendrites and increased spine density [208]. Astrocytes are also involved in metabolizing the endogenous anticonvulsant adenosine by the enzyme adenosine kinase (ADK). Interestingly, the reactive gliosis observed in ADK-deficient mice after kainate-induced neuronal damage is not accompanied by development of spontaneous recurring seizures [209].

The contribution of astrocytes in modulating epileptogenesis and ictogenesis could be greatly altered in presence of brain lesions, such as those observed in TLE [8] and animal models [50]. Several lines of evidence indicate that astrocytes are lost in several brain regions in pilocarpine-treated rats (Table **1**) [50, 71, 178, 179]. The presence of injuries to astroglial network has been found even after early diazepam administration [50]. In line with other investigations [210], we also observed areas of glial lesion in kainate-treated rats (Table **1**) [50]. Although the areas of neuronal damage were approximately equivalent to those of astrocytic loss in pilocarpine-treated rats, neurodegeneration was more widespread in the kainate model. Areas of suppressed GFAP immunostaining were consistently observed in the CA3b sector and medial entorhinal cortex deep layers in kainate-treated animals [50]. In addition, anterior olfactory nuclei and piriform cortex frequently displayed focal abolition of the GFAP immunostaining after kainate (Table **1**). Involvement of astrocytes in pilocarpine-induced damage was also a frequent finding in piriform cortex (Table **1**). This phenomenon is certainly not related to apoptotic mechanisms, al-

though they were found to be involved in astroglial cell death following SE [179], but it is probably explained by the occurrence of multiple events of focal pannecrosis [211], as found in the CA3 of pilocarpine-treated rats [71]. The nature of these pannecrotic lesions is still incompletely determined, but an excessive glutamate accumulation may play a role [212]. On the other hand, the astroglial lesion found in the CA3 stratum lacunosum-moleculare of pilocarpine-treated rats appeared to be of vascular origin [71].

3.2. Microglia

Microglia, the immune cells of the brain, are characterized by ramified processes that constantly survey their environment [213]. Microglia express various neurotransmitter receptors as well as a panoply of molecules that can modulate neuronal transmission [214], suggesting that microglia play a major role in the control of neuronal activity. Ramified microglia have been shown to modulate rapidly their contact with synaptic elements during neuronal activity [215]. Live-imaging experiments performed in zebrafish larva demonstrated that microglia rapidly make contact with the synapses of the most active neurons, which result in a rapid decrease in the frequency and amplitude of calcium events from these neurons [216]. Moreover, neurons treated with conditioned-medium from microglia cultures shown an increase in amplitude and duration of N-methyl-D-aspartate (NMDA) receptor-induced currents [217, 218]. These data clearly indicate that microglia can modulate neuronal activity. Many factors have been proposed to play a role in this neuronal transmission modulation. First, it is well known that a communication between neurons and microglia exists. Indeed, microglia express C-X3-C motif (CX3C) chemokine ligand 1 (CX3CL1) receptor (CX3CR1) and cluster of differentiation (CD) 200 receptor (CD200R); CX3CL1 and CD200 are secreted/expressed by neurons. Interestingly, animals knockout either for CX3CR1 or CD200 display impaired long term potentiation [219, 220], suggesting an involvement of both signaling pathways in synaptic plasticity. This is further supported by data showing that treatment of either neuronal cultures or acute hippocampal slices with CX3CL1 induces a strong modulation of neuronal calcium currents and a reduction of excitatory postsynaptic currents in CA1 [221, 222].

During stress or injury, microglia become rapidly activated: their processes become thicker and shorter and they secrete a large repertoire of cytokines, chemokines and growth factors [223], which could alter the control on neuronal transmission. For example, brain-derived neurotrophic factor (BDNF) secreted by activated microglia provokes a shift in the chloride gradient of nociceptive neurons leading to an increased excitability of these neurons through a $GABA_A$-mediated depolarization and ultimately causing allodynia and chronic pain [224, 225]. In epilepsy, morphologically-activated microglia have been observed in both animal models as well as human patients, suggesting that microglia play a prominent role in the disease [24]. Cytokines like interleukin (IL)-1β and tumor necrosis factor α (TNFα), which are expressed by activated microglia, have been shown to increase neuronal excitability in brain slices and, thus, might be involved in the development of epileptic activity [226-229]. Activation of Toll-like receptor 4 (TLR4) on microglia, which happens during bacterial infections but also through endogenous TLR4 ligands [230], was shown to increase the frequency of spontaneous 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) postsynaptic currents in CA1 [231], and also to increase local neuronal excitability leading to seizures [232].

4. BLOOD-BRAIN BARRIER

Lesions and changes in permeability of the blood-brain barrier in experimental epilepsies have been described in the late 60's [233, 234]. This blood-brain barrier leakage is characterized by the entry into the brain of various tracers during experimental epilepsy [235], as well as proteins such as serum albumin [236]. It was recently shown that serum albumin induces the release of transforming growth factor β (TGF-β) by astrocytes that, by an autocrine mechanism, can activate TGF-β receptor II, resulting in the downregulation of the inward rectifying potassium channel Kir4.1 [237]. This can lead to impaired extracellular potassium regulation, which disturbs osmotic balance and water regulation and increases chance of seizure occurrence [238]. This is further demonstrated in conditional knockout mice or mice carrying mutations in Kir4.1 channels that are associated with epilepsy [239, 240]. Moreover, potassium entry into the cells is accompanied by entry of water through the glial water channel aquaporin-4 (AQP4) to maintain osmotic balance. Interestingly, AQP4 expression is reduced in the kainate model of epilepsy, which might impair water delivery to the extracellular space and increase excitability [241]. This is further confirmed in mice lacking AQP4, in which seizure susceptibility is increased [242], and in patients affected by mesial TLE, where astrocytic AQP4 is downregulated [243]. Overall, studies have shown that blood-brain barrier leakage could be implicated in induction of seizures and progression of epilepsy [244-246].

