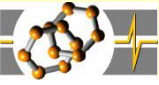


Effects of inhibition of glutamate uptake on the electrophysiological, optical and neurochemical characteristics of low-[Mg²⁺]-induced epileptiform activity in juvenile rat hippocampal slices

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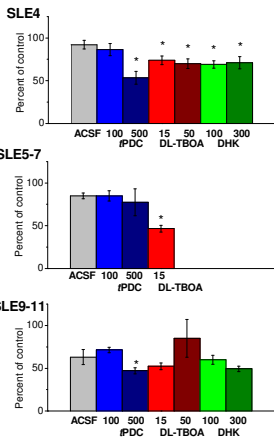


INTRODUCTION

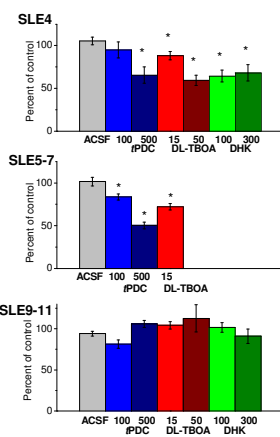
Ambient concentration of the major excitatory transmitter Glu is tightly controlled by sodium dependent, high affinity transporters located on astrocytes and neurons (EAAT1,2,3) in the hippocampus (Danbolt 2001). Failure of Glu uptake has been reported to evoke epileptic activity both *in vitro* (Danbolt 2001, Demarque et al., 2004, Cattani et al., 2007). Moreover, altered expression of Glu transporters have been reported in chronically epileptic animals (Ueda et al., 2001, Danbolt 2001). In contrast, Glu uptake transporters function in the reversed direction during ischemic conditions (Rossi et al., 2000). The low-[Mg²⁺] model of experimental epilepsy exhibits recurrent seizure-like events (SLEs) with a characteristic temporal pattern (Lasztóczy et al., 2006). We report on the effects of Glu uptake inhibitors L-trans-pyrrolidine-2,4-dicarboxylate (tPDC), DL-threo-b-benzyloxyaspartate (DL-TBOA) and dihydrokainic acid (DHK), representing different transporter specificity and membrane permeability profiles, on recurrent seizure-like events (SLEs) evoked in hippocampal slices by low-[Mg²⁺] condition.

RESULTS

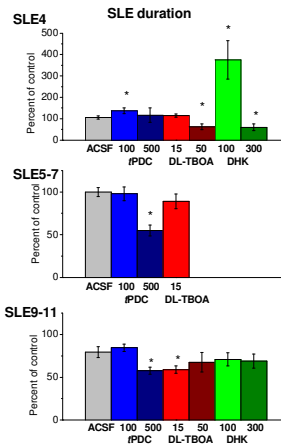
2. SLE onset time decreased



3. SLE amplitude decreased

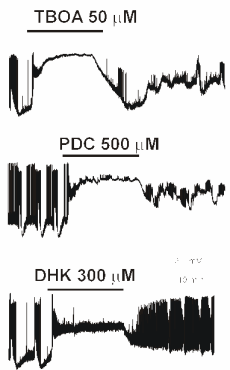


4. SLE duration either increased or decreased



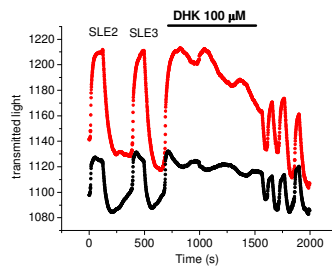
Effects of Glu uptake inhibitors on the onset time, the amplitude and the duration of the first (SLE4) and additional (SLE5-7) SLEs induced by low-[Mg²⁺] condition. Grey bars: control slices; blue bars 100 μM tPDC (n=10); dark blue bars: 500 μM tPDC (n=6), red bars: 15 μM DL-TBOA (n=10); wine bars: 50 μM TBOA (n=5); green bars: 100 μM DHK (n=8), dark green bars: 300 μM DHK (n=3). Values are expressed as percentile of control SLEs measured before drug administration. Asterisks indicate significant differences as compared to control slices at p < 0.05 level.

