BRIEF COMMUNICATION

Metals and electrolytes in sclerotic hippocampi in patients with drug-resistant mesial temporal lobe epilepsy

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Summary

An altered metal and electrolyte profile has been implicated in the pathologic mechanisms of chronic epilepsy; however, no study has comprehensively measured hippocampal concentrations of these elements in patients with mesial temporal lobe epilepsy and hippocampal sclerosis (mTLE-HS). We therefore analyzed hippocampi of 24 patients with drug-resistant mTLE-HS (mean age 35.6 ± 9.4 years) who underwent anterior temporal lobe resection and amygdalohippocampectomy and 17 hippocampi obtained by autopsy from 13 controls (mean age 40.5 ± 12.9 years), using inductively coupled plasma optical emission spectrometry (ICP-OES). Epileptic hippocampi showed significantly lower concentrations (μg/g of tissue) of copper (HS: 2.34 ± 0.12; control [C]: 3.57 ± 0.33; $p < 0.001$), manganese (HS: 0.205 ± 0.030; C: 0.409 ± 0.064; $p = 0.004$), and potassium (HS: 2,001 ± 59; C: 2,322 ± 61; $p < 0.001$), and increased sodium levels (HS: 1,131 ± 22; C: 1,040 ± 25; $p = 0.010$). Zinc, iron, calcium, and magnesium levels did not differ in HS and controls. In summary, copper and manganese levels are deficient, whereas iron level is unchanged in hippocampi from patients with mTLE-HS. Our results provide a basis for understanding the potential involvement of different metals and electrolytes in the pathology of HS.

KEY WORDS: Mesial temporal epilepsy, Hippocampal sclerosis, Copper, Manganese, Electrolytes.

Mesial temporal lobe epilepsy (mTLE) associated with hippocampal sclerosis (HS) is probably the single most frequent human focal epilepsy and the one most assiduously investigated. A number of animal and in vitro studies and some clinical data imply an important role of metals in mTLE-HS pathophysiology, but the levels of metals and electrolytes in epileptic human hippocampi have never been extensively analyzed. Altered levels of zinc and manganese in the hippocampus have been implicated in seizure development.[1–3] Copper deficiency in the brain is proposed to play an essential role in the development of seizures in some genetic disorders.[4] HS represents a common feature of epilepsy and neurodegenerative conditions, such as Alzheimer's disease (AD). It has been recognized that epilepsy- and neurodegeneration-related HS differ in their cellular features.[5] However, it is not known whether HS in epilepsy is accompanied by iron accumulation, which is found in AD hippocampi.[6] Finally, the level of primary electrolytes (sodium, potassium, calcium, and magnesium) could be affected by seizures or by changes in structure and cellular population of sclerotic hippocampus.

In the present study we have examined metal and electrolyte profile of sclerotic hippocampi of patients with drug-resistant mTLE-HS, using inductively coupled plasma optical emission spectrometry (ICP-OES). Epileptic hippocampal concentrations of metals and electrolytes were compared with the profile of control hippocampi.

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Methods

We analyzed hippocampi of 24 patients with drug-resistant (≥2 antiepileptic drug failures) mTLE-HS (mean age 35.6 ± 9.4 years; range 18–56 years; mean duration of epilepsy 22.5 ± 12.8 years; 12 with left HS; m/f = 12/12). In all patients, MRI findings were consistent with HS, which was confirmed by histopathologic analysis and classified according to International League Against Epilepsy (ILAE) classification of HS in patients with temporal lobe epilepsy (for further clinical and histologic details see Table S1).[7] All patients were rendered "seizure free" following surgery (median follow-up 25 months). Control hippocampi (n = 17) were acquired from 13 cadavers (mean age 40.5 ± 12.9 years; range 24–62; nine left hippocampi; m/f = 8/5), that were limited to sudden death without medical intervention, prolonged agonal periods, and secondary diagnosis of drug/alcohol dependency. Iatrogenic metal intake was negligible in both groups, that is, no micronutrient supplements, chelating agents, MRI contrasts, or metal-containing pharmaceuticals were prescribed/applied within the 2-year period before sample collection. The research was performed in accordance with the Declaration of Helsinki of the World Medical Association and has been approved by the Clinical Center of Serbia Ethics Committee on Human Research. Written informed consent was obtained from every subject or family member.

