Surviving CA1 pyramidal cells receive intact perisomatic inhibitory input in the human epileptic hippocampus

L. Wittner,1 L. Eröss,2 S. Czirják,3 P. Halász,4 T. F. Freund1 and Zs. Maglóczky1

Institute of Experimental Medicine, Hungarian Academy of Science, 2Neurosurgical Department, MAV Hospital Budapest, 3National Institute of Neurosurgery, H-1577 and 4National Institute of Psychiatry and Neurology, Epilepsy Center, Budapest, Hungary

Correspondence to: Dr. T. F. Freund, Institute of Experimental Medicine, Hungarian Academy of Sciences, POB 67, H-1450 Budapest, Hungary
E-mail: freund@koki.hu

Summary
Temporal lobe epilepsy (TLE) is known to be linked to an impaired balance of excitation and inhibition. Whether inhibition is decreased or preserved in the human epileptic hippocampus, beside the excess excitation, is still a debated question. In the present study, quantitative light and electron microscopy has been performed to analyse the distribution, morphology and input–output connections of parvalbumin (PV)-immunopositive interneurons, together with the entire perisomatic input of pyramidal cells, in the human control and epileptic CA1 region. Based on the degree of cell loss, the patients with therapy-resistant TLE formed four pathological groups. In the non-sclerotic CA1 region of TLE patients, where large numbers of pyramidal cells are preserved, the number of PV-immunopositive cell bodies decreased, whereas axon terminal staining, and the distribution of their postsynaptic targets was not altered. The synaptic coverage of CA1 pyramidal cell axon initial segments (AISs) remained unchanged in the epileptic tissue. The somatic inhibitory input is also preserved; it has been decreased only in the cases with patchy pyramidal cell loss in the CA1 region (control, 0.637; epileptic with mild cell loss, 0.642; epileptic with patchy cell loss, 0.424 μm synaptic length/100 μm soma perimeter). The strongly sclerotic epileptic CA1 region, where pyramidal cells can hardly be seen, contains a very small number of PV-immunopositive elements. Our results suggest that perisomatic inhibitory input is preserved in the epileptic CA1 region as long as pyramidal cells are present. Basket and axo-axonic cells survive in epilepsy if their original targets are present, although many of them lose their PV content or PV immunoreactivity. An efficient perisomatic inhibition is likely to take part in the generation of abnormal synchrony in the non-sclerotic epileptic CA1 region, and thus participate in the maintenance of epileptic seizures driven, for example, by hyperactive afferent input.

Keywords: perisomatic inhibition; GABA; basket cell; chandelier cell; selective vulnerability

Abbreviations: AIS = axon initial segment; CA1–3 = regions of the cornu Ammonis; PV = parvalbumin; TLE = temporal lobe epilepsy


Introduction
Epilepsy is thought to be related to a changed balance between excitation and inhibition (Avoli, 1983; Mody et al., 1992). In epilepsy, the CA1 region was shown to receive an enhanced excitatory input from the CA3 region in the rat hippocampus (Buzsáki et al., 1989; Rafiq et al., 1993; Bragin et al., 1997), from the dentate gyrus and/or CA2 region in the human hippocampus (Wittner et al., 2002) and from the sprouting CA1 pyramidal cell axons in rats (Esclapez et al., 1999; Lehmann et al., 2001) and humans (Lehmann et al., 2000). It is still not clear whether inhibition is decreased or preserved in the human epileptic CA1 region. There is evidence for interneuronal cell death (de Lanerolle et al., 1988, 1989; Maglóczky et al., 2000), as well as for the preservation of glutamic acid dehydrogenase (GAD)-positive cells (Babb et al., 1989).

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Since CA1 pyramidal cells provide the cortical output connections of the hippocampus (Ramón y Cajal, 1909–1911; Lorente de Nó, 1934; Maclean, 1992) and are known to be the most vulnerable in epilepsy (Sommer, 1880; Corsellis, 1955; Falconer, 1968), alterations in the inhibitory network in the CA1 region may be crucial in the pathophysiology of the epileptic temporal lobe.

