



Arg! Post-Translational Modifications in Mitochondrial Proteins After Status Epilepticus

Post-Translational Oxidative Modification and Inactivation of Mitochondrial Complex I in Epileptogenesis.

Ryan K, Backos DS, Reigan P, Patel M. *J Neurosci* 2012;32(33):11250–11258.

Mitochondrial oxidative stress and damage have been implicated in the etiology of temporal lobe epilepsy, but whether or not they have a functional impact on mitochondrial processes during epilepsy development (epileptogenesis) is unknown. One consequence of increased steady-state mitochondrial reactive oxygen species levels is protein post-translational modification (PTM). We hypothesize that complex I (CI), a protein complex of the mitochondrial electron transport chain, is a target for oxidant-induced PTMs, such as carbonylation, leading to impaired function during epileptogenesis. The goal of this study was to determine whether oxidative modifications occur and what impact they have on CI enzymatic activity in the rat hippocampus in response to kainate (KA)-induced epileptogenesis. Rats were injected with a single high dose of KA or vehicle and evidence for CI modifications was measured during the acute, latent, and chronic stages of epilepsy. Mitochondrial-specific carbonylation was increased acutely (48 h) and chronically (6 week), coincident with decreased CI activity. Mass spectrometry analysis of immunocaptured CI identified specific metal catalyzed carbonylation to Arg76 within the 75 kDa subunit concomitant with inhibition of CI activity during epileptogenesis. Computational-based molecular modeling studies revealed that Arg76 is in close proximity to the active site of CI and carbonylation of the residue is predicted to induce substantial structural alterations to the protein complex. These data provide evidence for the occurrence of a specific and irreversible oxidative modification of an important mitochondrial enzyme complex critical for cellular bioenergetics during the process of epileptogenesis.

Commentary

It is not hard to imagine how mitochondria may play a role in epilepsy, given their diverse functions: ATP generation, calcium storage, cell death signaling, and mediators of oxidative stress. Thus, it is no surprise that mitochondrial dysfunction has been strongly linked to the pathogenesis of central nervous system disorders. For example, one form of early-onset Parkinson disease is caused by mutations in key proteins such as the mitochondrial serine/threonine protein kinase PINK1 (PTEN-induced putative kinase 1). One mitochondrial function, ATP production, should have obvious importance in sustaining abnormal neuronal activity, which is widely believed to be critical for persistent seizure activity. Another potential line of evidence linking epilepsy and mitochondria comes from the high-fat, low carbohydrate ketogenic diet, which has been used to treat seizures in patients with complex I, II, and IV defects, suggesting that the diet provides some compensation for this pathology (1). In addition, the ketogenic diet increases transcription of genes encoding electron transport chain subunits (2). These data, however, do not prove that mitochondrial

dysfunction results from (or is the result of) ongoing seizure activity (3).

More direct evidence of epilepsy pathology caused by mitochondrial abnormalities is provided by patients with mutations in mitochondrial DNA and thus, abnormalities in electron transport chain proteins (e.g., MERRF or myoclonic epilepsy with ragged red fibers). Mitochondrial toxins such as the succinate dehydrogenase suicide inhibitor 3-nitropropionic acid also cause seizures. However, the role of mitochondria in patients with epilepsy who do not have mutations in electron transport chain proteins or mitochondrial toxin exposure remains undefined. Of interest, complex I function is decreased in tissue resected from patients with temporal lobe epilepsy (4), but the mechanism of this dysfunction is unknown. Finding a link between abnormal complex I function and epilepsy may point to a potential target for therapeutic intervention. Ryan et al. have now shown how epileptogenesis can cause a post-translational modification in a complex I protein, concurrent with inhibition of complex I activity.

Using the subcutaneous kainic acid injection model of status epilepticus, rats were studied at 48 hours, 1 week, and 6 weeks after injection. These time periods were selected to represent the acute, latent, and chronic stages of epileptogenesis. Seizure activity was monitored by video recording, and only rats with overt motor seizures were considered to have epilepsy. Of interest, carbonylation (i.e., addition of a



functional group containing carbon monoxide) of hippocampal (but not neocortical or cerebellar) mitochondrial proteins increased during the acute and chronic stages but not during the latent phase. Also, carbonylation did not increase in rats treated with kainic acid that did not have overt convulsions. The biphasic pattern of carbonylation is similar to the authors' prior work showing elevated levels of H_2O_2 at these time points (5). Hippocampal (but not neocortical or cerebellar) complex I activity also was decreased at the acute and chronic (but not latent phase) time points. When complex I proteins were examined, increased carbonylation was noted in the 75 kDa subunit. The authors then used mass spectroscopy to identify the specific residue that is modified (Arg76 in the 75 kDa subunit of complex I to glutamic semialdehyde [GSA76]) in all of the acute and chronic tissue. In contrast, this modification was noted only 25% of the time in tissue collected during the latent phase. Further analysis indicated the Arg 76 residue is highly conserved between prokaryotic and eukaryotic species, highlighting its importance. A molecular modeling program indicated that this residue is close to the NADH binding site and an Fe-S center that plays a role in electron transfer. Modeling of the conversion of Arg76 to GAS76 showed a shift in the position of the Fe-S center away from the NADH binding site and an additional shift of two peptide segments in the complex I 51 kDa subunit. The latter resulted in decreased interaction of the 75 kDa and 51 kDa subunits.

Ryan et al. thus address two unanswered questions. First, the data suggest a molecular pathological consequence of oxidative stress after kainic acid-induced status epilepticus. Although by-products of oxidative stress have been well documented, their effects are protean. Thus, finding a specific post-translational modification of an arginine residue predicted to be near the active site of complex I is novel. Second, this study and this group's prior work (5) together show that kainic acid-induced status epilepticus has a time-specific negative impact on complex I function, thus providing a link between oxidative stress, the electron transport chain, and epileptogenesis. The ability to reverse these changes, however, remains unclear because the carbonylation reaction is covalent and therefore irreversible. This makes the prevention of epileptogenesis the only viable means of preventing similar modifications.

This work raises clinically important questions. What is the exact impact of Arg76 carbonylation on mitochondrial function (i.e., in terms of electron transfer, redox state, ATP generation, proton leak via uncoupling proteins, mitochondrial membrane potential, and so on)? What is the relationship between carbonylation of complex I at the acute and chronic time points, given the lack of a difference during the latent period (i.e., when significant changes leading to epileptogen-

esis presumably occur)? Can these findings be generalized to other models of temporal lobe epilepsy, status epilepticus, or both? Do other oxidative stress-induced post-translational modifications functionally amplify or mitigate the findings shown here? Is there a way to intervene clinically during the latent phase to minimize the impact of oxidative stress-induced post-translation covalent modification of proteins? What role do post-translation changes play in paradigms of recurrent single seizures, rather than status epilepticus? Are the current findings dependent on chronological age, given the variable sensitivity to oxidative stress at either end of the age spectrum (6, 7)? Ultimately, would prevention of carbonylation of the Arg76 residue in the 75 kDa subunit of complex I prevent the development of epilepsy?

In summary, Ryan et al. have shown that the acute and chronic stages of epileptogenesis in the kainic-acid model are associated with oxidative stress-induced post-translational modification in the complex I 75 kDa subunit, which may alter conformational relationships with other subunits and one Fe-S active site. These data advance knowledge of the role played by oxidative stress in epilepsy and open new avenues of investigation into potential treatments for the chronic changes induced by recurrent seizures.

by Adam L. Hartman, MD

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