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Correlation between calbindin expression in granule cells of the resected hippocampal dentate gyrus and verbal memory in temporal lobe epilepsy

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ABSTRACT

Calbindin expression of granule cells of the dentate gyrus is decreased in temporal lobe epilepsy (TLE) regardless of its etiology. In this study, we examined the relation between reduction of calbindin immunoreactivity and the verbal and visuo-spatial memory function of patients with TLE of different etiologies. Significant linear correlation was shown between calbindin expression and short-term and long-term percent retention and retroactive interference in auditory verbal learning test (AVLT) of patients including those with hippocampal sclerosis. In addition, we found significant linear regression between calbindin expression and short-term and long-term percent retention of AVLT in patients whose epilepsy was caused by malformation of cortical development or tumor and when no hippocampal sclerosis and substantial neuronal loss were detected. Together with the role of calbindin in memory established in previous studies on calbindin knock-out mice, our results suggest that reduction of calbindin expression may contribute to memory impairments of patients with TLE, particularly, when neuronal loss is not significant.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common form of intractable focal epilepsies with complex partial seizures. In many patients (48–75%), therapy-resistant TLE is associated with sclerosis of the medial temporal lobe structures [1,2]. Less frequently, TLE is related to malformation of cortical development (MCD; 13–45%) and tumors (27–33%) of the central nervous system [3]. Mesial temporal sclerosis includes hippocampal sclerosis (HS) with severe pyramidal cell loss in CA1 and CA3 regions of Ammon's horn and subiculum as well as loss of hilar mossy cells in the dentate gyrus [4–7]. In addition to neuronal cell death, reorganization of synaptic circuits, including sprouting of mossy fibers and axons of other hippocampal neurons, is a common finding in both human TLE and experimental TLE [8–15]. In contrast to mesial temporal sclerosis, neurons of the hippocampal formation are mostly preserved in MCD and in tumor-related epilepsy (TUE) when the tumor does not invade the hippocampal formation [16–19]. However, in lesion-induced TLE, including TUE, a few previous studies suggested the involvement of the hippocampal formation in the epileptogenesis, even in cases when the epileptogenic focus was located outside the hippocampal formation and when no pathological alteration was visible in the hippocampus [20–23]. Recently, we have shown a common histological alteration in the hippocampal dentate gyrus in all forms of TLE regardless of the etiology of seizures [19]. Despite the absence of considerable neuronal death, granule cells of the dentate gyrus revealed diminished calcium-binding protein calbindin-D28k (CB) immunoreactivity in MCD and TUE, similar to what was observed earlier in HS [19,24]. In addition, the decrease of CB expression in the granule cells correlated negatively with the age of epilepsy onset and correlated positively with the duration of epilepsy [19].

Calbindin acts as a calcium-ion sensor and buffer, and it is a potential eliminator of intracellular calcium in cases when the cell is overloaded, e.g. in neuronal injuries. However, the role of CB in neuronal cell death in TLE is unclear; both neuroprotective and deleterious effects have been reported [25–28]. In knock-out mice lacking functional CB, neuronal loss following ischemia was not more severe than in wild-type controls. In contrast, reduced level of CB protein causes impairment of memory formation in experimental animals [29]. Results from CB-deficient mice indicated that CB plays an important role in long-term potentiation (LTP) and synaptic consolidation of hippocampal memory [29,30]. Further support for the possible role of CB in memory formation is that expression of CB by the granule cells and their axons during postnatal development correlates with the functional development of the dentate

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gyrus and behavioral maturation of rats [31,32]. Studies in humans show that morphological maturation of granule cells as indicated by their CB immunoreactivity is a long-lasting event, and it comes to an end by the time children are able to solve hippocampal-dependent memory tasks similar to spatial navigation tasks used in rodent studies [33,34].

In addition to spatial memory, the hippocampal formation plays an important role in declarative memory, which is suitable for intentional recall of learned information of facts and events [35,36]. In patients with chronic TLE, impairment of declarative memory has been demonstrated, and epilepsy severely affects long-term delayed recall of visual and verbal information [37,38]. Some patients with a focus in the left temporal lobe could not recall autobiographical memories from their childhood [39]. Left HS correlated with impairments in verbal episodic memory while visuo-spatial working memory was affected in right HS [40,41]. In addition, deficiency of verbal memory in left HS and deficiency of visual memory in right HS were observed in patients with early-onset, long-term duration and high-frequency of seizures [42]. Proton magnetic resonance spectroscopy (^{1}H MRS), which is assumed to be suitable for providing information about neuronal loss and gliosis, revealed correlation between ^{1}H MRS values and verbal memory scores [43]. Postoperative histological studies showed that pyramidal cell loss in the removed hippocampal formation significantly correlated with preoperatively detected memory impairments of patients [44–49]. Loss of hilar neurons and granule cells as well as lower proliferative capacity of neuronal precursor stem cells of the subgranular layer of the dentate gyrus was correlated with impaired memory function [44,45,47,49,50].