The calcium-binding protein parvalbumin (PV) is known to be present exclusively in perisomatic inhibitory interneurons in the hippocampus of rodents (Kosaka et al., 1987; Katsumaru et al., 1988b; Holm et al., 1990; Nitsch et al., 1990), monkeys (Seress et al., 1991; Ribak et al., 1993) and humans (Braak et al., 1991; Seress et al., 1993). Two perisomatic inhibitory cell types, basket and chandelier or axo-axonic cells, were demonstrated to be PV-immunopositive in all examined species, including humans (Seress et al., 1993; for a review see Freund and Buzsáki, 1996) and to control the output of principal cells (Miles et al., 1996). The survival of PV-immunopositive interneurons in epilepsy, as well as their role in the pathophysiological mechanisms of seizures and cell death are still controversial questions. Some authors have described the preservation (Sloviter et al., 1991; Arellano et al., 2003) and others the vulnerability (Zhu et al., 1997; Wittner et al., 2001) of these cells in the human epileptic hippocampus, and a decrease in the number of PV-immunopositive interneurons has been found in the epileptic neocortex (DeFelipe et al., 1993; Marco et al., 1997; DeFelipe, 1999). PV immunoreactivity is very sensitive to epileptic and ischaemic conditions and might disappear from neurons that are otherwise preserved (Johansen et al., 1990; Tortosa and Ferrer, 1993; Bazzett et al., 1994; Scotti et al., 1997a, b). Since PV immunostaining probably reveals only part of the originally PV-immunopositive cells, which in turn represent only one of the at least two basket cell types (the other being the cholecystokinin-containing cells; for review see Freund, 2003), perisomatic inhibition cannot be fully characterized by the analysis of PV-immunopositive interneurons. Controversial results have been found in different rat models of epilepsy and human studies concerning the perisomatic inhibitory input of hippocampal principal cells. In the dentate gyrus, loss of axo-axonic and axo-somatic inhibitory inputs of granule cells was found in the kindling (Sayin et al., 2003) and pilocarpine models (Kobayashi and Buckmaster, 2003), but the preservation of perisomatic inhibitory input (Wittner et al., 2001) and an increased rather than decreased recurrent inhibition of granule cells (Isokawa-Akesson et al., 1989) has been shown in the dentate gyrus of epileptic patients. Perisomatic GABAergic input of CA1 pyramidal cells remained unchanged in the kainate model (Morin et al., 1999), whereas in the pilocarpine model dendritic but not somatic inhibition is decreased (Cossart et al., 2001), together with a slight decrease also in axo-axonic input (Dinocourt et al., 2003). These contradictory results also show that an extensive comparison and correlation with data obtained from human epileptic tissue is needed to validate predictions from animal models.

No detailed electron microscopic analyses have been done to date investigating the perisomatic inhibitory input of CA1 pyramidal cells in the human hippocampus. Here we analysed the distribution, morphology and input–output characteristics of PV-immunopositive interneurons, as well as the inhibitory synaptic input of CA1 pyramidal cell somata and axon initial segments (AISs) of human control subjects and epileptic patients.

**Material and methods**

PV-containing elements were examined in the CA1 region of 32 temporal lobe samples surgically removed from epileptic patients, and in seven control hippocampi. Patients with intractable TLE were operated on in the National Institute of Neurosurgery and in the MAV Hospital in Budapest; standard anterior temporal lobectomies were performed (Spencer and Spencer, 1985). The anterior third of the temporal lobe was removed together with the temporomedial structures. Control brain samples were obtained from a 37-year-old woman who died by accident, from 47- and 48-year-old women and 47-, 51- and 56-year-old men who had a cardiac arrest, and from a 53-year-old man who died by suffocation. None of the control subjects had a record of any neurological disorder. Brains were removed 2–4 h after death; the dissection was performed in the Institute of Pathology and Forensic Medicine, Semmelweis University, Budapest. In all cases, the patient’s consent was obtained according to the Declaration of Helsinki, and the ethical regulations of the Hungarian Ministry of Health were followed.

After surgical removal, the epileptic tissue was immediately dissected into 2 mm thick blocks, and immersed in a fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4), as described earlier (Maglóczy et al., 1997). The fixative was changed every hour to a fresh solution during constant agitation for 6 h, then the blocks were post-fixed in the same fixative overnight. Four of the control hippocampi were subjected to the same procedure. Three of the control brains (C2, C10 and C11) were removed from the skull 2 h after death and both internal carotid and vertebral arteries were cannulated; the brain was perfused with physiological saline (21 in 30 min) followed by a fixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB (51 in 3.5 h). The hippocampus was removed after perfusion and cut into 2 mm thick blocks, which were post-fixed in the same fixative solution overnight.

**Immunocytochemistry**

Vibratome sections (60 μm thick) were cut from the blocks and, following washing in PB, they were immersed in 30% saccharose for 1–2 days, then frozen three times over liquid nitrogen. Sections were processed for immunostaining against PV, as follows. Sections were transferred to Tris-buffered saline (TBS, pH 7.4), then endogenous peroxidase was blocked by 1% H2O2 in TBS for 10 min. TBS was used for all the washes (3 × 10 min between each sample) and dilution of the antisera. Non-specific immunostaining was blocked by 5% milk powder and 2% bovine serum albumin. A monoclonal mouse antibody against PV (1 : 5000; Sigma, St Louis, MO) was used for 2 days at 4°C. The specificity of the antibody has been thoroughly tested by the manufacturer. For visualization of immunopositive elements, biotinylated anti-mouse immunoglobulin G (IgG) (1 : 300, Vector) was applied as secondary antiserum followed by
avidin-biotinylated horseradish peroxidase complex (ABC; 1 : 300, Vector). The immunoperoxidase reaction was developed by 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) dissolved in Tris buffer (TB, pH 7.6) as a chromogen. Sections were then osmicated (1% OsO4 in PB, 40 min) and dehydrated in ethanol (1% uranyl acetate was added at the 70% ethanol stage for 40 min) and mounted in Durcupan (ACM, Fluka). The control hippocampi were processed in the same way.

Controls of the method included the incubation of sections in the same way, but the primary antibody was replaced by normal mouse serum. No specific immunostaining could be detected under these conditions. Non-specific staining visualized large numbers of lipofuscin granules in the cells, which were therefore considered as background.

After light microscopic examination, areas of interest were re-embedded and sectioned for electron microscopy. Ultrathin serial sections were collected on Formvar-coated single slot grids, stained with lead citrate, and examined with a Hitachi 7100 electron microscope.