The epileptic hippocampi were removed en bloc, in the course of anterior temporal lobe resection and amygdalohippocampectomy. Control hippocampi were removed en bloc during the autopsy, maximally 10 h following death. The postmortem specimens were free from brain injury. Posterior parts of the head of both sclerotic and cadaveric hippocampi were immediately immersed in liquid nitrogen and stored at −80°C. Prior to preparation of the coronal hippocampal slices, which was conducted in −20°C chamber, choroidal plexi and/or parts of parahippocampal gyri (subiculum) were removed if present. For the purpose of metal and electrolyte analysis, coronal hippocampal slices (200 mg of wet tissue) were digested using ultra-pure HNO_3 (Sigma-Aldrich, St. Louis, MO, U.S.A.), H_2O_2 (Carlo Erba Reagents, Milano, Italy), and deionized 18 MΩ water, as described previously.[8] Upon digestion, the concentrations of Na, K, Ca, Mg, Fe, Cu, Mn, Zn, P, and S were determined by ICP-OES (SpectroGenesis EOP II; Spectro Analytical Instruments GmbH, Kleve, Germany).

Results

The profile of metals and electrolytes in hippocampi of patients with mTLE-HS differed considerably from the control hippocampal profile (Table 1). The increased level of sodium and lower concentrations of potassium, copper, and manganese are evident. Similar trends were observed when data for left and right hippocampi were processed separately, although in some cases (Na and K in right hippocampi) there was no significant difference between mTLE-HS and controls. This could be explained by the lower number of samples per group, or it might result from baseline characteristics of normal right hippocampus, which showed slightly lower Na and K concentrations compared to control left hippocampi. Concentrations of iron, calcium, and magnesium in HS were next to identical as in control hippocampi. The level of zinc in sclerotic hippocampi was increased compared to controls but no statistical significance was found. ICP-OES analysis provided the concentrations of two nonmetal macro elements: P and S. Phosphorus concentration was almost identical in two groups: 3,181 ± 470 in controls, and 3,126 ± 523 μg/g of tissue in HS. The concentration of sulfur was higher in HS (2,587 ± 294 μg/g) compared to control values (2,361 ± 516 μg/g; $p = 0.007$). Similar results were obtained separately for left (2,524 ± 347 vs. 2,359 ± 691; nonsignificant, $p = 0.08$) and right (2,652 ± 227 vs. 2,363 ± 253; $p = 0.025$) hippocampi. Spearman's rank correlation coefficients (r_s) were calculated to determine dependency between concentrations of investigated elements. Significant correlations were found for the following pairs: Ca–Mg ($r_s = 0.668$, $p = 0.005$), Mg–Mn ($r_s = 0.509$, $p = 0.044$), Na–Fe ($r_s = 0.603$, $p = 0.004$), K–Mg ($r_s = 0.606$, $p = 0.013$), K–Mn ($r_s = 0.762$, $p < 0.001$), K–P ($r_s = 0.583$, $p = 0.014$), Mg–P ($r_s = 0.505$, $p = 0.039$), and P–S ($r_s = 0.814$, $p < 0.001$) in control hippocampi; and Ca–Mg ($r_s = 0.450$, $p = 0.047$), Mn–Zn ($r_s = 0.662$, $p = 0.002$), Cu–P ($r_s = 0.411$, $p = 0.046$), and P–S ($r_s = 0.809$, $p < 0.001$) in epileptic hippocampi.

Discussion

Presented values for hippocampal metal and electrolyte profile are in accordance with previous results on metal concentrations in some other brain regions.[9] Of note, the age, which represents a factor that affects brain metal profile (e.g., the level of iron increases with age),[6] was not significantly different in two groups. Our results appear to be in generally good agreement with available data and concepts in epilepsy research.

HS is characterized by lower neuron counts, which is usually compensated by gliosis, granule cell dispersion, mossy fiber sprouting, and neurogenesis.[5] However, it appears that atrophy prevails over tissue remodeling. Increased concentration of sodium (the key extracellular electrolyte) and the fall in the level of potassium (the main intracellular electrolyte) imply that the number of cells or at least intracellular/extracellular volume ratio is decreased in HS.