4.1. Clinical Evidence of Blood-Brain Barrier Dysfunction in Epileptogenesis

Clinical data indirectly suggest a role for blood-brain barrier leakage in epileptogenesis [247]. The most frequent causes of blood-brain barrier dysfunction are stroke and traumatic brain injury. In the case of stroke, blood-brain barrier leakage was found in one third of patients acutely investigated with the tracer gadolinium [248]. The observation that stroke is the most frequent cause of epilepsy in aged people is indeed suggestive for a role of blood-brain barrier leakage in epileptogenesis [249]. Traumatic brain injuries are also frequently followed by development of epilepsy. It has been reported that the incidence of epilepsy ranges from 7% to 39% for closed-head injuries, and it reaches 57% in the case of penetrating injuries (reviewed in [250]). The mechanisms involved in disruption of the blood-brain barrier following stroke or closed-head injuries are still unclear, but they involve the release of matrix metalloproteases 2 and 9, reactive oxygen species, aquaporins and inflammatory mediators [249]. An indirect marker of blood-brain barrier dysfunction is represented by protein S100B [251], that belongs to the family of calcium binding proteins. This protein is specific for the central nervous system and is found in a variety of cell types [252]. Interestingly, it has been reported that plasma S100B levels are elevated in patients affected by

TLE compared with healthy controls, suggesting a malfunction of blood-brain barrier in the chronic phase of the disease [253]. Unfortunately, no prospective studies are currently available on protein S100B plasma levels in subjects at risk to develop TLE after a primary injury, so that it is not possible to rule out a role of blood-brain barrier disruption in the course of epileptogenesis.

5. NEUROINFLAMMATION

Inflammation can occur as a defense/repairing reaction to damage of proper cellular components of a tissue, or it can be caused by injuries to blood vessels vascularizing that tissue. Thus, inflammation can originate from blood vessels and then extend toward the interstitium or by the reverse process. For decades, the brain has been considered a special protected environment in which an inflammatory reaction could exceptionally occur, mainly in response to neuronal injuries [254]. The reason for which this opinion has not been challenged for a long time is that the blood-brain barrier represents a sort of wall separating inflammatory cells and pathogens circulating in the blood from the central nervous system. However, recent evidence suggests that a breach in the blood-brain barrier is not strictly required to allow an inflammatory process to take place in neural tissue [255]. Notably, clinical studies have shown that a long-lasting, chronic neuroinflammatory reaction can occur in regions far from the initially injured one, as well documented in the case of stroke [256] and of traumatic injury [257]. These data raised the hypothesis that, in certain circumstances, neuroinflammation is triggered directly within the neural tissue, probably because of a dysregulation, maybe in the functioning of microglia [254, 255]. This hypothesis is at odds with the more traditional view of inflammation, which is based on blood vessel changes, first involving vasodilation and loss of integrity in the separation between blood and interstitium, connoted by plasma extravasation, and followed by leukocyte migration through the vessel wall, as also documented in models of TLE [258].

5.1. Inflammatory Mechanisms in Epileptogenesis

In the last decade, inflammation has become a fundamental factor in seizures initiation and maintenance. As previously stated, both activated microglia and reactive astrocytes are observed in epileptic patients and animal models, and, through the release of inflammatory cytokines, can alter neuronal excitability and synchronization, thus increasing seizures probability. Both cell types can release pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα, which are thought to be responsible for the neuronal cell death occurring after SE [24]. This neuronal cell death leads to major network reorganization believed to be involved in development of chronic epilepsy [24, 28, 29]. However, inflammation is normally directed toward resolving the problems occurring in the tissue and coming back to homeostasis. Furthermore, microglia and astrocytes can secrete chemokines, such as C-C-motif chemokine ligand 2 (CCL2), CCL3 and CCL4, which are believed to be involved in the recruitment of peripheral inflammatory cells [259]. These molecules endow glial cells with powerful modulatory properties on neuroinflammatory reaction.

The observation coming from clinical studies supporting the view that microglia are persistently activated after a brain injury [256, 257] makes these glial cells a crucial player in triggering a complex cascade of events that definitely leads to a persistent inflammatory state. Interestingly, investigation in an animal model of focal cerebral ischemia showed that microglia directly activated in and around a lesion presented surface markers different from microglia activated in remote brain regions [260]. In the lesion core, microglia/macrophages expressed CD8, behaved as phagocytes and removed cell fragments. At variance, in regions of secondary degeneration, microglia activation occurred after days of latency, it was accompanied by upregulation of complement receptor 3 (CD11b), major histocompatibility complex class II and CD4 markers, with a scarce phagocytic activity. Although not well understood, these different phenotypes may be characteristic of functional states critical to the development and maintenance of neuroinflammation, as found in patients affected by stroke [256] or traumatic brain injury [257]. On the other hand, the lack of similar studies on patients affected by TLE does not allow any speculation on the prominent role of microglia in epilepsy.

Alternatively to microglia, a primary role of leukocytes in promoting seizures has been proposed [259, 261]. Indeed, brain infiltration of lymphocytes, monocytes, macrophages and neutrophils is observed in epileptic patients and animal models [261-264]. Particularly, Fabene and coworkers [261] demonstrated a role of leukocytes in mediating seizures in mice pretreated with antibodies against intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), or other mediators of interaction between inflammatory and endothelial cells, prior to pilocarpine injection. Interestingly, pilocarpine itself was able to increase the expression of VCAM-1, which further increased along with ICAM-1 and cognate molecules in response to seizures. Mice lacking the enzymes involved in processing the functionally active adhesion molecules for inflammatory cells generally did not respond to pilocarpine and did not develop spontaneous recurrent seizures. As these molecules mediate steps leading to the arrest and rolling of leukocytes, preliminary phenomena to vessel wall crossing, an involvement of inflammatory processes in acute seizures has been suggested. Less clear, instead, is the meaning of these phenomena in chronic epilepsy, in which glia could play a more important role [17, 244].