Quantitative analysis

Cell counting was performed in three control (C6, C10 and C11) and in six epileptic (P16, P22, P31, P40, P75 and P79) brains. Each PV-immunopositive cell together with the outlines of the CA1 region were drawn by camera lucida from two to five sections per patient. The area of the CA1 region was measured using the analySIS program, and the number of PV-immunopositive cells per unit area (mm²) was determined in the same area. The numbers of cells derived from different sections of the same subject were summed and divided by the sum of the areas of the same sections. The averages of the different pathological groups (control, mild, patchy and sclerotic) were determined by calculating the average of data from all patients in each group.

The target elements of PV-immunopositive axon terminals were examined in four control brains (C2, C6, C10 and C11) and in four epileptic patients (P15, P31, P40 and P54). Based on light microscopic examination, sections parallel to the plane of the CA1 pyramidial cell apical dendrites were chosen for the electron microscopic analysis. The entire width of CA1 stratum pyramidale including the zone of AISs located at the border of stratum oriens was re-embedded from PV-immunostained sections; the size of the blocks was similar. Serial sections were made from the blocks, and PV-containing terminals were analysed in every tenth section in order, following the rules of systematic random sampling, to avoid sampling of the same axon terminals. Photographs were taken from every PV-immunopositive synaptic terminal in each section, and the distribution of the postsynaptic target elements of the PV-immunopositive terminals was determined. In all electron microscopic analyses, the means of the different pathological groups (control versus epileptic, mild and patchy groups) were determined by calculating the average of the data of every patient belonging to that group.

The synaptic inputs of CA1 pyramidal cell AISs and somata were analysed in three control hippocampi (C2, C6 and C10) and in five epileptic patients (AIS, P15, P16, P40, P54 and P79; cell body, P15, P31, P40, P54 and P79). The sampling of the AISs of pyramidal cells was made as described above; each AIS observed in the stratum pyramidale and the bordering zone of the stratum oriens was digitized from every tenth section using a MegaView II camera. About 40–80 AISs were analysed in every subject. The border zone of the stratum oriens was included in the examined area to analyse the whole length of the pyramidal cell AISs. The AISs were obliquely cut, and the average perimeter was similar in all control and epileptic subjects. This varied between 7.38 and 13.64 µm, and no statistical differences were found between the groups of AISs of different subjects [Kruskal–Wallis ANOVA (analysis of variance), P > 0.05]. For the analysis of synaptic input of CA1 pyramidal cell bodies, serial sections were made from two blocks from each subject containing the entire width of the stratum pyramidale. About 20 pyramidal cells were digitized from each block (~40 cells per subject). The perimeters of the pyramidal cell AISs and somata and the length of the synapses they receive were measured by the analySIS program. The synaptic coverage (µm synaptic length/100 µm of AIS or soma perimeter) of every AIS and soma has been recorded. Statistical analysis (Kruskal–Wallis ANOVA and Mann–Whitney U test) were performed using the Statistica 6.0 program. The number of synapses (terminals forming simple and perforated synapses) per 100 µm perimeter was calculated in every patient by dividing the total number of synapses by the total perimeter recorded. Asymmetric somatic synapses were only occasionally found, and were not included in the calculation.

Dependence of PV immunostaining on fixation and post-mortem delay

In a preliminary experiment, we examined 12 control brains with different post-mortem delays and age in both genders (Wittner et al., 2002). Briefly, the progression of age influences the quality and quantity of immunostaining, i.e. most of the antisera showed weaker staining and a lower number of cells in aged subjects. Therefore, the subjects older than 60 years have been excluded from the detailed analysis. For electron microscopic studies, subjects were chosen with a post-mortem delay of <4 h, because longer post-mortem delays (6–24 h) resulted in poor ultrastructural preservation, and a decrease in immunoactivity. The electron microscopic analysis of these tissues revealed acceptable ultrastructural preservation even in the immersion-fixed control (C6), although it was inferior to the perfused tissue. The preservation of the 2–3 h post-mortem perfused controls (C2, C10 and C11) was comparable to the immediately fixed epileptic samples and to the perfused animal tissues. The PV staining in the control subjects included in the study was similar to each other and to previously described human or monkey PV immunostaining (Braak et al., 1991; Seress et al., 1991, 1993; Sloviter et al., 1991; Ribak et al., 1993). Therefore, we concluded that the differences found between control and epileptic tissues in the present study are likely to be caused by epilepsy.

Pathological classification of the tissue samples

All patients examined in the present study had therapy-resistant epilepsy of temporal lobe origin. The seizure focus was identified by multimodal studies including video-EEG monitoring, single photon emission computed tomography (SPECT) and/or PET. Only patients without gross temporal lobe damage or tumour were included in the study. The patients had different degrees of hippocampal atrophy and/or sclerosis. Similarly to recent results (de Lanerolle et al., 2003), we established four groups of epileptic patients based on the principal cell loss and interneuronal changes examined at the light microscopic level as follows (Table 1). (i) Type 1 (mild): similar to control, no considerable cell loss in the CA1 region, pyramidal cells are present, layers are visible, their borders are clearly identified. There is a slight loss in certain interneuron types, mostly in the hilus and the stratum oriens of the CA1 region. (ii) Type 2 (patchy): pyramidal cell loss in patches of the CA1
pyramidal cell layer, but these segments of the CA1 region are not atrophic. Interneuron loss is more pronounced. (iii) Type 3 (sclerotic): the CA1 region is shrunken, atrophic, >90% cell loss, occasionally scattered pyramidal cells remained in the CA1 region, separation of the layers is impossible. Only the stratum lacunosum-moleculare is present in the CA1 region as a distinct layer; the other strata could not be separated from each other due to the lack of pyramidal cells and shrinkage of the tissue. This remaining part should contain the layers determined in the control as strata oriens, pyramidale and radiatum. Mossy fibre sprouting and considerable changes in the distribution and morphology of interneurons can be observed in the samples of this group. (iv) Type 4 (gliotic): the whole hippocampus is shrunken, atrophic, not only the CA1 region, the loss of all cell types can be observed, including the resistant neurons (granule cells, calbindin-positive interneurons). The number of samples belonging to this group was small and therefore they were not included in this study.

