Hippocampal interneurons in human temporal lobe epilepsy:
differential changes of perisomatic and dendritic inhibition

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INTRODUCTION

Based on the definition of the World Health Organisation, epilepsy is a chronic functional disorder of the brain by different etiology, characterised with recurrent seizures. The epileptic seizure is the stereotype paroxysmal disorder of the mind, behaviour, emotions, motor and sensory functions, provoked by the opportune, sudden, fast and excessive firing of the gray matter, requiring increased neuronal excitement and hypersynchrony [1]. The frequency of epilepsy in the general population regardless of age is 1 to 2% [2]. Temporal lobe epilepsies are classified as symptomatic partial epileptic syndromes, with simple and complex partial epileptic seizures. The epileptogenic area, or focus, is located in the temporal lobe, most often in the hippocampus. In the majority of cases hippocampal sclerosis can be confirmed [3].

The hippocampus is part of the archicortex, the most primordial cortex of the mammal brain. It plays a significant role in learning and memory processes, as well as in the modifications of these mechanisms influenced by emotions. As a consequence of hippocampal damage, cognitive abilities and information processing are disturbed [4].

Hippocampal interneurons have an important functional role in the formation of network activity patterns, and form a very heterogeneous group of cells. In general, their cell bodies can be located in every region and layer of the hippocampus, their dendritic tree is diverse, their axons give local collaterals, and the neurotransmitter of many types is the inhibitory -amino-butyric acid (GABA). The inhibitory interneurons, due to their extended local axonal tree, have the ability to affect the activity of large groups of principal cells at the same time. Three functionally different interneuron groups exist in the hippocampus: one innervates the perisomatic region of principal cells (basket and axo-axonic cells), controlling their output, the second gives synapses to the dendritic region of principal cells, and might control synaptic plasticity and dendritic electrogenesis. The third group specifically innervates interneurons in the rat hippocampus, and gives synaptic contacts to both interneurons and principal cells in humans (see for review: [5]).

In most cases hippocampal sclerosis has been found in the surgically removed tissue of temporal lobe epileptic patients [6]. Hippocampal sclerosis consists of a shrunken hippocampus with a characteristic cell death pattern in the pyramidal layer of Ammon’s horn and the endfolium. Vulnerability of hippocampal principal cells to temporal lobe epilepsy has been examined since 1880 [7], whereas the vulnerability of non-principal cells has been recently investigated, since immunohistochemical techniques can be used to distinguish different interneuron types.
OBJECTIVES

Epilepsy is thought to be related to a changed balance between excitation and inhibition. The increased level of excitation might play a role in epileptogenesis, as well as in the generation and maintenance of epileptic seizures [8, 9]. The sprouting of mossy fibers and the supramammillary afferent pathway provide an excess excitation to the dentate gyrus [10, 11], whereas the CA1 region receives an enhanced excitatory input from the CA3 region and from the sprouting of CA1 pyramidal cell axons [12]. It is still not clear whether inhibition is decreased or preserved in the human epileptic hippocampus. There is evidence for interneuronal cell death, as well as for the preservation of GAD-positive cells [13, 14].

The changes of two functionally different interneuron types in the dentate gyrus and CA1 region of human epileptic hippocampus with light and electron microscopy were investigated in this thesis. Calbindin (CB)-positive interneurons are known to be dendritic inhibitory cells [15], and thereby control the input of principal cells [16]. Furthermore, they are preserved in the epileptic hippocampus [17]. Parvalbumin (PV)-positive interneurons are perisomatic inhibitory neurons (basket and axo-axonic cells) [15, 18] controlling the output of principal cells [5, 16], but their vulnerability to epileptic injury is controversial in the literature. The dentate gyrus is known to have an important role in the epileptogenesis and is preserved in epilepsy, whereas the CA1 region gives the output of the hippocampus and is vulnerable to epileptic injury. To reveal the changes of interneuron populations controlling the input and output of principal cells in these two regions might help in understanding the complex processes taking place in the epileptic hippocampus.

The experiments focussed on the following questions:

What are the changes in the morphology and distribution of CB- and PV-positive interneurons in the dentate gyrus and the CA1 region?

Do CB- and PV-immunoreactive interneurons take part in the epileptic synaptic reorganisation, and what kind of plastic changes do they show to epilepsy?

How does the synaptic input of CB-positive interneurons change in the CA1 region?