Whether inflammation is beneficial or has adverse effects in the context of epilepsy is still unclear. A pro-epileptogenic role of inflammation is evidenced by the inhibition of microglia activity by minocycline, which reduces seizures susceptibility in young animals [265]. Also, inhibition of leukocytes-vascular interaction and depletion of neutrophils reduce markedly the number of seizures and prevent the development of chronic epilepsy [261]. Similarly, SE onset, animal mortality, and blood-brain barrier damage are significantly reduced following splenectomy or in mice lacking perforin, a regulator of natural killer cells and cytotoxic T lymphocytes, indicating that T cells immunosuppression could be beneficial in epilepsy [266]. Moreover, blockage of IL-1β signaling system, either with receptor antagonists, IL-1β converting enzyme (ICE) inhibitor ICE/caspase-1, or by knocking down ICE/caspase-1 gene, reduces seizure suscep-

tibility [267-269]. On the contrary, evidence for a beneficial role of inflammation in epilepsy also exists. For example, induction of TLE in IL-6 knockout animals leads to an increase in oxidative stress, neuronal cell death, and enhancement of the severity of seizures [270]. Depletion of macrophages affects the dentate gyrus granule cell survival in the kainate model of epilepsy [264]. In mice lacking both T and B lymphocytes, neurodegeneration is aggravated following kainate-induced seizures and mice exhibit earlier spontaneous recurrent seizures [264]. Finally, specific depletion of hippocampal microglia results in no changes in acute seizure sensitivity compared to normal animals, but pre-conditioning with lipopolysaccharide prior to acute seizure induction provokes greater seizure activity and increased mortality in absence of microglia, indicating that activated microglia may have a protective function during SE [271].

5.2. Inflammatory Brain Diseases and Epilepsy

Apart from studies based on animal models, evidence coming from clinical studies is still scarce to support a role of neuroinflammation in TLE. In the case of multiple sclerosis, a recent study was aimed to describe the prevalence of epileptic seizures in this diffused inflammatory disease of the central nervous system [272]. This investigation identified only 36 out of 2,300 patients with epileptic seizures and multiple sclerosis co-occurrence. Thus, the prevalence of epilepsy in the population of patients suffering from multiple sclerosis was 1.5%, very close to that reported in the general population [273]. On the other hand, patients affected by multiple sclerosis appeared to frequently (72%) develop recurrent seizures after the first convulsive episode. In addition, the mean annual relapse rate, the mean expanded disability status scale score, and the ratio of patients with pediatric onset were higher in patients with multiple sclerosis and seizures, suggesting interaction between the two diseases. Finally, according to multiple logistic regression analysis, age at multiple sclerosis onset and the mean expanded disability status scale score were found to predict seizure occurrence [272].

The role of autoimmunity in epilepsy has been recently reviewed [274] by addressing a number of epileptic syndromes associated with immunological alterations and responding to immunomodulatory agents [5]. Among these syndromes, Rasmussen encephalitis was associated with antibodies against the neuronal glutamate receptor subunit 3. A role for neuroinflammation is suggested in Lennox-Gastaut, West, and Landau-Kleffner syndromes, which have been found to respond to treatment with corticosteroids or intravenous immune globulin. Interestingly, epilepsy has also been found in immunological disorders, such as paraneoplastic neurological syndromes, systemic lupus erythematous, stiff-person syndrome, Parry-Romberg syndrome, and Hashimoto's encephalopathy. More relevant to this review, autoantibodies against glutamic acid decarboxylase were found in patients affected by TLE [275-277]. Although these reports are suggestive for a role of neuroinflammation in TLE, more evidence is required to draw definite conclusions.

6. NEUROPROTECTION

One of the questions addressed in the case of mesial TLE is whether epileptogenesis can be prevented by neuroprotection. According to results obtained in models of SE based on chemoconvulsants [76], preconditioning prior SE induction may prevent neuronal loss but not epileptogenesis. However, these data are in contrast with findings obtained in kindling experiments, in which it was consistently shown that animals do not develop chronic epilepsy apart from the case of prolonged stimulation protocols that are associated with neuronal loss [21]. Another recent work in which epileptogenesis and brain damage have been analyzed in a model of posttraumatic epilepsy, by cooling the area of trauma it was possible to counteract the development of seizures but not tissue injury [278]. A combination of diazepam and the NMDA receptor blocker MK-801, administered 90 minutes after SE, lessened damage in the hippocampus and piriform cortex. However, this neuroprotection failed in preventing the development of spontaneous recurrent seizures [279]. Thus, current data do not allow a clear interpretation of the relationship between neuroprotection and epileptogenesis. On the other hand, neuroprotection is mandatory in patients exposed to SE, and more severe seizures are usually observed in the presence of a large damage in the brain [78]. This last point is particularly important in the light of what is the most significant problem of TLE, i.e. pharmacoresistance. Of the various hypotheses proposed to explain pharmacoresistance in mesial TLE, the presence of damage is probably that receiving more *consensus* in the clinical practice [280, 281]. Thus, it can be conceived that neuroprotection is required to prevent the most severe types of TLE [282]. However, to confirm this hypothesis, it is important to distinguish between drugs that provide neuroprotection because they are able to counteract seizures from those that can provide, instead, a direct neuronal rescue independent of anticonvulsant effects. Only these last agents can be considered as real neuroprotective drugs and can be used to test the hypothesis that neuroprotection modulates epileptogenesis.