**Results**

The distribution and morphological features of PV-immunopositive cells were examined at light and electron microscopic levels in the CA1 region derived from human controls and TLE patients. The number and distribution of cells proved that the antigenicity was retained for PV in the post-mortem controls included in this study (see Material and methods: considerations of the quality of post-mortem tissue). Samples taken from four control brains (three of them were perfused, see Material and methods) as well as from epileptic patients with mild and strong sclerosis were analysed further in the electron microscope to obtain data about the distribution of PV-positive axon terminals, their postsynaptic targets, the synaptic input of PV-positive interneuron cell bodies and dendrites, as well as the perisomatic synaptic input of CA1 pyramidal cells.

**Light microscopy of PV-positive interneurons**

In describing different regions of the hippocampal formation, we used the nomenclature of Lorente de Nó (1934) and Rosene and Van Hoesen (1987) with the proposed modifications of Seress concerning the CA3c region (Seress, 1988) (see also Fig. 1).

In the human control CA1 region, PV-immunopositive interneurons were located in the strata oriens and pyramidale (Fig. 2A), as was reported in previous publications (Braak

### Table 1 Patient data and general characterization of cell loss of the hippocampus

<table>
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<th>Path. group</th>
<th>Patient number</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Age at onset of epilepsy (year)</th>
<th>Duration of epilepsy (years)</th>
<th>CA1 sclerosis</th>
<th>Thickness of the granule cell layer</th>
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Semi-quantitative scale: 0 = no damage; 1 = 0–20%; 2 = 20–50%; 3 = >50%; 3* = 100% of the pyramidal cells of the region are missing.
Path. group = pathological group; M = male; F = female.
Large multipolar cells can be seen in the stratum pyramidale; their long dendrites run radially through the region and terminate in the stratum lacunosum-moleculare. Horizontal PV-immunopositive interneurons are situated in the stratum oriens; their dendrites arborize mostly in this layer (Fig. 2A). As it was described in the rat hippocampus (Fukuda and Kosaka, 2000), we often found long dendritic segments originating from different cells, attached to each other. Such dendritic juxtapositions were seen most frequently among radially running dendrites in the strata radiatum and lacunosum-moleculare (Fig. 2A, arrowheads) and the horizontal dendrites of the stratum oriens. Dense PV-immunopositive terminal staining was observed in the stratum pyramidale (Fig. 2A, insert). The PV-immunopositive interneurons in the human control CA1 region are known to belong to the populations of basket and axo-axonic cells (Braak et al., 1991; Seress et al., 1993; Sloviter et al., 1991), and terminate within the stratum pyramidale.

In the non-sclerotic epileptic CA1 region with mild cell loss (type 1), the distribution and number of PV-immunopositive interneurons did not change considerably; only a slight decrease was observed in the number of the horizontal stratum oriens cells (Fig. 2B, Table 2). Interestingly, the dendrites of PV-immunopositive interneurons were shorter than in the control tissue. Dense axonal staining of a density similar to, if not more than, that of controls was observed in the stratum pyramidale (Fig. 2B, insert); basket-like formations (small arrow) and vertically oriented bouton rows characteristic of chandelier cells can also be distinguished (double arrows). In the CA1 region with patchy cell loss (type 2), a considerable decrease was found in the number of PV-immunopositive cells (Fig. 2C, Table 2), with the preservation of a dense axonal staining (Fig. 2C, insert). The termination pattern of PV-immunopositive cells did not change; the axonal cloud was confined to the stratum pyramidale. It was distributed homogeneously in the pyramidal cell layer, and also covered the patches with no pyramidal cells visible at the light microscopic level (Fig. 2C, insert). In the sclerotic epileptic tissue (type 3), the number of PV-immunopositive elements was dramatically decreased (Fig. 2D, Table 2). PV-immunopositive cells and axons can hardly be found; only a few PV-immunopositive dendrites were present in the stratum pyramidale.
**Fig. 2** Light micrographs showing PV-immunostained elements in the human control (A) and epileptic (B, C and D) CA1 region. (A) In the control CA1 region, large PV-positive multipolar cells are located in the stratum pyramidale and horizontal cells in the stratum oriens (arrows). Long radial dendrites are running in the stratum radiatum and terminate in the stratum lacunosum-moleculare. Long dendrite segments originating from different cells were often attached to each other (arrowheads). A dense terminal staining can be observed in the stratum pyramidale (insert). (B) In the epileptic CA1 region with mild cell loss, a slight loss of PV-positive cells was observed, mostly in the stratum oriens. PV-positive dendrites seem to be shorter. The axon terminal cloud (insert) is similar to the control in density; basket- (small arrows) and chandelier-like (double arrows) formations can be observed. (C) In the epileptic tissue with patchy cell loss, the decrease in number of PV-immunostained cells is more pronounced. A dense terminal staining (insert), similar to the control, can be found even in those regions where no PV-positive cells are present. (D) In the strongly sclerotic epileptic tissue, the number of PV-immunoreactive cells is dramatically decreased; usually a few dendrites (arrowheads) are present in the region. Axon terminals can hardly be found (insert). s.o. = stratum oriens; s.p. = stratum pyramidale; s.r. = stratum radiatum. Scales, 50 μm; inserts, 15 μm.
Table 2 Density of PV-positive cells in the human control and epileptic CA1 region

