



Chopping Out CHOP Chops the Fate of Neurons

CHOP Regulates the P53–MDM2 Axis and is Required for Neuronal Survival After Seizures.

Engel T, Sanz-Rodriguez A, Jimenez-Mateos EM, Concannon CG, Jimenez-Pacheco A, Moran C, Mesuret G, Petit E, Delanty N, Farrell MA, O'Brien DF, Prehn JHM, Lucas JJ, Henshall DC. *Brain* 2013;136:577–592.

Hippocampal sclerosis is a frequent pathological finding in patients with temporal lobe epilepsy and can be caused by prolonged single or repeated brief seizures. Both DNA damage and endoplasmic reticulum stress have been implicated as underlying molecular mechanisms in seizure-induced brain injury. The CCAAT/enhancer-binding protein homologous protein (CHOP) is a transcriptional regulator induced downstream of DNA damage and endoplasmic reticulum stress, which can promote or inhibit apoptosis according to context. Recent work has proposed inhibition of CHOP as a suitable neuroprotective strategy. Here, we show that transcript and protein levels of CHOP increase in surviving subfields of the hippocampus after prolonged seizures (status epilepticus) in mouse models. CHOP was also elevated in the hippocampus from epileptic mice and patients with pharmacoresistant epilepsy. The hippocampus of CHOP-deficient mice was much more vulnerable to damage in mouse models of status epilepticus. Moreover, compared with wild-type animals, CHOP-deficient mice subject to status epilepticus developed more spontaneous seizures, displayed protracted hippocampal neurodegeneration and a deficit in a hippocampus-dependent object–place recognition task. The absence of CHOP was associated with a supra-maximal induction of p53 after status epilepticus, and inhibition of p53 abolished the cell death-promoting consequences of CHOP deficiency. The protective effect of CHOP could be partly explained by activating transcription of murine double minute 2 that targets p53 for degradation. These data demonstrate that CHOP is required for neuronal survival after seizures and caution against inhibition of CHOP as a neuroprotective strategy where excitotoxicity is an underlying pathomechanism.

Commentary

We all seem to know that “seizures beget seizures,” but do we really know why? Seizure-induced neuronal cell death may play a contributing role under certain circumstances (1, 2), and a variety of endogenous neuroprotective mechanisms are in place to limit neuronal injury (3–5). Neuronal cell loss in epilepsy is not straightforward and can depend upon spatial patterns, cell types, and timing of pathways that can either promote or reduce injury (3). Prolonged seizures are thought to induce excessive glutamate receptor activation, leading to the disruption of intracellular calcium homeostasis, oxidative stress, DNA damage, and dysfunction of intracellular organelles—among which, the unfolded protein response and associated endoplasmic reticulum stress might play a crucial role. This response can trigger apoptosis through the proto-oncogene CCAAT/enhancer-binding protein homologous protein (CHOP), which is a transcription factor induced by all three differing pathways triggered by the unfolded protein response (6). The precise role of CHOP in cell death pathways has been highly controversial. Based on findings that CHOP-enhances neuronal cell death in models of neurodegeneration

and acute brain injury, the therapeutic inhibition of CHOP has been suggested as a neuroprotective strategy (7). That said, CHOP was shown to promote neuronal survival after endoplasmic reticulum stress, supporting a neuroprotective role of CHOP (8). Since CHOP expression in the hippocampus is induced by seizures (9), the question arises whether this increase promotes neuronal cell death in epilepsy or whether an increase in CHOP is an adaptive response to limit neuronal cell loss. The distinction between those possibilities is of obvious relevance for the development of neuroprotective strategies in epilepsy.

The study by Engel and colleagues was designed to gain mechanistic and functional insight into the role of CHOP in seizure-induced neuronal cell death. The authors used a mouse model of focal onset status epilepticus (SE), triggered by unilateral injection of the excitotoxin kainic acid (KA) into the basolateral amygdala. This treatment leads to acute neuronal cell loss in region CA3 of the ipsilateral hippocampus, followed by development of spontaneous seizures. Using quantitative analyses, they first showed that CHOP protein expression was increased in the whole hippocampus 8 hours after the SE, whereas CA3-selective neuronal cell loss was found 24 hours after the SE. Likewise, CHOP expression was found to be increased in all subfields of the hippocampus 8 hours following a seizure preconditioning stimulus—a well-established neuroprotective strategy—or 8 hours after combining the SE with