6.1. Antiepileptic Drugs

Antiepileptic drugs may also possess neuroprotective properties, as evidenced in models of cerebral ischemia [282]. This is typically the case of diazepam that, consistently with findings in stroke models (reviewed in [282]), when administered during SE was found to attenuate seizures, reduce neuronal loss and lessen the severity of spontaneous recurrent seizures in the chronic phase [71, 77, 78]. To this regard, one of the possible mechanisms involved in neuroprotection afforded by antiepileptic drugs could be the induction of trophic factors, such as BDNF, which was found to be massively induced by counteracting seizures with diazepam and phenobarbital during pilocarpine-induced SE [283]. Other antiepileptic drugs have also been found to display neuroprotective properties in models of cerebral ischemia, as in the case of diazepam [284]. These drugs were extensively reviewed by others [285]. Although the possibility that antiepileptic drugs may possess neuroprotective properties received consistent confirmation in animal models, clinical trials attempted until now to prevent epilepsy after a primary injury were not conclusive for a beneficial role of antiepileptic drugs administration as prophylactic treatment [286, 287]. In particular in posttraumatic epilepsy,

none of the drugs (phenytoin, phenobarbital, carbamazepine, valproate) administered for prophylaxes showed a positive effect on late seizures, although some of them, such as phenytoin and carbamazepine, were successful in suppressing early seizures (reviewed by [288]).

6.2. Anti-Inflammatory Drugs

A number of studies addressed the effects of anti-inflammatory therapy in models of epilepsy [289-292]. All of them focused on the enzyme cyclooxygenase-2 (COX-2), which is responsible for the production of pro-inflammatory prostaglandins, a key-target of anti-inflammatory therapy in epilepsy. The rational for this approach is based on the evidence that inflammation may contribute to epileptogenesis and to neuronal injury in the brain exposed to epileptic seizures [24]. Thus, anti-inflammatory treatments with selective COX-2 inhibitors may represent a novel approach for neuroprotection and anti-epileptogenesis. First investigations resulted in positive findings, demonstrating that the COX-2 inhibitor celecoxib is able to partially preserve cognitive functions, assessed by evaluating spatial and non-spatial tasks in kainate-treated rats [289]. In addition, celecoxib reduced the occurrence of spontaneous recurrent seizures after pilocarpine-induced prolonged seizures, prevented neuronal death and microglia activation in the hilus of dentate gyrus and CA1, and counteracted the appearance of ectopic granule cells in the hilus and gliosis [290]. In a further study, treatment with parecoxib, a precursor of the potent and selective COX-2 inhibitor valdecoxib, reduced neuronal loss in the hippocampus and piriform cortex, but did not affect incidence, frequency or duration of spontaneous seizures developed after SE, neither the behavioral and cognitive alterations associated with damage. However, the severity of spontaneous seizures was reduced, indicating a beneficial effect [291]. Finally, in developing rat pups in which SE was induced by lithium-pilocarpine, a blocker of IL-1 receptors (IL-1ra) and the COX-2 inhibitor CAY 10404, when combined together, attenuated acute injury in CA1. The same combination administered in 3-week-old rats for 10 days following SE, reduced the development of spontaneous recurrent seizures and limited the extent of mossy fiber sprouting [292].

6.3. Mammalian Target Of the Rapamycin (mTOR) Inhibitors

The mTOR pathway regulates cell proliferation, growth, and differentiation by phosphorylating several translational regulators, such as ribosomal S6 kinase and initiation factor 4E binding protein 1 (4EBP1) [293, 294]. In view of its critical role, the mTOR pathway is regulated by growth factors, cytokines, neurotransmitters, and hormones [293, 295, 296]. Mutations of genes able to inhibit the mTOR pathway are clinically very relevant and lead to abnormal cell proliferation and differentiation, such as those occurring in tuberous sclerosis [297]. Recent findings suggest that mTOR dysregulation could be involved in the pathophysiology of TLE [298-300]. In particular, it has been found that seizures induced by kainate in rats produce a biphasic activation of the mTOR pathway, characterized by an increase in phospho-S6 expression 1 hour after seizure onset, peaking at 3-6 hours, and returning to baseline by 24 hours, both in hippocampus and cerebral cortex. A second increase in phospho-S6 was observed few days after SE, specifically in the hippocampus; it persisted for several weeks and, thus, correlated with the development of epileptogenesis. Interestingly, pretreatment with the mTOR inhibitor rapamycin prevented the changes in mTOR activation and, by this way, displayed neuroprotective properties and blocked the development of spontaneous seizures. Accordingly, treatment after termination of SE failed in preventing neuronal death and epilepsy, although spontaneous seizures were less frequent in rapamycin-treated rats [298]. However, when tested in pilocarpine-treated mice rapamycin did not affect the frequency of spontaneous seizures, in spite of decreased sprouting of mossy fibers in the dentate gyrus [126]. Interestingly, the lack of effect of rapamycin in this model was reflected in the absence of neuroprotection, since hilar neuronal loss was similar in epileptic controls and rapamycin-treated mice. These discrepancies were interpreted as related to the different species used, but a further study in rats also failed in demonstrating any antiepileptogenic effect of rapamycin [301]. In this last study, rats were treated for two weeks starting one day after SE induction, which was caused by electrical stimulation of the amygdala. No differences were found in mossy fiber sprouting between rapamycin- or vehicle-treated rats, but neuronal loss was not evaluated.

Adverse effects of rapamycin treatment were also evidenced in immature animals [302]. In this experiment, rapamycin-pretreated rats were injected with pilocarpine or kainate at 3-4 weeks of age and compared with vehicle-pretreated age-matched rats. Notably, rapamycin increased the seizure severity and mortality in the pilocarpine but not in the kainate group of treatment. However, in this work the possible effects on neuronal survival were not investigated. In view of the contradictory findings reported with rapamycin on neuroprotection and epileptogenesis, it appears interesting the approach to brain damage studied in a different work, in which rapamycin was chronically administered starting 4 hours after electrically induced SE [300]. In this paradigm, rapamycin-treated rats resulted to be refractory to develop spontaneous seizure, were partially protected from neuronal loss and mossy fiber sprouting, presented upregulation of inflammation markers (CD11b/c and CD68) as in controls but, notably, the blood-brain barrier leakage was minimal with respect to the control group. In addition, a positive correlation between blood-brain barrier disruption and seizure severity was observed. Although the investigators did not clarify the mechanism by which rapamycin maintained the blood-brain barrier integrity, this phenomenon was indeed associated with a lower propensity to develop seizures in a way independent of the modulation of neuroinflammation, since glial cell reactivity and macrophage invasion was similar in vehicle and rapamycin groups of treatment [300]. For this reason, it could be interesting to investigate whether pharmacological approaches aimed at preserving the integrity of blood-brain barrier could be antiepileptogenic.

