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DISTINCT CA^{2+}-DEPENDENT MECHANISMS CONTRIBUTE TO THE OPPOSITE EFFECTS OF SEIZURES ON THE EXPRESSION OF TWO ISOFORMS OF THE HYPERPOLARIZATION-ACTIVATED CYCLIC NUCLEOTIDE-GATED (HCN) CHANNEL

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Rationale: Seizures in the immature hippocampus alter the expression of specific HCN channel isoforms (Brewster et al., 2002; 2005) and these changes may be responsible for the hyperexcitability of the hippocampal circuit. However, the molecular mechanisms underlying the activity-dependent regulation of HCN genes are still unclear. We have used organotypic hippocampal slice cultures to probe the role of seizure-evoked cellular Ca^{2+} influx, and specifically of Ca^{2+} dependent protein phosphorylation and de-phosphorylation, via Cam Kinase II and the Ca^{2+}-activated phosphatase Calcineurin, respectively in HCN channel expression. Our studies suggest that seizure-evoked downregulation of HCN1, but not seizure-evoked enhancement of HCN2 mRNA expression required Cam Kinase II activation. Therefore, we examine here whether seizure-evoked upregulation of HCN2 mRNA levels is mediated by Calcineurin.

Methods: *In vitro* seizure-like events ('seizures') lasting 3 hours were induced in organotypic cultures (prepared from P8 rats and cultured for 3 days) using kainic acid (KA, 6 μM). The requirement for Calcineurin activation was tested by using a specific blocker of this enzyme, FK506 (1 μM or 5 μM) together with KA. Cultures were harvested 48 hours later, a timepoint where effects of *in vivo* seizures on channel expression are well defined. mRNA expression of HCN1 & HCN2 were determined by quantitative *in situ* hybridization, comparing cultures that were main-tained in normal medium or subjected to seizures, in the presence or absence of FK506. Analyses were performed 'blindly'.

Results: As found *in vivo*, seizures in the organotypic cultures reduced HCN1- and increased HCN2 mRNA levels (Brewster et al., 2002; 2005). When seizures were induced in the presence of FK506 (both 1 μM and 5 μM), they were no longer able to increase HCN2 mRNA levels. The specificity of this effect (i.e., the effect of the Calcineurin inhibitor on seizure-evoked HCN1 expression) are still under study, as are experiments where the antagonist is added after the termination of the seizures. Blocking the activation of Cam Kinase II did not influence seizure-evoked upregulation of HCN2 mRNA expression.

Conclusions: Seizure activity-evoked upregulation of HCN2 mRNA levels requires the actions of the calcium dependent phosphatase, Calcineurin, but not of Cam Kinase II. This is in contrast to the mechanisms governing activity-dependent regulation of the HCN1 isoform, suggesting that the two isoforms are under separate molecular regulatory cascades. (Supported by NIH NS35439 (TZB); NS47993 (ALB).)