What are the postsynaptic targets of CB-stained axon terminals (interneurons and excitatory afferents) in the CA1 region, and how does it change in epileptic tissue?
What are the postsynaptic targets of PV-positive axons in the dentate gyrus and the CA1 region, and how does it change in the epileptic tissue?

How does the perisomatic inhibitory input of principal cells (granule cells and pyramidal cells) change in the two observed regions in epilepsy?

**METHODS**

CB- and PV-containing elements were examined in 7 human control hippocampi and in 34 temporal lobe samples surgically removed from epileptic patients. The seizure focus of therapy resistant temporal lobe epileptic patients was determined by video-EEG monitoring, SPECT and/or PET, and standard temporal lobectomy was performed. The control brains were removed 2 to 4 hours after death, the dissection was performed in the Semmelweis University, Budapest, and the regulations of the Hungarian Ministry of Health were followed.

*Immunohistochemistry*

After surgical removal the epileptic tissue was immediately dissected into 3 to 4 mm thick blocks, and immersed into a fixative solution. Four of the control hippocampi were subjected to the same procedure. Three of the control brains were removed from the skull 2 to 4 hours after death, both internal carotid and vertebral arteries were cannulated, and the brains were perfused, the hippocampus was removed and dissected into 3 to 4 mm blocks. After fixation 60\(\mu\)m thick vibratome sections were cut from the blocks, and immunostaining was performed. In our experiments, the following primary antibodies were used: monoclonal mouse anti-PV, mouse anti-cholecystokinin (CCK), rabbit anti-neuropeptide Y (NPY), rat anti-somatostatin (SOM) and polyclonal rabbit anti-CB. The sections were incubated in the primary antibodies for 2 days, at 4\(^\circ\)C, then in biotinilated secondary antibodies for 2 hours. This was followed by incubation with avidin-biotin peroxidase complex solution for 1,5 hours. The immunoperoxidase reaction was developed with 3,3'-diaminobenzidin-4HCl (DAB) as a chromogen. The sections were then osmicated and dehydrated in ethanol grade and propylene oxide, then embedded in Durcupan. After light microscopic examination, areas of interest were reembedded and sectioned for electron microscopy, and observed with a Hitachi 7100 electron microscope.
For double immunofluorescent staining the primary antibodies rabbit anti-CB was used together with mouse anti-PV. Cy3-conjugated donkey anti-rabbit and FITC-conjugated goat anti-mouse secondary antibodies were used to visualise the immunopositive elements.

**Quantitative analysis**

**Cell counting**

To determine the number of PV-positive cells per unit area, dentate gyrus sections with every PV-stained cell bodies were drawn with the aid of a camera lucida. The area of the sections was measured, the number of cells per $1\,\text{mm}^2$ was determined in each the str. granulosum + moleculare, and hilus.

**Distribution of postsynaptic target elements**

For the analysis of target elements of PV-and CB-positive axon terminals blocks of similar size were reembedded, and serial sections were cut. Every 10th section was systematically scanned, a photo was taken from every positive terminal forming synaptic contact, and the distribution of postsynaptic target elements was determined. In the case of CB-positive terminals the ratio of terminals giving symmetric and asymmetric synapses, as well as the proportion of CB-immunoreactive targets were determined.

**Synaptic coverage**

The synaptic input of dentate granule cell somata and axon initial segments (AIS) was analysed. The sampling of the AISs was performed as described above, every AIS was digitized from every 10th section. The perimeter of the AISs, and the length of every synapse were measured. The synaptic coverage ($\mu\text{m synaptic length/ } 100\mu\text{m AIS perimeter}$) and the number of synapses/ $100\mu\text{m AIS perimeter}$ has been recorded. In every control and epileptic samples the sum of the number or the length of the synapses were taken and divided by the sum of the AIS perimeters. For the analysis of synaptic input of granule cell somata, fifty neighbouring granule cells were analyzed from one ultrathin section, and the number of symmetric synapses was determined.

The analysis of the CA1 pyramidal cell AIS input was performed the same way, as described above. For the analysis of the somata, from PV-stained sections of each sample an average of 40 cell bodies were digitized, as well as every terminals giving synapses to the cell bodies, regardless to the PV-content of the terminal. The perimeter of the cell bodies and the length of the synapses were measured. The synaptic coverage ($\mu\text{m synaptic length/ } 100\mu\text{m soma perimeter}$) and the number of synapses/ $100\mu\text{m soma perimeter}$ has been recorded. The sum of the number or the length of the synapses were taken and divided by the sum of the cell body perimeters. Statistical analyses were performed to check whether the synaptic coverage of cell bodies and AISs are different from the
control in each epileptic cases, as well as in the different groups of epileptic patients. The data derived from different control subjects did not differ statistically, and were pooled. The epileptic samples were compared to the pooled control data.