6.4. Growth Hormone Secretagogues

The discovery of ghrelin and its receptor, the growth hormone (GH) secretagogue (GHS) receptor (GHS-R) type 1a, introduced new players in the regulation of GH and insu-

lin-like growth factor-1 (IGF-1) production [303, 304]. Ghrelin is a 28 amino acid peptide hormone acylated at Ser-3, synthesized primarily in the stomach. Besides its GH releasing ability by a pathway independent from that regulated by GH releasing hormone (GHRH) receptor, ghrelin influences a broad range of biological processes such as food intake, energy expenditure, cell proliferation, cardiovascular and immune functions, by interaction with GHS-R1a, which is characterized by a high degree of constitutive signaling activity. The other isoform of the receptor, GHS-R1b, does not bind ghrelin but modulates GHS-R1a activity by forming heterodimeric complexes [305].

Overall, GH, GHSs, like ghrelin and cognate molecules, and IGF-1 have been found to exert neuroprotective effects in different models [306, 307], including kainate-induced SE [308, 309], the pilocarpine model [310], cerebral ischemia [311, 312], and models of neurodegenerative diseases [313]. Notably, rats in which IGF-1 was administered intracerebrally together with kainate, no signs of degeneration were detected in the contralateral hemisphere, whereas ipsilateral cell loss, assessed by Fluoro-Jade B staining, was consistently reduced, in association with less reactive gliosis [314]. Interestingly, treatment with ghrelin was also able to significantly reduce neuronal cell death caused by kainate in CA1 and CA3 regions of the hippocampus. These effects were clearly dependent on activation of GHS-R1a [308]. Ghrelin also counteracted the induction of proinflammatory mediators such as TNFα, IL-1β, and COX-2, definitely preventing microglia and astrocytes activation. The prevention of microglia and astrocyte reactivity was associated with the inhibition of matrix metalloproteinase-3 expression and neuroprotection. Ghrelin displayed neuroprotective effects also in the pilocarpine model. In particular, Nissl-stained neuronal cell counts were higher in ghrelin-treated rats exposed to pilocarpine-induced SE [310]. In this model, ghrelin was able to downregulate the phosphatidylinositol-3-kinase and protein kinase B pathway, reversed the decreased ratio of B cell leukemia/lymphoma 2 (Bcl-2) to Bcl-2-associated X protein (Bax) induced by seizures and prevented activation of caspase-3.

Although this evidence suggests that GHSs possess promising protective properties in models of TLE, including prevention of glia activation, no data were published on the relationship with the vascular damage that we recently assessed both in the pilocarpine and kainate models [50]. Alterations in levels of laminin immunoreactivity have been reported in blood vessel basal lamina, following cerebral ischemia [315] or, respectively, quinolinic acid [316], kainate [50, 317-319] and pilocarpine [50, 71] treatments, and even in consequence of mechanical lesions [55, 56]. In pilocarpine-treated rats experiencing a SE lasting for 30 minutes, we documented the presence of vascular damage by electron microscopy in areas of the CA3 region denoted by loss of GFAP immunostaining [71]. This finding was paralleled by increased laminin immunoreactivity in blood vessel basal lamina, a phenomenon observed 3 and 7 days after SE that was followed by normalization one week later. The increased laminin immunostaining could be explained by an improved accessibility of laminin epitopes in damaged blood vessels [55, 56, 320]. An increased activity of tissue-type plasminogen activator, which is also able to stimulate matrix metalloproteinase 9 [321], was progressively observed in the CA3 stratum lacunosum-moleculare in rats during SE [318]. Induction of tissue-type plasminogen activator was associated with increased laminin immunostaining in blood vessels also in a model of cerebral ischemia [315]. Thus, degradation of basal lamina of blood vessels could justify the observed changes of laminin immunoreactivity in pilocarpine-treated rats, as reported in (Table **1**). It has also been suggested that the upregulation of laminin immunoreactivity observed after a mechanical damage could depend on disconnection of the gliovascular junctions that take part in the blood-brain barrier [55, 56]. According to these authors, gliovascular junctions could limit the accessibility of the basal lamina of blood vessels to antibodies, thus "hiding" the laminin epitopes. The cleavage of β-dystroglycans, which are laminin receptors, by matrix metalloproteinases may lead to disconnection of the gliovascular junctions making laminin accessible to antibodies [316, 322]. An interesting new finding is the strong relationship between glial and vascular changes found in piriform cortex of rats treated with pilocarpine or with kainate [50]. A similar relationship was also found in neostriatum after local injection of endothelin-1, suggesting the involvement of ischemic mechanisms [50]. Definitely, the overall evidence suggests that the concomitant damage to astrocytes and blood vessel could be due to a vascular dysfunction, in particular in the piriform cortex of animals exposed to SE. This hypothesis is supported by experiments that showed increased expression of endothelin-1 in endothelial cells of blood vessels located in piriform cortex damaged by pilocarpine-induced SE [323]. It is well known that endothelin-1 can cause local ischemia due to persistent vasoconstriction when injected into the brain [324, 325], and this mechanism could provoke an ischemic damage in piriform cortex during SE. However, it is unclear why astrocytes are apparently more affected than neurons (Table **1**). In models of cerebral ischemia it has been found that GFAP and other glial proteins are downregulated before neuronal cell death [326, 327]. On the other hand, diazepam is a neuroprotective drug shown to be very effective in models of cerebral ischemia [284]. Thus, it is possible that pharmacological treatments aimed at neuroprotection may interfere with mechanisms leading to neuronal cell damage but not with those involved in astrocytic lesions, as it probably happens in the case of diazepam (*cf.* [50]). In view of these limitations, protective drugs with a wide spectrum are warranted in addressing prevention of lesions in the brain affected by a primary precipitating injury.

