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SEIZURES REDUCE THE EXPRESSION OF THE HYPERPOLARIZATION-ACTIVATED CYCLIC NUCLEOTIDE-GATED CATION (HCN) CHANNEL 1 VIA Ca^{++}/CALMODULIN DEPENDENT PROTEIN KINASE II ACTIVATION

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Rationale: HCN channels, mediating I_h, are crucial determinants of intrinsic neuronal properties and neuronal network excitability. Seizure activity in the developing hippocampus led to a long-lasting hyperexcitable state of the hippocampal circuit that was associated with modifications in I_h properties and altered expression of specific HCN isoforms (reduced HCN1 and enhanced HCN2 levels). Because the seizure-induced alteration of the HCN repertoire may contribute to network hyperexcitability, understanding the mechanisms by which seizures alter HCN mRNA expression is important.

Methods: We employed the organotypic hippocampal slice cultures, where seizure duration can be controlled, to test the hypothesis that the changes in HCN channel expression are mediated by seizure-evoked Ca^{++} influx and the consequent activation of Ca^{++}/calmodulin dependent protein Kinase II (CaM Kinase II). Seizures (lasting 3 hours) were induced in the cultures (prepared from postnatal day 8 rats and cultured for 3 days) using low [Mg^{++}] (0.4 mM) or kainic acid (6 μM). The requirement for CaM Kinase II activation was tested by using the specific blocker of this enzyme, KN-93 (10 μM) together with kainic acid and for 24 hours after the seizures. Cultures were harvested 48 hours later, a timepoint where effects of *in vivo* seizures on channel expression were found (Brewster et al., 2002, 2005). Quantitative *in situ* hybridization was employed to determine mRNA expression of HCN1 and HCN2.

Results: Seizures reduced mRNA and protein levels of GluR2 already within hours, creating Ca^{++} permeable AMPA channels, and leading to augmented entry of Ca^{++} into hippocampal pyramidal cells, as measured using an assay based upon kainate stimulated uptake of Co^{++} ions (Yin et al., 1996), and providing a likely route for the enhanced Ca^{++} entry. Seizures *in vitro* resulted in HCN expression changes comparable to those observed *in vivo* (Brewster et al., 2002, 2005), i.e. reduced HCN1 (by 22%) and increased HCN2 mRNA levels (by 20%). When seizures were provoked in the presence of the CaM Kinase II inhibitor, they were no longer capable of reducing HCN1 mRNA levels, compared to the robust reduction of transcript levels in sister cultures treated with kainic acid or low [Mg^{++}] alone.

Conclusions: Seizure-evoked down-regulation of HCN1 channel expression involves activation of Ca^{++}/calmodulin dependent protein Kinase II, that is probably consequent to seizure-provoked Ca^{++} influx. (Supported by NS35439 (TZB); NS47993 (ALB).)