Hippocampal tissue in drug-resistant mTLE-HS showed copper deficiency. This could be placed into the context of some previous findings: (1) Copper is a cofactor of a number of metalloenzymes (dopamine β-hydroxylase, Cu-Zn superoxide dismutase [SOD], cytochrome *c* oxidase). It is

Table 1. Hippocampal metals and electrolytes in patients with mTLE-HS and controls

	Hippocampi		Left hippocampi		Right hippocampi	
Metals (μg/g of tissue)	Controls (n = 17)	HS (n = 24)	Controls (n = 9)	HS (n = 12)	Controls (n = 8)	HS (n = 12)
Na	1,040 ± 25	1,131 ± 22	1,076 ± 32	1,152 ± 28	999 ± 35	1,111 ± 34
	p = 0.010		p = 0.049		n.s. (p = 0.09)	
K	2,322 ± 61	2,001 ± 59	2,370 ± 103	1,960 ± 70	2,267 ± 58	2,041 ± 97
	p < 0.001		p = 0.004		n.s. (p = 0.08)	
Ca	104.6 ± 14.1	101.0 ± 14.6	105.2 ± 16.2	108.6 ± 20.8	110.6 ± 23.7	94.6 ± 21.1
	n.s.		n.s.		n.s.	
Mg	118.2 ± 4.4	106.8 ± 3.5	117.4 ± 6.5	102.9 ± 6.6	119.2 ± 6.5	110.8 ± 2.1
	n.s.		n.s.		n.s.	
Zn	10.97 ± 1.03	13.97 ± 1.51	11.74 ± 1.74	15.35 ± 3.25	10.00 ± 0.84	13.05 ± 1.39
	n.s.		n.s.		n.s.	
Fe	61.9 ± 10.7	62.2 ± 5.1	72.4 ± 17.4	63.0 ± 7.7	50.1 ± 11.4	61.4 ± 7.1
	n.s.		n.s.		n.s.	
Cu	3.57 ± 0.33	2.34 ± 0.12	3.47 ± 0.51	2.06 ± 0.14	3.68 ± 0.43	2.62 ± 0.17
	p < 0.001		p = 0.003		p = 0.025	
Mn	0.409 ± 0.064	0.205 ± 0.030	0.429 ± 0.085	0.220 ± 0.052	0.386 ± 0.093	0.190 ± 0.030
	p = 0.004		p = 0.016		p = 0.048	

All values are given as means ± standard error (SE).
Statistical analyses were conducted using STATISTICA 8.0 (StatSoft Inc., Tulsa, OK, U.S.A.). p-Values were determined using nonparametric two-tailed Mann-Whitney *U* test. Results were taken to be statistically different if p < 0.05; n.s., nonsignificant.

important to note that dopamine β-hydroxylase knockouts generally fail to respond to valproate;[10] (2) copper is released into synaptic cleft after neuronal depolarization and plays a physiologic role in activity-dependent modulation of synaptic activity. Furthermore, a decrease of copper concentration promotes *N*-methyl-D-aspartate (NMDA) receptor excitotoxicity in hippocampal neurons.[4] In relation to this, copper deficiency in the brain is proposed to play an essential role in the development of seizures and neuron loss in Menkes disease, an inherited disorder of copper transport.[4] (3) It has been shown that exogenously applied copper can reduce/inhibit NMDA-mediated potentials/currents in hippocampal neurons.[4]

Iron is not accumulated in sclerotic hippocampi of patients with mTLE, which is in contrast to neurodegenerative settings.[6] Hence, prooxidative/cytotoxic activity of iron is unlikely to be involved in epileptic HS. Our finding is in accordance with previous notions that hippocampal atrophy in epilepsy is substantially different compared to neurodegenerative diseases. This could be related to regenerative capacities of hippocampus that are employed in epilepsy and involve mossy fiber sprouting, granule cell dispersion, and neurogenesis,[5] but which are absent or compromised in neurodegenerative conditions.[11]

Low manganese concentration in human HS that was observed here might explain severely decreased expression of hippocampal glutamine synthetase in mTLE that has been reported previously.[12] Glutamine synthetase is the main manganese metalloprotein in the brain (predominately located in astrocytes), and its suppression leads to recurrent seizures.[12] It has been reported that brain manganese level is decreased in model animals after exposure to seizure-inducing stimuli.[2] It is tempting to speculate that a feedback loop might be present develop between the induction of seizures, manganese level, and glutamine synthetase activity. In addition, low hippocampal manganese level in HS might reflect the loss of neurons, which are rich in mitochondrial antioxidative enzyme–manganese SOD.

In close, low manganese level in HS is likely to represent the net outcome of a number of pathologic and pathophysiologic events.

Zinc is the most intensively investigated metal in epilepsy research. Studies on animal models of seizures and epilepsy have found a decrease of hippocampal zinc, as well as alleviating/promoting effects of zinc loading/deficiency, respectively, on susceptibility to seizures.[3] However, data presented here show clearly that the total level of zinc is not decreased in human epileptogenic hippocampi. Nevertheless, a previously proposed concept on the essential role of zinc distribution (and not concentration per se)[1] has gained a lot of attention. Histologic studies on models and human samples have documented that zinc accumulation or depletion develops in specific hippocampal cells and regions.[3] A complex role of zinc in synaptic vesicles, extracellular compartment, and intracellular stores is also under investigation.[13,14] Therefore, further investigation of specific localization of zinc in epileptic hippocampi (for example, in the regions showing mossy fiber sprouting) are needed.