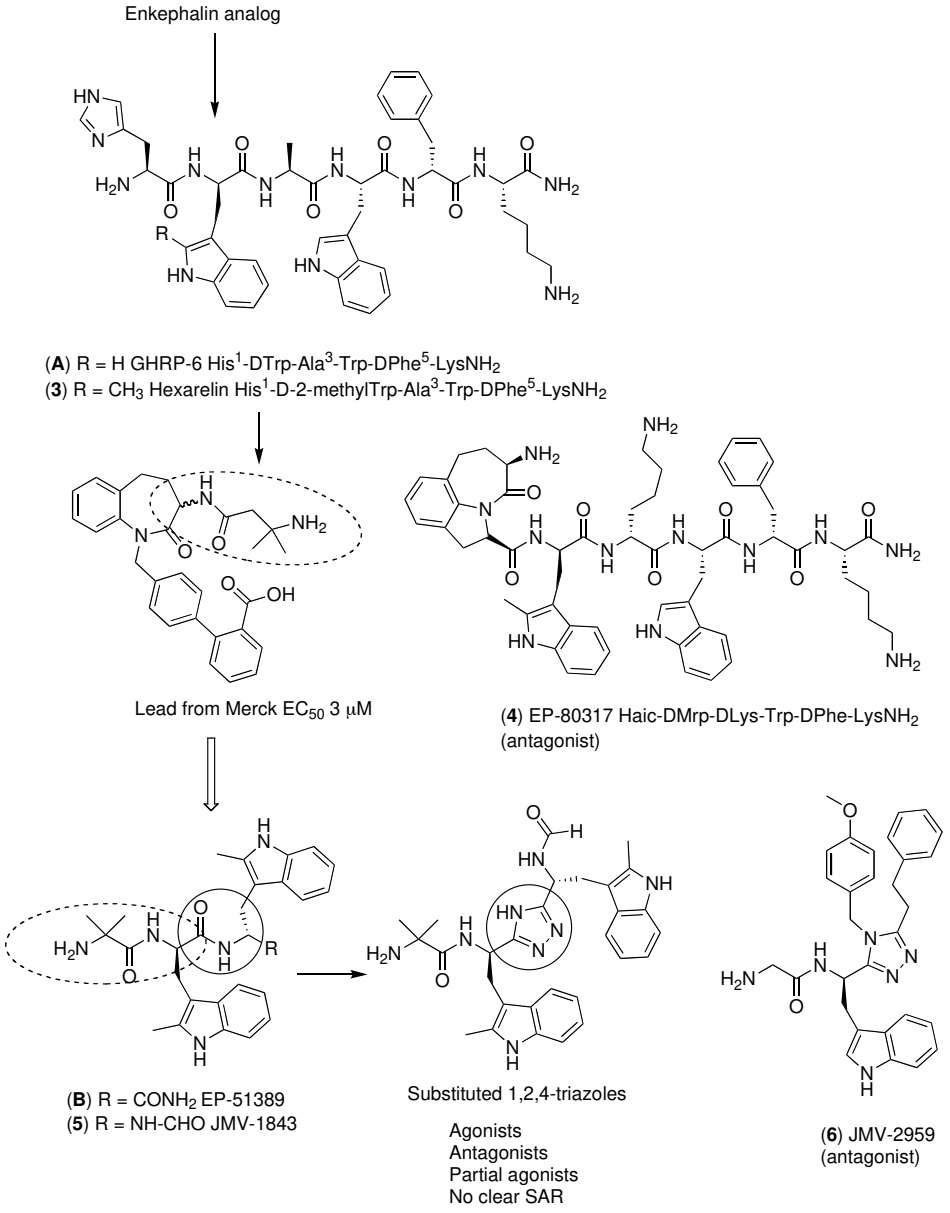
6.4.1. Protective Effects of GHS-R1a Agonists on Vascular Damage

Several compounds have been discovered, acting as agonists, antagonists, or inverse agonists at GHS-R1a. Starting from the assessment of C-amidated Met- and Leu-enkephalins for GH releasing activity from pituitary incubates, the first peptidic GH secretagogue, GHRP-6 (compound (**A**), Scheme **1**), was discovered, followed by hexarelin (compound (**3**), Scheme **1**). Subsequently, it was characterized the receptor involved in GHRP-6 and hexarelin activities, GHS-R1a, and finally the natural ligand, ghrelin. On the basis of structure-activity relationship (SAR), Merck performed a focused screening that led to the discovery for

the first time of peptidomimetics of GHRP-6 (whose lead structure is reported in Scheme **1**), compounds characterized by the presence of a aminoisobutyric amide [328].

On these bases, several research groups discovered various chemotypes able to modulate GHS-R1a [335]. Worth of note are EP-51389 ((**B**), Scheme **1**) and its derivative JMV-1843 (EP-1572, AESZ-130) ((**5**), Scheme **1**), that originated the widest series of GHS-R1a ligands. (Scheme **1**) reports the structures and the design process of the ghrelin modulators reported in the present article. In fact, starting from JMV-1843, a series of substituted 1,2,4-triazoles, acting as agonists, partial agonists, or antagonists, were synthesized and pharmacologically evaluated, but no clear correlations between *in vitro* and *in vivo* results have been obtained [335].