5. Sustained depolarization of CA3 pyramidal neurons



Effects of Glu uptake inhibitors on the membrane potential of CA3 pyramidal neurons

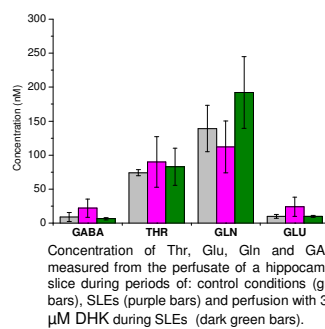
6. Elevation of light transmittance



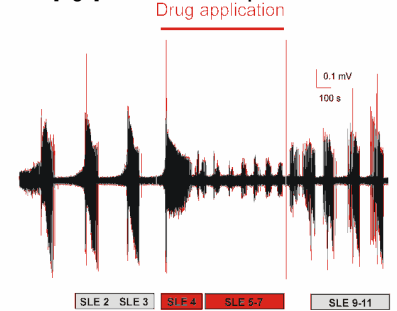
Effects of DHK on the light transmittance measured from CA3 region of the hippocampal slices with a CCD camera (Micromax, Princeton Instruments). Red symbols: stratum pyramidale, black symbols: stratum radiatum

7. Measurement of amino acid concentrations from the superfusate of hippocampal slices

Mass spectrometric measurements were run on an Applied Biosystems 3200QTrap MSMS coupled to a Perkin Elmer Series200 LC system. Free amino acids were separated on a Phenomenex Synergi Hydro RP column (150x3mm) using 0.5% heptafluoro-butyric acid as ion pairing agent in water and acetonitrile



1. Perfusion of juvenile (P10-13) rat hippocampal slices with low-[Mg²⁺] ACSF resulted in spontaneous SLEs



Representative field potential recording of an SLE from the CA3 pyramidal layer of a hippocampal slice from a P12 rat.

DISCUSSION

- Glu uptake inhibitors decreased the onset time of SLE4, indicating that cellular uptake of Glu – when functional – delay the SLE onset.
- Surprisingly, Glu uptake inhibitors decreased the amplitude of SLE4, and additionally, the higher inhibitor concentrations (TBOA 50 μM, tPDC 500 μM, DHK 300 μM) decreased the duration of SLE4 also.
Possible mechanisms:
 - mGluR activation
 - depolarization-induced inactivation of neurons.
 - rundown of transmitter pools (Glu, GABA)
- DHK 100 μM and tPDC 100 μM increased the duration of SLEs. Mechanisms:
 - decreased astroglial potassium uptake by DHK
 - mGluR activation by tPDC
- Sustained depolarization of pyramidal neurons and sustained elevation of light transmittance suggest contribution of depolarization induced inactivation of neurons.

CONCLUSIONS

- Inhibition of the cellular uptake of Glu precipitated a major role for the ambient Glu in determining the onset time and amplitude of epileptiform discharges. Thus the pre-ictal build-up of extracellular Glu, which is in turn delayed by Glu uptake mechanisms under control conditions, contributes to ictogenesis.
- Variable effects of tPDC, DL-TBOA and DHK on SLE duration may reflect differences in subtype-specificity or mechanism of action (transportable vs. non-transportable) of the inhibitors.

METHODS

Transverse 400 μm thick hippocampal slices were prepared from juvenile (P10-13) male Wistar rats. To evoke spontaneous recurrent SLEs, juvenile (P10-13) rat hippocampal slices were bathed in low-[Mg²⁺] ACSF. Extracellular electrodes were filled with ACSF and placed in the CA3 pyramidal layer. For current clamp recordings, pipettes (3-5 MΩ) contained (in mM) 135 KCl, 10 NaCl, 0.05 CaCl₂, 2 ATP, 1 EGTA and 10 HEPES). Traces were low-pass filtered at 2 kHz and digitized at 10 kHz with a Digidata 1320A A/D board (Axon Instruments, Foster City, CA) controlled by a computer running pClamp8 (Axon Instruments, Foster City, CA, USA). Statistical significance of the differences was assessed by Student's independent t-test or ANOVA as appropriate, with P < 0.05 taken as significant. All data are expressed as mean ± S.E.

Acknowledgements

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