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Ultrastructural changes to rat hippocampus in pentylenetetrazol- and kainic acid-induced status epilepticus: A study using electron microscopy

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ABSTRACT

A pentylenetetrazol (PTZ)-induced status epilepticus model in rats was used in the study. The brains were studied one month after treatment. Ultrastructural observations using electron microscopy performed on the neurons, glial cells, and synapses, in the hippocampal CA1 region of epileptic brains, demonstrated the following major changes over normal control brain tissue. (i) There is ultrastructural alterations in some neurons, glial cells and synapses in the hippocampal CA1 region. (ii) The destruction of cellular organelles and peripheral, partial or even total chromatolysis in some pyramidal cells and in interneurons are observed. Several astrocytes are proliferated or activated. Presynaptic terminals with granular vesicles and degenerated presynaptic profiles are rarely observed. (iii) The alterations observed are found to be dependent on the frequency of seizure activities following the PTZ treatment. It was observed that if seizure episodes are frequent and severe, the ultrastructure of hippocampal area is significantly changed. Interestingly, the ultrastructure of CA1 area is found to be only moderately altered if seizure episodes following the status epilepticus are rare and more superficial; (iv) alterations in mitochondria and dendrites are among the most common ultrastructural changes seen, suggesting cell stress and changes to cellular metabolism. These morphological changes, observed in brain neurons in status epilepticus, are a reflection of epileptic pathophysiology. Further studies at the chemical and molecular level of neurotransmitter release, such as at the level of porosomes (secretory portals) at the presynaptic membrane, will further reveal molecular details of these changes.

1. Introduction

The post-status epilepticus (SE) model is one of the most informative and broadly used animal models for epilepsy studies. In many cases it closely mimics the clinical manifestations of mesial temporal lobe epilepsy in humans, the most common type of epilepsy in adults (Sharma et al., 2007; Vermoesen et al., 2010; Zhang et al., 2014). The post-SE model is usually provoked by systemic or local injection of a single convulsive dose of epilepsy-produced drug or electrical stimulation. The triggering process is typically followed by latency period with subsequent development of generalized seizures, enhanced neuronal excitability and specific biochemical, molecular and structural alterations which are developed mainly in epileptogenic regions of brain (Sharma et al., 2007; Zhang et al., 2014). The extent, to which these regions have been changed, differs depending on the chemical nature of these drugs or the character of stimulation.

While behavioral, electrophysiological and molecular alterations accompanying post-SE have been studied intensively, corresponding structural modifications were described only in a few studies. However it is generally accepted that to optimize the treatment of epilepsy and complications of SE, it is important to study not only the efficacy of different anticonvulsant/antiepileptic drugs in preventing behavioral and, electrophysiological seizures or corresponding molecular changes, but also to determine how effectively they prevent seizure-induced neuronal damage

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(Pitkanen et al., 1996; Wong, 2005; Wong and Guo, 2013). For this reason the clarification of structural alterations provoked by different forms of epilepsy is crucial.

Neuroanatomical approaches have helped significantly to clarify the mechanisms of epilepsy. Various morphological alterations, such as cell loss, astrogliosis or reorganization of dendrites and axons of specific cells, were detected. Besides, using immunohistochemical methods, the neurochemical identity of vulnerable neurons, upregulation or downregulation of specific neuropeptides, neurotransmitters and their receptors as well as other molecular changes, accompanying different forms of epilepsy, have been identified (Fritschy, 2008; Krishnakumar et al., 2014; Sperk et al., 2009; Toth and Magloczky, 2014; Zhvaniia et al., 2007). Based on such data, it was proposed that only severe neuronal damage cannot explain the consequential epileptic activity, so, subcellular structural, molecular and nano modifications, facilitating abnormal firing and seizure episodes, should be present (Ryan et al., 2014; Otahal et al., 2014). Different approaches could be used to reveal such fine modifications. One of such approaches, electron microscopy (EM), gives the possibility to detect alterations that may develop on ultrastructural level of different cells, organelles, suborganelles, synapses and existing neuronal circuits as a result of epileptic activity.

Earlier we described behavioral changes and corresponding structural and ultrastructural modifications, which develop in the hippocampus and piriform cortex some weeks and months after single injection of convulsive dose of epilepsy-produced drug, kainic acid (KA) (Kotaria et al., 2013; Zhvaniia et al., 2007; Zhvania et al., 2014). In the present EM study we continue the analysis of long-lasting structural changes provoked by SE. Specifically, our aim is the clarifications of ultrastructural alterations provoked by pentylenetetrazol (PTZ)-induced SE in the hippocampus, brain area, which is known to be actively involved in epileptogenesis.