We have recently characterized the properties of different GHSs (compounds **1**-**6** whose pharmacological characteristics are reported in Table **2**) in the pilocarpine model of induced seizures and SE, reporting various beneficial effects of ghrelin analogues such as hexarelin (**3**), EP-80317 (**4**) and desacyl ghrelin (**2**) on convulsions, whereas other GHS-R1a ligands were ineffective, as in the case of JMV-1843 (**5**) and JMV-2959 (**6**) [345]. Concerning the animals, those that, independently of drug treatment, experienced SE after the pilocarpine injection were the ideal subjects to assess the presence of a protective effect of GHS-R1a agonists, as suggested by previous reports [308, 310]. In particular, we investigated damage to blood vessels in the piriform cortex, one of the key cerebral regions for ictogenesis [59]. The piriform cortex is also one of the most susceptible brain regions to induced damage that follows SE in the kainate and



(Scheme **1**). Design of the compounds reported in the present review interacting with ghrelin receptors: non-peptidic GHSs and aminoisobutyric amide/triazole derivatives [328-334]. The structure of the antagonists EP-80317 and JMV-2959 is also displayed. Abbreviations: SAR: structure-activity relationship.

Table 2. Pharmacological Characteristics of Compounds 1-6, A and B

Compound	GHS-R1a Binding Assay IC_{50} (nM)	GHS-R1a Functional Assay		GH Release	CD36[i] IC_{50} Binding (mM)	Ref.
		EC_{50} (nM)	% Activity at 10 mM relative to ghrelin			
Ghrelin[l] (**1**)	0.25[a]	32[b]	(100)	Compound/hexarelin GH release 0.65[c]		[336]
Desacylghrelin[e] (**2**)	>10,000[a]	>10,000[b]	41	100-fold less potent than ghrelin.		[337]
GHRP-6 (**A**)	6.08[d]	10			1.82	[338]
Hexarelin[m] (**3**)	15.9[d]	10			2.08	[338]
EP-80317 (**4**)	750[d]	ghrelin antagonist			1.11	[334, 338]
EP-51389 (**B**)	62.9[d]			Compound/hexarelin GH release 0.89[c]	>100	[338, 339]
JMV-1843 (**5**)	22.9[f]			Compound/hexarelin GH release 1.31[c] Orally active in humans		[329]
JMV-2959[h] (**6**)	32[g]	0 at 10 mM Kb:19 nM ghrelin antagonist		GH release (ng/mL) after s.c. injection at 80 mg/kg: 9.85 (saline 5.24, hexarelin at 160 mg/kg: 170.10)		[330, 340]

Abbreviations: a: [^{35}S]MK-0677 binding assay. IC_{50} reflects concentration of peptide at 50% specific binding; b: Aequorin bioluminescence assay. EC_{50} is the concentration of peptide at 50% maximum calcium accumulation; c: Result normalized as a ratio of GH release stimulated by 300 mg/kg of test compounds and GH release stimulated by 300 mg/kg of hexarelin; d: Competitive binding assay in presence of [^{125}I]ghrelin; e: Desacylghrelin, the unacylated precursor of ghrelin at Ser-3 OH, has its own receptors, and acts as a potent functional inhibitor of ghrelin [341]. Its neuroprotective effects do not appear to be mediated through activation of GHS-R1a, as antagonism of this receptor fails to block the neuroprotective effects against oxygen-glucose deprivation insult; on the contrary, at least some neuroprotective effects of ghrelin are mediated by GHS-R1a [342]; f: [^{125}I-Tyr^4]ghrelin human pituitary membranes; IC_{50} for [^{125}I-His^9]ghrelin cloned human GHS-R1a: 123 nM; hexarelin 18.0 nM (human pituitary membranes); ghrelin: 9.8 nM and 0.39 nM for human pituitary membranes and GHS-R1a cloned receptor, respectively; g: [^{125}I-His^9]ghrelin; h: This compound had no effect on GH secretion or food intake when administered alone, but it was able to suppress hexarelin-induced food intake but not to inhibit GH secretion promoted by hexarelin. All the findings suggest the existence of ghrelin receptor subtypes or a particular mechanism of action for GH secretion or food intake [330]; i: The activity on the receptor CD36, a multifunctional B-type scavenger receptor, was discovered on the basis of the cardiovascular effects of GHRP-6 and hexarelin. This receptor mediates the cardiovascular action of these compounds, and emerged as an interesting target with potential therapeutic implications in the treatment of atherosclerotic conditions or in presence of a marked angiogenesis; l: for articles dealing with ghrelin neuroprotection, see [313, 343]. Recently, it has been shown that the anticonvulsant effects of ghrelin are mediated via the GHS-R1a, by means of inverse agonism and desensitization/internalization of the GHSR [344]; m: for neuroprotection exerted by hexarelin, see ref. [342].

pilocarpine models of TLE [48, 49]. Damage to piriform cortex arises earlier than in other regions, since it is observed in layers II and III of the piriform cortex at approximately 8 h after kainate- and pilocarpine-induced SE, when neurodegeneration has still to occur in other injured regions such as the amygdala and the hippocampus [346]. Usually, the piriform cortex develops a severe edema with remarkable neuronal and astrocytic damage [48, 53, 347, 348], such as that found in the CA3 stratum lacunosum-moleculare of pilocarpine-treated rats [71]. As shown in (Fig. **3**), by quantifying the increase in laminin immunoreactivity 4 days after the pilocarpine treatment, we observed that a single injection of the GHS-R1a full agonist JMV-1843 (**5**) was able to restrain the changes in laminin immunoreactivity and, thus, blood vessel damage. This finding was at odds with the results obtained by injecting ghrelin that, probably for the less advantageous pharmacokinetics [349, 350], did not prevent blood vessel damage. These data are consistent with the observed effects of (**5**) JMV-1843 in the CA3 of pilocarpine-treated rats [351].