The granule cells of the dentate gyrus are stimulated by entorhinal excitatory afferents and form the postsynaptic site of the first synapse of the trisynaptic hippocampal circuit; therefore, they are essential for memory formation. Moreover, granule cells express CB, a calcium-binding protein that is critical in hippocampal learning processes [29,30]. In our present study, we analyzed the possible correlation between the decrease of CB expression in the granule cells of surgically resected hippocampal formations and different presurgical learning and memory functions of patients with drug-resistant TLE.

2. Methods

2.1. Patients

Surgically removed tissues of the hippocampal formation of adult patients with medically-refractory TLE (n = 17) were used. In this study, we only included those patients who underwent extensive psychological testing before the operation. Ten of these patients had HS verified by MRI. In five patients, epilepsy was attributed to MCD, and in three of these patients, no pathological alterations could be detected in the hippocampal formation with MRI. Two patients suffered from epilepsy caused by tumor located in the temporal lobe, but the hippocampal formation was not invaded. The same cohort of epilepsy patients was used in our previous study describing the relationship between CB loss and etiology, but behavioral studies were not included [19]. Demographic data and clinical findings of the patients are listed in Table 1.

Epilepsy patients were evaluated in the Department of Epileptology, Neurology Clinic at the University of Pécs. Video/EEG monitoring was performed on all patients, and the temporal epileptic focus was identified. All patients underwent high-resolution brain MR imaging performed on a 3-T MR machine (Siemens Trio, Siemens AG, Erlangen, Germany) using a special protocol for TLE focusing on the temporal lobe and especially on the hippocampal formation.

2.2. Neuropsychological testing

Neuropsychological assessment included IQ, verbal intelligence quotient (VQ) and performance quotient (PQ) testing using the Wechsler Adult Intelligence Scale. Verbal skills and naming ability were tested using the verbal fluency test and Boston Naming test. In the phonemic version of the verbal fluency test, the subjects had to produce words beginning with the letters F, A, and S in one minute. In the categorical version of the verbal fluency test, the subjects produced animals in one minute. In the Boston Naming test, the subjects named pictures of 60 line-drawing objects. Verbal attention was measured with the forward version of the digit span task. Visual attention was assessed using the Trail Making test and the forward version of the Corsi Block-Tapping task [51]. Visual construction ability and memory were assessed using the Rey–Osterrieth Complex Figure (ROCF) test. After copying the ROCF, the patient had to draw it from memory in delayed recall (30 min). In the ROCF test, a standard Taylor's scoring system was administered with a maximum of 36 points over copying and memory versions. Each figure was divided into 18 different blocks. When the subject drew properly placed, correct blocks, 2 points were given. Properly placed and distorted or poorly placed and correct blocks were rated with 1 point. Distorted, poorly placed blocks were scored with half point. In case of absent or unrecognizable blocks, no points were given [52]. Verbal learning and memory were tested using a Hungarian version of the Rey auditory verbal learning test (AVLT). Auditory verbal learning test measures

Table 1
Summary of the clinical data of the patients with TLE.

Case no.	Age (Y)	Gender	Age of onset (Y)	Duration (Y)	Seizure frequency	Side of focus	MRI diagnosis
1	48	F	2	46	1–2/mo	L	HS
2	42	F	6	36	10–16/mo	R	HS
3	24	F	14	10	4–5/mo	R	HS
4	32	M	24	8	1/mo	R	HS
5	45	M	6	39	15/mo	L	HS
6	43	F	5	38	4–5/mo	L	HS
7	46	F	8	38	1–3/mo	L	HS
8	47	M	1	46	1–2/mo	L	HS
9	40	M	2	38	6–10/mo	R	HS
10	52	M	18	34	1–2/mo	R	HS
11	28	M	12	16	8–10/mo	R	Cortical heterotopia; HF is MRI negative
12	34	M	7	27	4–8/mo	R	Cortical dysgenesis
13	50	F	28	22	3–4/mo	R	Cortex and HF are MRI negative
14	31	F	12	19	No data	R	Cortex and HF are MRI negative
15	17	F	10	7	2–3/mo	R	Cortical and hippocampal dysgenesis
16	28	M	13	15	1/year	R	Tumor
17	27	M	18	9	No data	R	Tumor

Abbreviations: Y, years; mo, months; MCD, malformation of cortical development; R, right; L, left; HS, hippocampal sclerosis; M, male; F, female; TLE, temporal lobe epilepsy; MRI, magnetic resonance imaging; HF, hippocampal formation.