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Cell density (no. of cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>3.85</td>
</tr>
<tr>
<td>C11</td>
<td>6.81</td>
</tr>
<tr>
<td>C6</td>
<td>4.97</td>
</tr>
<tr>
<td>Average</td>
<td>5.21</td>
</tr>
<tr>
<td>Epileptic (mild)</td>
<td></td>
</tr>
<tr>
<td>P40</td>
<td>3.06</td>
</tr>
<tr>
<td>P79</td>
<td>4.97</td>
</tr>
<tr>
<td>Average</td>
<td>4.02</td>
</tr>
<tr>
<td>Epileptic (patchy)</td>
<td></td>
</tr>
<tr>
<td>P16</td>
<td>1.01</td>
</tr>
<tr>
<td>P31</td>
<td>0.89</td>
</tr>
<tr>
<td>Average</td>
<td>0.95</td>
</tr>
<tr>
<td>Epileptic (sclerotic)</td>
<td></td>
</tr>
<tr>
<td>P75</td>
<td>1.10</td>
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<tr>
<td>P22</td>
<td>1.15</td>
</tr>
<tr>
<td>Average</td>
<td>1.12</td>
</tr>
</tbody>
</table>

The sclerotic CA1 region (Fig. 2D, insert). The border between the sclerotic CA1 region and the prosubiculum was clearly visible; this latter was not sclerotic, a large number of pyramidal cells were present and the PV staining was similar to the control (Fig. 1). Since very small numbers of PV-immunopositive elements were found in the sclerotic CA1 region, samples from only the non-sclerotic epileptic hippocampi (with mild or patchy cell loss) were chosen for further quantitative analyses performed at the electron microscopic level. Cell counting confirmed the loss of PV-immunopositive cells in the epileptic CA1 region (Table 2). Three controls and two patients from each pathological group were chosen for the analysis. The area of the CA1 region was measured and the number of PV-immunopositive cells per unit area was determined (see Material and methods). We found a decrease of PV-immunopositive cells that correlated well with the degree of hippocampal damage in general (Table 2), i.e. the number of PV-immunopositive neurons was similar to control in those hippocampi where no considerable pyramidal cell loss was found in the CA1 region (type 1), a severe decrease in the number of PV-immunopositive somata accompanied by the preservation of axonal staining in cases with patchy cell loss (type 2), and severe cell loss with no axonal staining for PV in the sclerotic hippocampi (type 3).

Electron microscopy of PV-positive elements

Cell bodies and dendrites

Five PV-immunopositive cells from the CA1 region of human controls C2, C6 and C11 were analysed at the electron microscopic level (Fig. 3A). Every examined cell body was located in the stratum pyramidale. PV-immunopositive interneuron cell bodies received a large number of asymmetrical (excitatory) synapses, and axon terminals forming symmetrical (inhibitory) synapses were rarely seen (Fig. 3A1–3). Ten to 12 synaptic contacts per section were observed to terminate on the cell body of PV-immunopositive interneurons, usually one or two of them giving symmetrical synapses. PV-stained dendrites were covered by a large number of asymmetrical synapses. Zonula adhaerentia were frequently observed between PV-immunopositive dendrites (Fig. 4A).

Four PV-immunopositive interneurons derived from epileptic tissue (P15, P40, P54 and P79) were analysed in the electron microscope (Fig. 3B). Only PV-immunopositive cells from the non-sclerotic CA1 region were analysed, since PV-immunopositive cell bodies can hardly be found in the sclerotic tissue. In the non-sclerotic epileptic CA1 region, the synaptic input of PV-immunopositive cell bodies and dendrites was similar to the control, a large number of terminals forming asymmetrical synapses and a few others giving symmetrical synapses terminated on the PV-immunopositive elements (Fig. 3B1–2). A small number of PV-immunopositive dendrites were also analysed in the sclerotic CA1 region (P21, P22, type 3, sclerotic tissue). These dendrites were also covered by asymmetrical synapses, and by glial elements (Fig. 4B). In the epileptic CA1 region, zonula adhaerentia were frequently found between PV-immunopositive dendrites, but PV-immunopositive and -negative dendrites also formed zonula adhaerentia with each other (Fig. 4B).

