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CHARACTERIZATION OF NEURONS IN THE CORTICAL WHITE MATTER IN HUMAN TEMPORAL LOBE EPILEPSY

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Abstract—The aim of the present work was to characterize neurons in the archi- and neocortical white matter, and to investigate their distribution in mesial temporal sclerosis. Immunohistochemistry and quantification of neurons were performed on surgically resected tissue sections of patients with therapy-resistant temporal lobe epilepsy. Temporal lobe tissues of patients with tumor but without epilepsy and that from autopsy were used as controls. Neurons were identified with immunohistochemistry using antibodies against NeuN, calcium-binding proteins, transcription factor Tbr1 and neurofilaments. We found significantly higher density of neurons in the archi- and neocortical white matter of patients with temporal lobe epilepsy than in that of controls. Based on their morphology and neurochemical content, both excitatory and inhibitory cells were present among these neurons. A subset of neurons in the white matter was Tbr-1-immunoreactive and these neurons coexpressed NeuN and neurofilament marker SMI311R. No colocalization of Tbr1 was observed with the inhibitory neuronal markers, calcium-binding proteins. We suggest that a large population of white matter neurons comprises remnants of the subplate. Furthermore, we propose that a subset of white matter neurons was arrested during migration, highlighting the role of cortical maldevelopment in epilepsy associated with mesial temporal sclerosis.

Key words: epileptic seizures, excitatory neurons, inhibitory neurons, neuronal network, subplate.

INTRODUCTION

During cortical development postmitotic neuronal precursors migrate through transient cortical layers including the subplate and the intermediate zone both forming the future white matter (WM) (Allendoerfer and Shatz, 1994). Around birth a subset of subplate neurons disappear by programed cell death while many others survive and are distributed in the subcortical WM (Chun and Shatz, 1989; Kostovic and Rakic, 1990; Suárez-Solá et al., 2009; Judaš et al., 2010). Integration of surviving subplate neurons into the neocortical circuitry can be assumed based on their axonal projections and their synaptic inputs (Kostovic and Rakic, 1980; Mrzljak et al., 1988). Morphological characterization of WM neurons in the control human cortex suggests that both excitatory and inhibitory neurons exist among them (Kostovic and Rakic, 1980; Mrzljak et al., 1988; Meyer et al., 1992).

The presence of subcortical WM and deeply localized interstitial neurons have been associated with a variety of neurological and psychiatric disorders including epilepsy (Hardiman et al., 1988; Emery et al., 1997; Kasper et al., 1999; Thom et al., 2001; Suárez-Solá et al., 2009; Liu et al., 2014). In temporal lobe epilepsy (TLE) several studies showed large numbers of neurons in the WM of the temporal cortex (Emery et al., 1997; Kasper et al., 1999; Thom et al., 2001; Liu et al., 2014). However other studies did not confirm the above-mentioned results (Opeskin et al., 2000; Bothwell et al., 2001; Loup et al., 2009). The discrepancies between these results might be caused by several factors. One factor could be the different staining methods that in some studies allowed only the identification of a subset of neurons with large pyramidal and multipolar shape (Emery et al., 1997; Kasper et al., 1999). Other factors could be the areas compared the diversity of patients regarding the type of epilepsy and the dissimilarity of methods used for quantification (Opeskin et al., 2000; Thom et al., 2001).

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Abbreviations: CA1, cornu Ammonis 1; CA3, cornu Ammonis 3; CB, calbindin; CR, calretinin; DAPI, 4′,6-diamidino-2-phenylindole; DG, dentate gyrus; EC, entorhinal cortex; F, female; FS, febrile seizure; GABA, gamma-butyric- amino acid; GFAP, glial fibrillary acidic protein; GM, gray matter; HS, hippocampal sclerosis; Ki-67, proliferation marker; L, left; M, male; m, month; MRI, magnetic resonance imaging; NA, not available; NeuN, neuronal nuclear antigen; PB, phosphate buffer; PC, perinatal complication; PET, positron emission tomography; PV, parvalbumin; R, right; SMI311R, neurofilament marker; SUB, subicular complex; Tbr1, T-box brain1 transcription factor; TLE, temporal lobe epilepsy; Y, year; WM, white matter.

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Due to the controversial data on the number of WM neurons their role in epileptic seizures and in the surgical outcome of patients remains debated (Hardiman et al., 1988; Eriksson et al., 2005; Kasper, 2005). Morphological and PET studies of the WM indicate the role of $GABA_A$ receptors and accordingly alteration of neuronal circuits in TLE patients (Loup et al., 2009). In addition the presence of an excess number of WM neurons without dyslamination of the cerebral cortex is an important entity in evaluating the neuropathological signs for TLE (Blümcke et al., 2009, 2011).