The synaptic input of CB-positive dendrites was also analysed. Photos were taken from every immunopositive dendrites in one ultrathin section, then the perimeter of the dendrites and the length of the synapses were measured. The total synaptic coverage (µm synaptic length/ 100µm dendrite perimeter) and the ratio of symmetric and asymmetric synaptic lengths relative to the total synaptic coverage (µm symmetric and asymmetric synaptic length/ µm total synaptic length, represented in percentage) were determined.

**RESULTS**

All patients examined in the present study had therapy resistant epilepsy of temporal lobe origin. Based on the characteristic morphological alterations of temporal lobe epilepsy four groups of patients were formed. The four groups were distinguished on the basis of principal cell death and interneuronal changes at light microscopic level: type 1 hippocampus similar to the control; type 2 patchy cell loss in the str. pyramidale of the CA1 region; type 3 hippocampus showing hippocampalis sclerosis and sprouting; type 4 the whole hippocampus is shrunken, not only the CA1 region, cell loss in each cell types. Due to the low number of samples, the hippocampi of the latest type were not included in this study.

*Changes in morphology and distribution of calbindin-containing elements*

**Dentate gyrus**

In the human control dentate gyrus calbindin is found in principal cells (granule cells) and in non-principal cells [15]. Fusiform and multipolar CB-positive interneurons with smooth dendrites can be found in the hilus, whereas str. moleculare contains a large number of CB-stained horizontal, and fewer multipolar cells. The dendritic tree is short, only the proximal dendrites are visible.

In the dentate gyrus derived from epileptic patients a large number of CB-positive interneurons are present, whereas part of the granule cells lost its CB-positivity [19]. The cells are stained with long dendrites, and large interneurons bearing somatic and/or dendritic spines can be seen in the hilus, which were never seen in the control tissue. In the majority of those epileptic cases where granule cells lost their CB-content, the axons of granule cells are CB-positive. The sprouting of granule cell axons gives a characteristic axonal network in the inner molecular layer [10, 20].
**CA1 region**

CB-positive interneurons can be found in every layer of the CA1 region of human control hippocampus, they possess long smooth dendrites. Horizontal CB-immunoreactive cells are present in the str. oriens, whereas in the strata pyramidale and radiatum the cells are radially oriented multipolar interneurons. In the mildly sclerotic group (type 1 and 2), the horizontal CB-immunoreactive cells in the str. oriens were less frequently seen, whereas in other layers their number appeared unchanged. Long radial dendrites disappeared in the strongly sclerotic cases (type 3), the CB-positive cells possessed a large number of short, distorted dendrites, which were usually spiny. Large cell bodies were more frequently present, and they were also often decorated with spines. The proportion of cells with abnormal morphology correlated with the degree of sclerosis: in the less sclerotic cases only a few were seen, whereas in the strongly sclerotic cases cells with normal morphology were hardly ever observed.

**Electron microscopy of calbindin-positive elements**

**Dentate gyrus**

In the epileptic dentate gyrus three types of CB-positive hilar interneurons were distinguished: giant cells (long diameter: 30-60\(\mu\)m, short diameter: 20-40\(\mu\)m), normal size neurons covered with glial processes (long diameter: 15-35\(\mu\)m, short diameter: 10-25\(\mu\)m, this is the usual size range of the control hilus), and neurons with somatic spines. All cells except those covered with glial processes received a large number of terminals forming asymmetric synapses. The presence of CB-positive and –negative mossy terminals giving synapses to CB-positive interneuron dendrites also confirmed the sprouting of mossy fibers.