Axon terminals and postsynaptic targets

The postsynaptic target distribution of PV-immunopositive boutons was determined in the CA1 region of four control (C2, A6, C10 and C11, n = 52–55) and four epileptic samples (P40 and P54, mild; and P15 and P31, patchy; Fig. 5, n = 52–107). In the CA1 region of patients with strong sclerosis, PV-immunopositive terminals were too sparse to study; in most areas they were absent. In the sample from P15, we distinguished patches containing (P15PC+) and lacking (P15PC−) pyramidal cells. Blocks were re-embedded from both regions, and the target distribution of PV-immunopositive terminals determined separately. Since the results deriving from the two separate blocks were similar, the data were pooled. PV-immunopositive terminals gave exclusively symmetrical synapses mostly to pyramidal cells in both control and epileptic tissue: they terminated on AISs (control, 14.5%; epileptic, 17.3%), pyramidal cell bodies (control, 14.8%; epileptic, 11.1%; Fig. 4D), proximal dendrites (control, 18.2%; epileptic, 23.1%), distal dendrites of pyramidal cells (control, 11.6%; epileptic, 6.4%) and spines (control, 17.6%; epileptic, 10.9%). They rarely established synaptic contacts on interneuron dendrites (control, 2.3%; epileptic, 1.9%), and even less frequently on PV-immunopositive interneuron dendrites. We also distinguished a category of unidentified dendrites with small diameter (control, 21.0%; epileptic, 29.4%). There was a larger variance within the groups of control and epileptic samples than between the control and the epileptic groups. Some of the targets were degenerating profiles in the epileptic samples from P15, P31 and P54 (P15, 54.2%; in P15PC+, 11.7%; in P15PC−, 92.2%; P31, 81.5%; P54, 60.7%; Fig. 4C).
Perisomatic inhibition of pyramidal cells

Cell bodies. Somatic inhibitory input of CA1 pyramidal cells was analysed in three control (C2, C6 and C10) and in five epileptic (P40, P54 and P79, mild; and P15 and P31, patchy) samples (Table 3, Fig. 6A). From each subject, we analysed on average 40 pyramidal cells from different areas (two separate blocks, ~20 cells each). Synaptic coverage (μm synaptic length/100 μm soma perimeter) and the number of synapses/100 μm soma perimeter were determined (see Materials and methods). Every symmetric synapse, irrespective of its PV content, was recorded and measured. Asymmetric synapses were only occasionally found and were not included in the analysis. In the control tissue, 16–29% of the terminals were PV immunopositive, giving symmetric...
synapses to the pyramidal cell bodies. This ratio was higher in certain epileptic cases (45–48%, in P79, mild; P15 and P31, patchy), and did not change in others (27%, in P40; P54, mild).

In both control and epileptic tissue, some small differences were found in the synaptically coverage and the number of terminals giving synapses to pyramidal cell somata in the different blocks derived from the same subject. After the

Fig. 4 PV-stained dendrites received a large number of asymmetric (excitatory) synapses (arrows) in both the control and epileptic CA1 region. Zonula adhaerentia (arrowheads) were often observed between PV-immunoreactive dendrites in the human control (A) and epileptic tissue. Zonula adhaerentia were found between PV-positive and -negative dendrites as well in the epileptic CA1 region (B). In the strongly sclerotic epileptic CA1 region, only a small number of PV-positive dendrites was found (B), and usually they were covered by glial processes (g). PV-immunoreactive axon terminals give symmetric synaptic contacts mostly to pyramidal cells in both the control and non-sclerotic epileptic tissue (with mild or patchy cell loss in the CA1 region). This figure shows two PV-stained boutons terminating on degenerating (C) and healthy pyramidal cell somata (D) deriving from an epileptic patient with patchy cell loss in the CA1 region. Scales: 1 μm.
data were pooled by subjects, very similar results were found within the different groups (control, mild and patchy epileptic, Table 3, Fig. 6A). In the epileptic group with mild cell loss the synaptic input of pyramidal cells was very similar to the control (synaptic coverage: 0.637 ± 0.043 (mean ± SEM) versus 0.642 ± 0.048 in control and epileptic with mild cell loss, respectively).

In the epileptic tissue with patchy cell loss (P15 and P31), we took samples from light microscopically identified patches that were rich or poor in (contained hardly any) pyramidal cells. At the electron microscopic level, we found that in ‘poor’ patches, pyramidal cells were still present, but the majority of them showed signs of degeneration (100% in P15; 81% in P31). Interestingly, all examined parameters (synaptic coverage, number of synapses/100 µm soma

Table 3 Synaptic input of CA1 pyramidal cell somata

<table>
<thead>
<tr>
<th>Subject</th>
<th>Synaptic coverage of pyramidal cell somata (µm synaptic length/100 µm soma perimeter, mean ± SEM)</th>
<th>No. of synapses (terminals)/100 µm soma perimeter</th>
<th>Ratio of PV-positive terminals (%)</th>
<th>Average synaptic active zone length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C2 (n = 40) 0.676 ± 0.086</td>
<td>3.36</td>
<td>29.07</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>C10 (n = 40) 0.633 ± 0.074</td>
<td>3.44</td>
<td>15.85</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>C6 (n = 40) 0.604 ± 0.070</td>
<td>3.59</td>
<td>24.42</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>Average 0.637</td>
<td>3.462</td>
<td>23.11</td>
<td>0.165</td>
</tr>
<tr>
<td>Epileptic</td>
<td>P40 (n = 40) 0.730 ± 0.097</td>
<td>3.95</td>
<td>26.67</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>P54 (n = 39) 0.583 ± 0.079</td>
<td>3.13</td>
<td>26.92</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>P79 (n = 40) 0.613 ± 0.075</td>
<td>3.63</td>
<td>46.07</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>Type 1 average 0.642</td>
<td>3.572</td>
<td>33.22</td>
<td>0.166</td>
</tr>
<tr>
<td></td>
<td>P15 (n = 39) 0.444 ± 0.076*</td>
<td>2.06</td>
<td>45.10</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>P31 (n = 40) 0.404 ± 0.075*</td>
<td>2.18</td>
<td>48.00</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td>Type 2 average 0.424*</td>
<td>2.117</td>
<td>46.55</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>Average 0.554</td>
<td>2.990</td>
<td>38.55</td>
<td>0.173</td>
</tr>
</tbody>
</table>

n = number of analysed pyramidal cell bodies, *P < 0.05.