7. CONCLUSION AND PERSPECTIVES

The most severe type of epilepsy in adults, TLE, is characterized by complex pathological changes whose relationship with refractoriness to therapy is unclear. In most of patients suffering from TLE, early brain damaging events, that are probably responsible for hippocampal sclerosis or for damage to other extrahippocampal regions, are frequently found. The dynamic of these events has been thoroughly investigated in animal models of TLE, which revealed a variety of alterations occurring in the brain affected by epilepsy. Although investigation in TLE animal models did not allow the clarification of the relationship between damage and epileptogenesis, epilepsy appears to be more severe both in experimental animals as well as in patients with extensive brain damage. Research on the use of antiepileptic drugs as

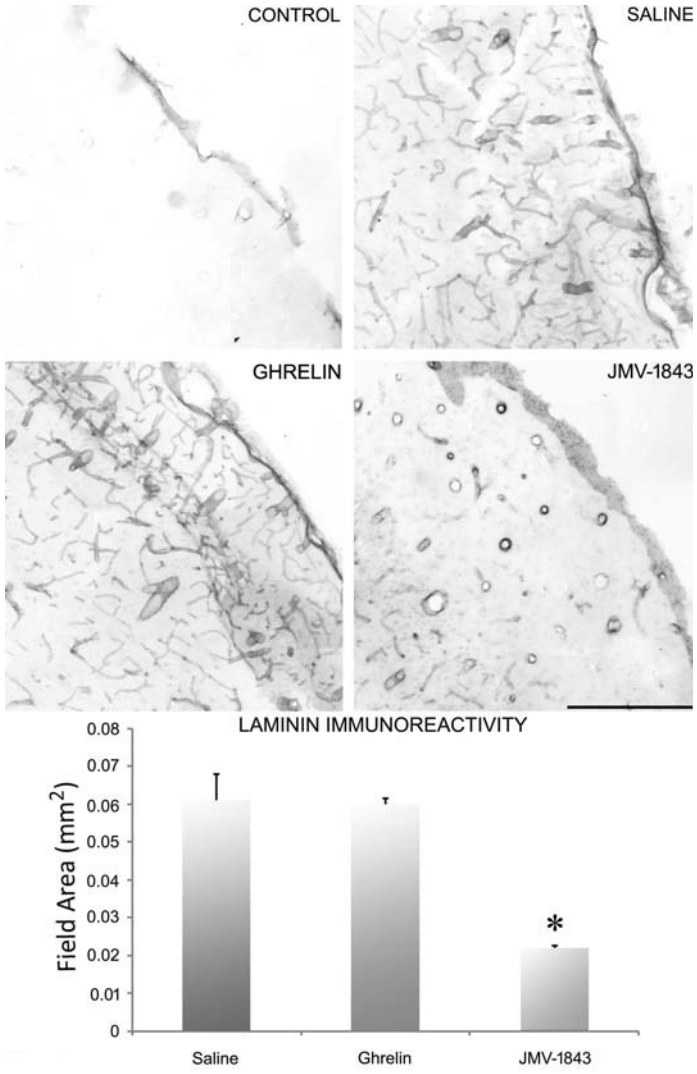


Fig. (3). Quantification of the protective effects of JMV-1843 in piriform cortex, evaluated by an antibody against laminin (methods as in [50]). Rats were pretreated with saline, ghrelin (1.5 mg/kg), or JMV-1843 (330 µg/kg), as previously reported [345]. Note that JMV-1843 significantly reduced the increase in laminin immunoreactivity. *= $p<0.05$ *vs.* saline and ghrelin groups, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. Scale bar, 350 µm.

neuroprotective agents, apart from limited cases such as diazepam, turned out to be frustrating, mainly because of the limited results on epileptogenesis or, even, for the absence of consistent findings. More recent evidence about the role of inflammation in acute and chronic seizures, the involvement of glial cells and damage to blood vessels have suggested new targets for protective, possibly preventive, therapies. Anti-inflammatory drugs such as COX-2 inhibitors and rapamycin were shown to counteract damage to multiple components of brain tissue, allowing a wide spectrum intervention. Drugs acting as GHSs present a straight vascular tropism that results in diminished neuronal and astrocytic damage in regions, such as the piriform cortex and CA3, in which vasogenic edema plays an important role in injury development. These findings suggest that new pharmacological tools can be taken into consideration as promising neuroprotective molecules and/or add on treatments in pharmacoresistant epilepsy.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

4EBP1	=	4E binding protein 1
ADK	=	Adenosine kinase

AMPA	=	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid
AQP4	=	Aquaporin-4
Bax	=	Bcl-2-associated X protein
Bcl-2	=	B cell leukemia/lymphoma 2
BDNF	=	Brain-derived trophic factor
CA	=	*Cornu ammonis*
CCL	=	C-C-motif chemokine ligand
CD	=	Cluster of differentiation
CD200R	=	CD200 receptor
COX-2	=	Cyclooxygenase-2
CX3C	=	C-X3-C-motif chemokine
CX3CL1	=	C-X3-C-motif chemokine ligand 1
CX3CR1	=	C-X3-C-motif chemokine receptor 1
DAPI	=	4',6-diamidino-2-phenylindole
GABA	=	γ-aminobutyric acid
GFAP	=	Glial fibrillary acidic protein
GH	=	Growth hormone
GHRH	=	Growth hormone releasing hormone
GHS	=	Growth hormone secretagogue
GHS-R	=	Growth hormone secretagogue receptor
ICAM-1	=	Intracellular adhesion molecule 1
ICE	=	Interleukin 1β converting enzyme
IGF-1	=	Insulin-like growth factor-1
IL	=	Interleukin
IL-1ra	=	Interleukin 1 receptor
mTOR	=	Mammalian target of rapamycin
NeuN	=	Neuron-specific nuclear protein
NMDA	=	N-methyl-D-aspartate
SAR	=	Structure-activity relationship
SE	=	*Status epilepticus*
TGF-β	=	Transforming growth factor β
TLE	=	Temporal lobe epilepsy
TLR4	=	Toll-like receptor 4
TNFα	=	Tumor necrosis factor α
VCAM-1	=	Vascular cell adhesion molecule 1

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