**CA1 region**

In the control CA1 region CB-positive interneuron cell bodies received a moderate number of synapses, small terminals giving both symmetric and asymmetric synapses were seen. The number and type of synapses contacting the cell bodies did not show any profound changes in the epileptic cases. In the control CA1 region CB-immunoreactive interneuron dendrites receive mainly asymmetric (presumed excitatory) synapses, whereas symmetric (presumed inhibitory) synaptic contacts were rarely seen. In the epileptic tissue more terminals gave symmetric synaptic contacts to the CB-positive interneuron dendrites. The perimeter of CB-positive interneuron dendrites and the length of symmetric and asymmetric synapses were measured and synaptic coverage (\(\mu\)m synaptic length/ 100 \(\mu\)m of dendrite perimeter) as well as the ratios of symmetric and asymmetric synaptic lengths relative to the
synaptic coverage (µm symmetric as well as asymmetric synaptic length/µm total synaptic length, in percentage) were determined. The total synaptic coverage (including symmetric and asymmetric synapses) did not change notably in the epileptic samples compared to the control tissues. Although the total synaptic coverage was not altered, in all epileptic samples (even in the cases with no obvious sclerosis) the ratio of symmetric synaptic lengths considerably increased. In strongly sclerotic cases (type 3) a large proportion of terminals contacting CB-immunoreactive dendrites were themselves CB-positive (asymmetric and symmetric synapses as well). This type of CB to CB contact was much less frequent in controls or mildly sclerotic cases (type 1, 2).

The distribution of the target elements of CB-immunostained terminals was determined in every layer of the CA1 region in each analyzed sample. The targets of CB-positive terminals giving asymmetric synapses are mainly pyramidal cell dendritic shafts and spines, as well as CB-negative interneurons. Asymmetric synapses contact primarily pyramidal cell spines, whereas boutons giving symmetric synapses terminate mostly on dendritic shafts. The proportion of CB-positive interneurons varied between 0 and 10% among the targets of CB-immunoreactive terminals.

A large number of CB-positive symmetric synapses were observed on axon initial segments in the str. pyramidale, which has not been reported in earlier human hippocampal studies. CB-PV double fluorescent immunostaining was carried out, and we did not find overlap in the CA1 region between the two markers. Furthermore, the morphology of these two cell types is different.

In the mildly sclerotic tissue (type 1 and 2) the target distribution of CB-positive terminals was similar to the control, whereas it changed considerably in the CA1 region of epileptic patients with a strong sclerosis: pyramidal cells are hardly present, therefore all target dendrites, spines and AISs likely belong to the surviving interneurons. The ratio of asymmetric and symmetric synapses established by CB-immunostained terminals was similar in the control and the strongly epileptic tissue, whereas it was considerably higher in the non sclerotic epileptic CA1 region (type 1, 2).

Changes in distribution and morphology of parvalbumin-positive elements

In the human hippocampus PV-immunoreactive interneurons belong to perisomatic inhibitory cells: basket and axo-axonic interneuron types [15, 18]. In the epileptic samples the number of PV-positive cells decreased in parallel with the degree of sclerosis in the CA1 region. In most cases some characteristic multipolar cells remained in the stratum moleculare of the dentate gyrus, whereas the hilus was usually devoid of stained cells. In the control tissue PV-positive axon terminals formed a homogenous network in the stratum granulosum, whereas in the epileptic samples dense axon terminal clouds alternated with sectors devoid of PV-positive fibers. In the control CA1 region large multipolar PV-stained interneurons are present in the str. pyramidale, their radial smooth dendrites usually
terminate in the str. lacunosum-moleculare. Horizontal PV-positive interneurons are located in the str. oriens. Long segments of PV-stained dendrites originating from different cells were often attached to each others. Dense axonal cloud can be seen in the str. pyramidale. In the non sclerotic epileptic CA1 region (type 1, 2) the number and distribution of PV-immunoreactive cells did not considerably differ from the control, only the cells located in the str. oriens seem to be less in number. In the very sclerotic CA1 region the number of PV-immunostained elements is dramatically decreased.

Electron microscopy of parvalbumin-positive interneurons

Dentate gyrus

The PV-positive interneuron cell bodies receive a moderate number of synaptic input both in the control and the epileptic tissue. Large numbers of asymmetric excitatory synapses terminated on PV-immunoreactive dendrites in the control dentate gyrus. In the epileptic tissue, both in the hilus and str. moleculare the PV-containing dendrites received input from mossy terminals as well.

The target distribution of PV-positive axon terminals was determined. The most frequent targets were granule cell somata and dendrites, AISs were less frequently found. In the epileptic tissue the ratio of AISs increased among the targets.