Epilepsy Currents, Vol. 13, No. 5 (September/October) 2013 pp. 219–220
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salubral, a neuroprotective drug that blocks the endoplasmic reticulum stress response. The latter treatment correlated with reduced injury in CA3. If increased CHOP relates to neuroprotection, then a genetic deletion of CHOP should render the brain more susceptible to seizure-induced neuronal cell loss. As the next logical step, Engel and colleagues subjected *Chop*^{-/-} mice to intraamygdaloid KA-injections. Importantly, the gene deletion did not seem to alter the acute seizures; however, SE exacerbated the neuronal injury in the mutant animals in a “dose-dependent” manner, with heterozygous mutants showing an intermediate injury level. Pharmacological induction of endoplasmic reticulum stress at a level that is neuroprotective in wild-type animals was found to exacerbate injury in the knockout animals, suggesting that CHOP might be involved in the neuroprotective preconditioning response.

To gain insight into putative pathways acting downstream of CHOP, Engel and colleagues profiled gene expression in SE-exposed wild-type and *Chop*^{-/-} mice using microarrays. Intriguingly, the tumor suppressor protein p53 was found to be increased in SE-exposed mutants, and the expression of several genes known to be regulated by p53 was likewise increased, suggesting that the increased expression of CHOP in SE-exposed wild-type animals prevented the activation of several cell death-promoting pathways. If this assumption is true, the blockade of p53 in *Chop*^{-/-} mice should reduce seizure-induced neuronal cell death to wild-type levels. Experiments with the p53 inhibitor pifithrin- α demonstrated the validity of this assumption. Physiological steady-state levels of p53 are largely governed by its degradation through the ubiquitin-proteasome system, which involves the mouse double minute 2 homolog (MDM2). Reporter experiments demonstrated that CHOP can drive MDM2 expression in wild-type mice, but increased SE-induced MDM2 expression was no longer possible in *Chop*^{-/-} mice; this suggests that CHOP activation by SE leads to a reduction of cell death-promoting pathways via MDM2 activation.

In summary, this elegant set of experiments suggests the following mechanism contributing to seizure-induced neuronal cell loss: SE \rightarrow induction of CHOP \rightarrow induction of MDM2 \rightarrow increased degradation of p53 \rightarrow reduced activity of p53-dependent injurious pathways, resulting in increased neuroprotection. Interestingly, the same pathway seemed to be maintained in epileptic *Chop*^{-/-} animals, which developed an aggravated epileptic phenotype compared to wild-type controls. This finding might be of relevance for human epilepsy because the authors also reported increased CHOP protein levels in the hippocampi of patients with temporal lobe epilepsy without hippocampal sclerosis. Together, these findings are relevant and suggest that therapeutic interventions based on CHOP inhibition might not be suitable approaches to decrease neuroprotection, at least within the context of seizure-induced neuronal cell loss.

Based on these data, it is tempting to speculate that increased expression of CHOP might be an endogenous neuroprotective mechanism that provides a brake within a vicious cycle of continued seizure-induced neuronal cell loss, as has nicely been demonstrated in the aggravated epileptic phenotype of *Chop*^{-/-} mice. If this assumption is true, should

we question the involvement of neuronal cell loss in “seizures beget seizures”? One limitation of this elegant study is the lack of immunohistochemical analyses of CHOP expression. Without any information on cell-type selectivity of SE-induced CHOP expression changes, the claim is tenuous that CHOP is upregulated in surviving neurons. If CHOP was overexpressed in astrocytes and reduced in neurons, a tissue homogenate might still show an “increase,” which is not necessarily equal to upregulation in surviving neurons. Interestingly, the current study was performed on the C57BL/6 background, a strain of mice known to be relatively resistant to KA-induced neuronal cell death (10). It remains to be determined how genetic trait loci might interact with the CHOP-dependent pathways described by Engel and colleagues. A word of caution also seems to be justified, based on the fact that CHOP is a known proto-oncogene, which may limit the therapeutic utility of CHOP as a new therapeutic target. Despite these caveats, the studies discussed here suggest that augmentation of CHOP function or stimulation of CHOP-dependent pathways might be a potential therapeutic strategy to attenuate seizure-induced neuronal cell loss, an exciting possibility that warrants further basic research in that direction.

by Detlev Boison, PhD

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