Fig. 5 Postsynaptic target distribution of PV-positive axon terminals in the CA1 region of human control and epileptic hippocampus. PV-immunostained terminals give symmetric synapses mostly to pyramidal cells: somata, AIS, proximal and distal dendrites and spines. The target distribution did not change systematically in the epileptic tissue; a high variability was found between the different subjects of the same groups (control, and epileptic with mild or patchy cell loss).

Fig. 6 Perisomatic synaptic input of CA1 pyramidal cells. (A) The synaptic coverage (100 µm synaptic length/100 µm soma or AIS perimeter) of CA1 pyramidal cell somata did not change in the epileptic CA1 region with mild cell loss, but it decreased notably in the epileptic tissue derived from patients with patchy cell loss in the CA1 region. (B) The synaptic coverage of CA1 pyramidal cell AISs did not change in the epileptic tissue compared with the control.
and microtubule fascicles was preserved. The synaptic coverage of degenerating AISs from both samples was decreased [synaptic coverage in P15, 3.60 ± 0.65 (mean ± SEM) versus 2.20 ± 0.53 of non-degenerating and degenerating AIS, respectively; synaptic coverage in P54, 3.98 ± 0.94 versus 3.69 ± 0.49 of non-degenerating and degenerating AIS, respectively]. However, the sample is too small (16 degenerating AISs from P15, and eight non-degenerating AISs from P54) to evaluate the significance of this result.

We found an increase of active zone length of synapses terminating on pyramidal cell somata and AISs (Tables 3 and 4), as it was reported earlier in an animal model of epilepsy (Nusser et al., 1998) and human epileptic tissue (Wittner et al., 2002).

## Discussion

### The number of PV-immunopositive cells is decreased in the epileptic CA1 region

Animal models and human studies have provided evidence for both the vulnerability (Best et al., 1993; Maglóczky and Freund, 1995; Buckmaster and Dudek, 1997; Zhu et al., 1997) and the preservation (Sloviter et al., 1991) of PV-immunopositive interneurons in epilepsy. In the present study, we observed a decrease in the number of PV-immunopositive interneurons in the human epileptic CA1 region, and this reduction was correlated with the degree of sclerotic cell loss. In the non-sclerotic epileptic CA1 region with mild cell loss (type 1), a slight decrease was observed in the number of PV-immunopositive interneurons (Fig. 2B), which was more pronounced in the CA1 region with patchy pyramidal cell loss (type 2). This phenomenon was observed in conjunction with the preservation of the dense terminal staining in the stratum pyramidale (Fig. 2C, Table 2). The distribution of postsynaptic target elements of PV-positive axon terminals remained unchanged.
(Fig. 5) and the perisomatic inhibitory input of CA1 pyramidal cells was preserved in the non-sclerotic epileptic CA1 region (Tables 3 and 4, Fig. 6). A reduction of PV immunoreactivity without cell loss was found in animal models of ischaemia and epilepsy (Johansen et al., 1990; Tortosa and Ferrer, 1993; Bazzett et al., 1994; Maglóczky and Freund, 1995; Scotti et al., 1997a), as well as in the human epileptic dentate gyrus (Wittner et al., 2001). We found that the loss of PV immunostaining from cell bodies and dendrites was accompanied by the preservation of their terminals as well as the inhibitory input of pyramidal cells, suggesting that here again we are dealing with the decrease of immunoreactivity rather than the death of the cells. In animal models of epilepsy, slower synthesis of PV or a change in conformation due to calcium overloading was suggested as a possible cause for the reduced PV immunostaining (Johansen et al., 1990; Scotti et al., 1997a). This might also operate in the human hippocampus.

In contrast to the non-sclerotic epileptic hippocampus, in the sclerotic CA1 region PV-immunopositive interneurons are lost, the number of PV-immunonegative elements has dramatically decreased and usually small numbers of dendrites are visible in the region (Fig. 2D). The nomenclature of the human hippocampal formation is still controversial, including the determination of the border between the CA1 region and subiculum and the existence of the prosubiculum (Rosene and Van Hoesen, 1987; Amaral and Insausti, 1990; Duvernoy, 1998). This might explain the contradictory observations concerning the preservation (Arellano et al., 2003) or disappearance (present work) of PV-immunopositive elements in the sclerotic CA1 region.