Perisomatic input of granule cells

PV-immunostaining might be sensitive to ischemia or epilepsy, PV might disappear from the cell body and dendrites of otherwise healthy surviving neurons [21, 22]. Although PV staining was preserved in most part of the axon terminals, the presence of patches containing few stained terminals raised the question of whether these cells degenerated or lost their PV-content. Therefore we analysed the input of granule cell somata and AISs regardless to the PV-content of the presynaptic terminals. The number of symmetric synapses terminating on the cell bodies did not change significantly in the epileptic dentate gyrus. The synaptic coverage of granule cell AISs significantly increased in every epileptic sample, and showed a positive correlation with the degree of sclerosis in the CA1 region.

CA1 region

Large numbers of asymmetric and a few symmetric synapses terminated on both PV-positive cell bodies and dendrites. The number of terminals giving asymmetric synapses to PV-stained somata was considerably higher in the CA1 region then in the dentate gyrus. Zonula adhaerentia were frequently found between PV-immunoreactive dendrites. No considerable changes were found in the synaptic input of PV-positive cell bodies and dendrites in the epileptic CA1 region. Zonula adhaerentia were frequently found between PV-containing dendrites, as well as between PV-immunopositive and –negative dendrites.
Postsynaptic target distribution of PV-immunoreactive axon terminals was determined in the control and the non-sclerotic epileptic CA1 region. They mostly terminate on pyramidal cell AISs, cell bodies, proximal dendrites. The postsynaptic target distribution of PV-positive terminals did not change notably in the epileptic CA1 region.

_Perisomatic input of pyramidal cells_

The synaptic coverage and the number of synapses/100 µm perimeter of pyramidal cell bodies and AISs were determined. In the CA1 region of the type 1 epileptic group the input of pyramidal cell somata was similar to the control, whereas it significantly decreased in the type 2 epileptic tissue. The synaptic input of AISs did not significantly changed in the epileptic tissue.

_Short summary of the results_

CB-positive interneurons are preserved in the epileptic hippocampus, but the morphology of individual cells is altered.

The input-output connections of CB-positive interneurons changed in the epileptic CA1 region: they received more inhibitory synaptic input, and parallel to the death of pyramidal cells they changed their targets, terminating on the surviving interneurons instead.

The number of PV-positive cells decreased in the epileptic hippocampus, but most of the cells survive in epilepsy. However, many of them lose their PV-content, or –immunoreactivity.

Perisomatic inhibition is preserved in the epileptic hippocampus, it does not change considerably in the CA1 region, while pyramidal cells are present, whereas granule cell AISs receive an increased inhibitory input.

**DISCUSSION**

_Changes of dendritic inhibitory calbindin-containing interneurons_

**CB-positive interneurons are preserved in epilepsy, but their morphology is changed**

The resistancy of CB-stained interneurons to epileptic damage is a well known phenomenon [11, 17]. A large number of CB-positive non-principal cells are preserved in the CA1 region of our human epileptic samples. In the epileptic hippocampus, the morphological features of the preserved CB-positive interneurons are characteristics of immature hippocampal neurons [23-25]. We assume that neurotrophins may play a role in the survival and alterations of CB-containing interneurons [26-30].
Changes in the synaptic input of calbindin-positive interneurons

The synaptic input of CB-positive interneurons is changed in the epileptic dentate gyrus: sprouted CB-positive and –negative mossy fibers terminate on CB-positive interneuron dendrites in the str. moleculare as well, which was never seen in the control tissue. A large number of excitatory synapses arrived to the spines of spiny hilar interneurons, whereas this was not observed in the case of CB-positive hilar cells covered with glial processes. The increased input of hilar interneuron cell bodies and dendrites might be a compensatory mechanism: the increased activity of inhibitory cells might decrease the excess activity of granule cells. In the CA1 region the total synaptic coverage of CB-containing interneuron dendrites did not change in the epileptic tissue, but the ratio of inhibitory synapses significantly increased. This was found even in the non-sclerotic CA1 region (type 1, 2) indicating an intense synaptic reorganization takes place before the death of considerable number of pyramidal cells. This increased inhibitory input of inhibitory cells suggests a potentially enhanced disinhibition that may result in hyperexcitability of pyramidal cells, due to a less efficient dendritic inhibition. This could be related to an abnormal synchrony and plasticity in the pyramidal cell network.