Correlation analysis revealed a general breakdown of metal and electrolyte homeostasis in epileptic hippocampi. All correlations between the concentrations of specific metals that are found in control hippocampi (where positive correlation between magnesium and manganese levels represents a general feature of brain tissue[9]) are lost in HS, except for Ca–Mg and P–S. On the other hand, HS is characterized by positive correlation between the levels of zinc

and manganese, which implies that a common pathologic factor might affect the concentrations of these two metals in HS.

In addition to metal and electrolyte profile, ICP-OES analysis showed that the concentration of sulfur is increased in HS compared to control tissue. This could represent an epiphenomenon of antiepileptic drug–related accumulation of homocysteine.[15] A strong positive correlation between hippocampal levels of phosphorus and sulfur that was observed in both control and sclerotic hippocampi, might be of importance for general (neuro)physiology.

The lack of data from the crime scene, that is, hippocampus, has been one of the drawbacks for understanding the role of metals in the pathologic mechanisms of mTLE-HS. Our results fill the gap and provide a relevant prerequisite for further research of the potential involvement of different metals in the pathology of HS. It should be noted that this is the first report on metal and electrolyte profile in HS. Hence, although our findings might have an impact on future medication (e.g., copper and manganese replacement therapy), further confirmation is mandatory in order to draw major conclusions.

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Disclosure

None of the authors has any conflict of interest related to this manuscript. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Coulter DA. Mossy fiber zinc and temporal lobe epilepsy: pathological association with altered "epileptic" gamma-aminobutyric acid A receptors in dentate granule cells. *Epilepsia* 2000;41 (Suppl. 6):S96–S99.
2. Gonzalez-Reyes RE, Gutierrez-Alvarez AM, Moreno CB. Manganese and epilepsy: a systematic review of the literature. *Brain Res Rev* 2007;53:332–336.
3. Mitsuya K, Nitta N, Suzuki F. Persistent zinc depletion in the mossy fiber terminals in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. *Epilepsia* 2009;50:1979–1990.
4. Schlief ML, West T, Craig AM, et al. Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. *Proc Natl Acad Sci USA* 2006;103:14919–14924.
5. Malmgren K, Thom M. Hippocampal sclerosis – origins and imaging. *Epilepsia* 2012;53 (Suppl. 4):19–33.
6. Stankiewicz J, Panter SS, Neema M, et al. Iron in chronic brain disorders: imaging and neurotherapeutic implications. *Neurotherapeutics* 2007;4:371–386.
7. Blümcke I, Thom M, Aronica E, et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* 2013;54:1315–1329.
8. Wang X, Zhang J, Zhao L, et al. Effect of subchronic exposure to arsenic on levels of essential trace elements in mice brain and its gender difference. *Biometals* 2013;26:123–131.
9. Krebs N, Langkammer C, Goessler W, et al. Assessment of trace elements in human brain using inductively coupled plasma mass spectrometry. *J Trace Elem Med Biol* 2014;28:1–7.
10. Schank JR, Liles LC, Weinshenker D. Reduced anticonvulsant efficacy of valproic acid in dopamine beta-hydroxylase knockout mice. *Epilepsy Res* 2005;65:23–31.
11. Winner B, Kohl Z, Gage FH. Neurodegenerative disease and adult neurogenesis. *Eur J Neurosci* 2011;33:1139–1151.
12. Eid T, Behar K, Dhaher R, et al. Roles of glutamine synthetase inhibition in epilepsy. *Neurochem Res* 2012;37:2339–2350.
13. Lavoie N, Peralta MR 3rd, Chiasson M, et al. Extracellular chelation of zinc does not affect hippocampal excitability and seizure-induced cell death in rats. *J Physiol* 2007;578:275–289.
14. Pan E, Zhang XA, Huang Z, et al. Vesicular zinc promotes presynaptic and inhibits postsynaptic long-term potentiation of mossy fiber-CA3 synapse. *Neuron* 2011;71:1116–1126.
15. Kim DW, Lee SY, Shon YM, et al. Effects of new antiepileptic drugs on circulatory markers for vascular risk in patients with newly diagnosed epilepsy. *Epilepsia* 2013;54:e146–e149.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical characteristics of the analyzed patients.