Pentylenetetrazol, also identified as pentylenetetrazol, metrazol, pentetrazol, pentamethylenetetrazol, Cardiazol or PTZ, mainly acts by binding to the picrotoxin-recognition site and benzodiazepine-binding site of the post-synaptic $GABA_A$ receptor (Bidmon et al., 2009; Huang et al., 2013). As a result of binding, the effect of endogenous GABA and other inhibitory transmitters is decreased, turning the system in a hyper-excitable state. There are three major PTZ models: (i) single administration of convulsive dose, (ii) kindling model, provoked by repeated injection of sub-convulsive dose and (iii) repeated injections of a low, but convulsive dose with seizure-free periods in between (typically described as repeated series of seizures) (Bidmon et al., 2009). Common features of all three models are: (i) convulsing effect occurs with a short latency and (ii) neurodegeneration is absent or is induced with delay. These features are the main advantages of PTZ models compared with others, (for example, with pilocarpin or KA models), in which neurodegeneration usually occurs with the initial seizure episode. Specifically, one chemical (PTZ) could be used to clarify two different mechanisms of epilepsy: (i) cellular response mechanisms induced by seizure episodes, which, at least at the early stages, develop separately from neuronal death and (ii) processes, mostly related to late neuronal degeneration, that have been detected in brain samples taken from patients with some forms of epilepsy; in many aspects these processes are comparable to situations observed during ischemia (Bidmon et al., 2009).

All three PTZ models are known to alter several neurotransmitter systems, such as GABAergic, adenosinergic or glutamatergic (Akula et al., 2008; Azanchi et al., 2014; Bidmon et al., 2009; Mulholland et al., 2008), the whole brain hydroxyl radicals (Rauca et al., 2004; Watanabe et al., 2013), the level of different proteins (Zhang et al., 2014), the level of free fatty acid (Erakovic et al., 2003) and induce other molecular modifications, preferably in epileptogenic areas of brain, including hippocampus (Amada et al., 2013; Malhi et al., 2014; Turker et al., 2014). Some data point to structural alterations, which develop in the hippocampus as a result of PTZ kindling (Huang et al., 2013; Zaitsev et al., 2014; Zhang et al., 2014). So, PTZ produces acute effect on the ultrastructural level of hippocampal neurons in slices (Schormair et al., 1993) and blood–brain barrier integrity (Orhan et al., 2014). However, data obtained on different PTZ models are often incomparable. Moreover, discrepancy could exist even concerning results, which were obtained using the same PTZ model. Several factors, such as species-specific differences, strain-specific differences, differences between individuals of a single strain or breeding group, the dose, duration or type of treatment, should provoke such inconsistency. Therefore, different aspects of PTZ-related pathologies still require further clarification and discussion.

In the present study we elucidate ultrastructural alterations, which take place in neurons, glial cells and synapses of hippocampal CA1 area 1 month after SE produced by single administration of convulsive dose of PTZ. As far as we know, there are no special studies concerning this subject.

2. Materials and methods

2.1. Animals and experimental design

Studies were carried out using 95–105 day (d) old male Wistar rats, weighing 220–230 g. During experiment the rats were housed under normal controlled environment (temperature 20–22 °C, humidity 55–60%, light 07.30–19.30). Standard food pellets and tap water were available.

The following groups of animals were used:

- *Rats were treated i.p. singly, with PTZ (Sigma)* dissolved in buffered saline (SAL) at a dose of 50 mg/kg ($n = 15$).
- *Rats were treated i.p. singly, with SAL*. These rats composed the first subgroup of control animals ($n = 5$).
- *Intact rats from ordinary vivarial conditions*. These rats composed the second subgroup of control animals ($n = 5$).

PTZ or saline was administered between 8.00 and 9.00 a.m., to diminish the effect of circadian rhythms.

Experimental procedures were approved by Animal Studies Committee, I. Beritashvili Center of Experimental Biomedicine and Committee of Ethics, Ilia State University.

2.2. Monitoring of SE

Animals were monitored immediately after PTZ or saline injection. Behavioral responses were recorded with a video system, during the 30 days (d) and analyzed according to revised Racine's scale for PTS-induced seizures (Luttjohann et al., 2009). The rats which developed the generalized tonic–clonic seizure with the loss of righting reflex during 10 min after PTZ injection were considered as animals with SE. Only these rats were included in subsequent EM analysis.

2.3. Perfusion and brain processing

Studies were done in the hippocampal CA1 area of rats 30 d after PTZ or saline injection and in normal rats. Following pentobarbital injection (100 mg/kg, i.p.) rats to have EM examination of their brains underwent transcardiac perfusion with 0.9% NaCl, followed by 500 ml of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M PB, pH – 7.4 at a perfusion pressure 120 mmHg. The brains were removed from skull and placed in the same fixative