Calbindin-positive chandelier cells in the human CA1 region

A high proportion of the CB-immunoreactive terminals giving symmetric synapses terminated on AISs within the str. pyramidale of the human control CA1 region. Thus, beside the CB-immunostained dendritic inhibitory cell types described previously [15, 17], our data suggest the existence of a CB-positive perisomatic inhibitory cell type in the human CA1 region. In our study CB-positive terminals forming multiple contacts on cell bodies were not found, therefore the CB-immunopositive boutons terminating on AISs should belong to a type of chandelier cells rather than to basket cells. These cells do not represent a subpopulation of PV-immunoreactive axo-axonic cells of the CA1 region, since CB and PV were not colocalized in this region.

Sprouting of CB-positive axons in the epileptic hippocampus

Dentate gyrus

The analysis of the input of CB-containing interneuron dendrites also demonstrated the well known phenomena: the mossy fiber sprouting [10] and the loss of CB from granule cells [19]. The sprouted mossy fibers as well as the sprouted CR-positive supramammillary afferents [11] give an extra excitation to granule cells [31].
Possible sources of the CB-immunoreactive excitatory terminals in the epileptic CA1 region

In the strongly sclerotic CA1 region CB-immunoreactive terminals forming asymmetric synapses are present at a similar ratio as in controls, even though CA1 pyramidal cells can be hardly found. We assume that CB-positive excitatory terminals are derived from granule cells and/or from CA2 pyramidal cells, since these are CB-positive principal cells, and are preserved in epilepsy. In the type 1 and 2 epileptic CA1 region, the CB-positive excitatory terminals might originate from CA1 pyramidal cells as well, which are present in large number in these samples [12]. Furthermore, we cannot exclude the possibility that a new extrahippocampal afferent pathway innervated the CA1 region [32, 33].

CB-positive interneuron terminals established new synapses in the epileptic CA1 region

In the CA1 region of the control human hippocampus the main targets of CB-positive terminals are pyramidal cell dendritic shafts and spines. In the strongly sclerotic tissue CB-immunoreactive terminals terminate on the morphologically altered but surviving interneurons and on each other. Sprouting and change in target selection of CB-immunostained terminals are the likely consequences of the death of pyramidal cells. These changes have not been observed in the non-sclerotic tissue where pyramidal cells are present, confirming that the loss of natural targets triggers these events.

Changes of perisomatic inhibitory parvalbumin-positive interneurons

The number of PV-positive interneurons decreased in epilepsy, but most of the cells survived, they lost their PV-content

There are controversial data about the vulnerability of PV-containing interneurons to epileptic damage [17, 34, 35]. Our results showed that the number of PV-immunostained interneurons decreased in the dentate gyrus and the CA1 region, in parallel with the degree of sclerosis. At the same time, the axon terminal cloud in not decreased, until the principal cells are present in the region. Since a decrease of somatic and dendritic PV-immunoreactivity was observed in animal models of ischaemia and epilepsy [21, 22] often with a preservation of terminal staining the loss of PV-immunopositive dendrites and somata is unlikely a consequence of the death of these cells. The preservation of perisomatic inhibitory input in both the dentate gyrus and the CA1 region indicates that similar mechanisms might operate in the human tissue as well.
The role of gap junctions between parvalbumin-positive cells in the synchrony of principal cells

Zonula adhaerentia were frequently observed between PV-positive dendrites both in the human control [15] and the epileptic CA1 region. The electrical coupling plays an important role in the synchrony generation of PV-immunoreactive cells [36]. These perisomatic inhibitory neurons may participate in the efficient arrangement of pyramidal cell firing patterns, and in the induction of large scale neuronal activities, like gamma [37] or theta oscillations [38]. In PV knock out mice, the lack of PV in perisomatic inhibitory cells enhanced the inhibition of principal cells by facilitating GABA-release [39]. Similar processes might occur in the epileptic human tissue, perisomatic inhibitory cells which lost their PV-content might increase the efficiency of inhibition on principal cells in a synchronous way, which might play a role in the generation and maintenance of epileptic seizures [40].

Plastic changes of parvalbumin-containing interneurons in epilepsy

Both in the dentate gyrus and the CA1 region the perisomatic input of principal cells was preserved in epilepsy, as long as they were present in the region, even though a considerable difference was found between the two regions. The perisomatic input of granule cells is preserved, and is significantly increased on the AISs, whereas it is preserved unchanged in the CA1 region. Only the CA1 region of non-, or mildly sclerotic epileptic cases were analysed, where pyramidal cells are present, in the very sclerotic CA1 region pyramidal cells were only occasionally found. Thus, the perisomatic inhibitory cells in the sclerotic CA1 region do not show plastic changes similar to the dentate gyrus, they require the presence of their original targets for surviving and participating in the synaptic reorganization.