Little is known about the composition of the neuronal population found in the epileptic cortical WM. Therefore, in the present study our aim was to characterize WM neurons in human TLE, using a wide variety of neurochemical markers of principal cells and interneurons.

The origin of WM neurons in TLE patients is still not clear. Theoretically, they can be remnants of the subplate, or neurons arrested during migration. While subplate neurons are among the first to develop, neuronal migration is a later event that lasts up to the 30th week of gestation (Kostovic and Rakic, 1980; Meyer et al., 2002). Characterization of WM neurons can help in answering the question regarding their origin. Therefore, in addition to the general neuronal markers such as neurofilaments and calcium-binding proteins, we studied immunoreactivity of the brain-specific T-box transcription factor (Tbr1). Tbr1 is highly expressed in early-born glutamatergic neurons and it plays a crucial role in the differentiation of the subplate (Bulfone et al., 1995; Hevner et al., 2001, 2006).

In addition, we compared the number and distribution of WM neurons in the archicortical subiculum and entorhinal cortex as well as in the temporal neocortex on surgically resected tissue sections of patients with therapy resistant TLE.

EXPERIMENTAL PROCEDURES

Patients

Surgically removed tissues of the temporal archi- and neocortices of medically refractory TLE patients (n = 14) were used in this study. In patient cases, hippocampal sclerosis had been verified with MRI and histopathology, and no malformation of cortical development was observed on MR images. Cortices of patients (n = 4) with brain tumor but without history of TLE served as controls. In addition, temporal lobe tissues from autopsy controls (n = 3) were also used. Demographics and clinical data of patients are summarized in Tables 1 and 2.

Patients were evaluated in the Epilepsy Center of the Department of Neurology of the University of Pécs, and surgeries were performed in the Department of Neurosurgery under general anesthesia, through a standard temporal craniotomy. Cortical tissue consisting of gray matter (GM) of the inferior, middle and superior temporal gyri and the underlying WM were obtained from resections performed for strictly therapeutic reasons. The resected cortical tissues were sent for both routine pathology and special histological studies.

For controls, surgically removed tissues were used from four patients suffering from brain tumor. Temporal lobectomy was indicated to guarantee safe and radical removal when the tumor was located in the medio-basal part of the temporal lobe, i.e. in the parahippocampal gyrus. Accordingly, intact temporal neocortex 20 mm to 40 mm distant from the tumor was removed. Histological diagnosis of the tumor was performed in the Department of Pathology at the University of Pécs Medical School. For diagnosis, immunohistochemistry with the routine use of antibodies such as Ki-67, glial fibrillary acidic protein (GFAP) and p53 was performed. In addition, GFAP immunohistochemistry was performed on vibratome sections of the cortical tissue blocks used in the present study. After sectioning with the vibratome, the remnants of the tissue blocks were embedded in paraffin. Ki-67 immunohistochemistry was performed on paraffin sections to detect the possible infiltration of the tumor. Only those sections were included that were not infiltrated by tumor cells. In the distant cortical areas used for the present study, neither diffusion-weighted MRI nor histological examinations revealed peritumoral tissue changes.

All examinations including tissue processing and histological examination followed the regulations of the Code of Ethics of the World Medical Association (Declaration of Helsinki) and were carried out with the adequate understanding and written consent of the patients as well as with the approval of the ethics committee of our institution.

Immunohistochemistry

After removal, the surgically resected archi- and neocortical tissues were immediately immersed in 4% paraformaldehyde solution buffered with PB (0.1 M, pH = 7.4). Then, approximately 0.8- to 1-cm wide tissue blocks were cut that contained the archicortical subicular complex and the entorhinal cortex. In addition, from the tissue containing temporal neocortical areas, the superior, middle and inferior temporal gyri were cut. Depending on the size of the tissue blocks removed during the surgery, 1–4 tissue slabs were cut from each area. The average numbers of the tissue blocks containing the archi- and the neocortex per patient were 1.9 ± 0.79 and 2.18 ± 1.11, respectively. These tissue blocks were fixed overnight in 4% buffered formaldehyde. Following autopsy, brains were fixed routinely in 10% formaldehyde solution for 3 weeks in the Department of Pathology at the University of Pécs. Temporal lobe tissue blocks of both origin were cut with Vibratome at 80 μm.

Pretreatment and indirect immunohistochemistry using the avidin–biotin peroxidase detection system (Vector, Burlingame, CA) were performed as described earlier and 3,3'-diaminobenzidine was used as chromogen (Abrahám et al., 2011).

For the colocalization study, the same pretreatment was used as described earlier (Abrahám et al., 2011). Following incubation with the primary antibodies, fluorescent