PV-immunopositive interneurons receive the highest number of synapses among the different interneuron types in the rat hippocampus, and they receive the smallest proportion of GABAergic input (Gulyás et al., 1999). No difference was observed in the synaptic input of PV-immunopositive cell bodies in the epileptic CA1 region compared with the control (Fig. 3), but the sprouting of mossy fibres in the dentate gyrus (Sutula et al., 1989; Houser et al., 1990; Represa et al., 1990), as well as the sprouting of a calbindin-positive excitatory pathway in the CA1 region (Wittner et al., 2002) is likely to increase the density of excitatory input to PV-immunopositive dendrites further (Blasco-Ibáñez et al., 2000; Maglóczky et al., 2000). Direct evidence for enhanced excitation in the CA1 region was shown only in animal models (Buzsáki et al., 1989; Rafiq et al., 1993; Bragin et al., 1997).

The overexcitation of these interneurons might play a role in the decrease of PV below the level of immunocytochemical detection (Bazzett et al., 1994), and/or in the excitotoxic death of these cells, as was shown earlier for CA1 pyramidal cells (Sano and Kirino, 1990; Meldrum, 1991, 1993). PV-immunopositive cell bodies located in the CA1 region receive a much larger excitatory input than those in the dentate gyrus (10–12 versus 4–5 synapses/cell body perimeter in one section, in the CA1 region or the dentate gyrus, respectively; Wittner et al., 2001). It is possible that these two mechanisms together, i.e. loss of original targets and overexcitation of the cells, play a role in the disappearance of PV-immunopositive interneurons in the sclerotic CA1 region.

**Perisomatic inhibition is preserved if pyramidal cells are present**

Basket cells and axo-axonic (or chandelier) cells are responsible for the perisomatic input of principal cells in the hippocampus of all examined species, including rat and monkey (Somogyi et al., 1983; Lewis and Lund, 1990; Seress and Frotscher, 1991; Freund and Buzsáki, 1996; Halasy et al., 1996; Martínez et al., 1996) and humans as well (Seress et al., 1993; Braak et al., 1991). The majority of these cells are PV immunopositive, but cholecystokinin-containing basket cells (Lotstra and Vanderhaeghen, 1987) and calbindin-positive chandelier cells (Wittner et al., 2002) have also been described in the human hippocampus.

Since the PV content of perisomatic inhibitory cells is sensitive to epileptic and ischaemic conditions (see above), some surviving PV-immunopositive cells may not be visible with PV immunostaining. Therefore, we examined the perisomatic inhibitory synapses on CA1 pyramidal cells irrespective of their PV immunoreactivity.

Analysis of synaptic coverage of pyramidal cell somata and AISs showed that perisomatic inhibitory input is preserved in the epileptic CA1 region, except in the samples belonging to the pathological group with patchy cell loss, where a slight, but significant decrease was observed (Table 3, Fig. 6). Furthermore, the distribution of postsynaptic targets of PV-positive terminals in the epileptic tissue was similar to that of the control (Fig. 5). These results suggest that perisomatic inhibitory cells may survive in epilepsy, if their targets are present in the region. 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.

In the rodent hippocampus, electrical coupling plays an important role in the synchronization of PV-immunopositive interneurons (Deans et al., 2001; Meyer et al., 2002). Gap junction-coupled ensembles of PV-immunopositive cells (Katsumaru et al., 1988a; Fukuda and Kosaka, 2000) synchronize the firing pattern of pyramidal cells and generate gamma oscillations in the neocortex and hippocampus (Penttonen et al., 1998; Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamás et al., 2000). Zonula adherentia have been observed between PV-immunopositive dendrites in the human control (Seress et al., 1993) and epileptic hippocampus (Fig. 4A and B), as well as in the vicinity of gap junctions in rats (Kosaka and Hama, 1985), and they are thought to ensure the mechanical connection of dendrites. Our results suggest that similar coupling mechanisms occur between PV-immunopositive interneurons in human tissue, which in turn probably lead to a more effective synchronization of pyramidal cells that may participate in the maintenance of epileptic seizures.
Conclusions

The balance of excitation and inhibition in the human CA1 region is considerably altered during epileptic changes. An excess excitation is provided by the sprouted CA1 pyramidal cell axons (Lehmann et al., 2000), as well as by the sprouting of afferents from the dentate gyrus and/or the CA2 region (Wittner et al., 2002), and/or from extrahippocampal pathways (Lehmann et al., 2000). Certain populations of dendritic inhibitory cells survive in epilepsy, and participate in the intense synaptic reorganization (Wittner et al., 2002), while others are lost (de Lanerolle et al., 1988, 1989; Maglóczky et al., 2000). Perisomatic inhibitory input is preserved in the non-sclerotic epileptic CA1 region, where pyramidal cells survive. The preserved perisomatic inhibition together with an excess excitation and an impaired dendritic inhibition might lead to an abnormal synchrony in output regions of the hippocampus, which still contain large enough numbers of projection neurons. This might have an important role in the generation and spread of epileptic seizures. In this group of patients, the preservation and possible increase in the efficacy of the perisomatic inhibitory network may be causally related to hypersynchrony during seizures. This may partly explain the difficulties in therapies that rely on the modulation of GABAergic transmission, that does not distinguish between perisomatic (controlling synchrony) and dendritic (controlling input plasticity) inhibition.

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References

Houser CR, Miyashiro JE, Schwartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber


Scotti AL, Kalt G, Bollag O, Nitsch C. Parvalbumin disappears from GABAergic CA1 neurons of the gerbil hippocampus with seizure onset while its persistence exists in the perforant path. Brain Res 1997b; 760: 109–17.