The enhancement of the strong excitatory input of PV-positive interneurons [41] might be participate in the excitotoxicity provoked cell death, as it was proved in CA1 pyramidal cells [42, 43]. The preservation in the dentate gyrus and the total lack of PV-positive elements as well as the perisomatic input in the CA1 region suggests, that the two phenomena – i.e. the high excitatory input and the loss of postsynaptic targets – might induce the cell death of PV-immunostained interneurons.

The perisomatic inhibitory input of hippocampal principal cells is preserved in epilepsy

The increased excitation has been attributed to sprouted mossy fibers, and hyperexcitation was thought to be responsible, at least in part, for the generation of epileptic seizures [31, 44]. Decrease of inhibition was also considered to take part in the hyperexcitability of granule cells, and thus, in the induction and maintenance of the seizures [13, 45]. In our samples perisomatic inhibition of principal cells is preserved in the epileptic dentate gyrus ad CA1 region, the inhibitory input of granule cell AISs
is even increased. We suppose that the axons of axo-axonic cells are able to sprout and form new synapses, regardless to their PV-content. The sprouting of inhibitory terminals contacting granule cell axon initial segments is an important element of the synaptic reorganization during epilepsy. Hyperinnervation of granule cell axon initial segments may facilitate the synchronization of their firing [46, 47]. Thus, beside a hyperexcitation, an enhanced inhibition may be instrumental for the generation of epileptic seizures. However, we cannot rule out the possibility that the sprouting of axo-axonic cell terminals is part of a compensatory mechanism designed to provide more effective inhibition of individual granule cells.

In the CA1 region the perisomatic inhibitory input remain unchanged as long as pyramidal cells are present. The lack of profound changes in perisomatic inhibitory input in the CA1 region suggests that other factors are likely to account for the selective vulnerability of pyramidal cells to epileptic injury.

CONCLUSIONS

Our results suggest that the inhibitory network, controlling the input and output of principal cells in the dentate gyrus and the CA1 region, is notably altered in epilepsy. An intense synaptic reorganisation takes place in the epileptic hippocampus, even in the non-sclerotic tissue, before the death of considerable number of pyramidal cells.

The sprouting of mossy fibers and the supramammillary afferent pathway provide an excess excitation to the dentate gyrus, whereas the CA1 region receives an enhanced excitatory input from the CA3 region and from the sprouting of CA1 pyramidal cell axons.

Both principal cells and certain functionally different interneuron types participate in the synaptic reorganisation which follows epileptic activity. These interneurons show plastic changes, not only survival or cell death in response to epileptic injury. CB-positive dendritic inhibitory cells, thought to control the efficacy and plasticity of principal cell input [16], survive in epilepsy, and participate in the synaptic reorganisation, they show plastic changes.

PV-positive perisomatic inhibitory cells, controlling the output of principal cells [16], are preserved in the dentate gyrus. Their axons sprout, but the most of the cells lose their PV-content. This results in an increase of inhibition arriving to the principal cells through the short term facilitation of GABA-release [39]. In the CA1 region most PV-positive interneurons are preserved without changes as long as their postsynaptic targets are present, but they disappear with the death of pyramidal cells.
In summary, the excitation of principal cells is increased and the dendritic inhibition is decreased, whereas the perisomatic inhibition is preserved.

The enhancement of perisomatic inhibitory input in the dentate gyrus, as well as the increase of inhibition originated from the decrease in PV-content of the cells might lead to a more efficient synchronisation of granule cells during gamma oscillations [39]. This enhanced synchrony, together with an excess excitation [10, 11] may facilitate the generation and maintenance of epileptic seizures. Furthermore, the hyperexcitability of CA1 pyramidal cells mediated by the sprouting of their axons [12] as well as by the disinhibition of CB-positive interneurons and by the presence of electrically coupled and synchronised perisomatic inhibitory cells, may participate in the arrangement of pyramidal cell firing patterns, and in the induction or maintenance of hypersynchronous population events and epileptic seizures.

Future strategies considering the possibility of both hyperexcitation and hyperinhibition might result in the development of new therapeutical approaches to prevent epileptic seizures.

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Saját közlemények jegyzéke

Jelen dolgozat témájához kapcsolódó közlemények:


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