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Changes in synapsin 1 phosphorylation and tubulin acetylation in mice deficient in protein L-isoaspartyl methyltransferase

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Protein L-isoaspartyl methyltransferase (PIMT) repairs damaged proteins that contain abnormal isoaspartyl (isoAsp) peptide bonds. PIMT knockout (KO) mice accumulate high levels of isoAsp damage in the brain and succumb to fatal epileptic seizures at 28–60 days after birth. Synapsin 1 and tubulin are major endogenous substrates for PIMT in brain, which could help explain the abnormal synaptic transmission and disorganized microtubules seen in the KO mice. To evaluate the effect of PIMT deficiency on the function of these proteins *in vivo*, we used Western blotting with modification-specific antibodies to assess the state of phosphorylation of synapsin 1 and acetylation of tubulin. In female mice, phosphorylation of synapsin 1 at the Ser-9 (protein kinase A) site was increased 145% in KO versus WT (wild type) mice. In males the increase was 22%. There was no change in phosphorylation at the Ser-603 (CaM kinase II) site in either male or female mice. Acetylation of α-tubulin at Lys-40 was decreased approximately 28% in both male and female KO mice. These results show that isoAsp accumulations in synapsin 1 and tubulin are associated with functional changes in these proteins *in vivo*. We propose that these changes contribute to the aberrant neurotransmission and abnormal microtubule organization caused by PIMT deficiency.

Key words: tubulin, synapsin

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Protein repair in the brain, proteomic analysis of endogenous substrates for protein L-isoaspartyl methyltransferase in mouse brain.

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Abstract

Protein L-isoaspartyl methyltransferase (PIMT) catalyzes repair of L-isoaspartyl peptide bonds, a major source of protein damage under physiological conditions. PIMT knock-out (KO) mice exhibit brain enlargement and fatal epileptic seizures. All organs accumulate isoaspartyl proteins, but only the brain manifests an overt pathology. To further explore the role of PIMT in brain function, we undertook a global analysis of endogenous substrates for PIMT in mouse brain. Extracts from PIMT-KO mice were subjected to two-dimensional gel electrophoresis and blotted onto membranes. Isoaspartyl proteins were radiolabeled on-blot using [methyl-(3)H]S-adenosyl-L-methionine and recombinant PIMT. Fluorography of the blot revealed 30-35 (3)H-labeled proteins, 22 of which were identified by peptide mass fingerprinting. These isoaspartate-prone proteins represent a wide range of cellular functions, including neuronal development, synaptic transmission, cytoskeletal structure and dynamics, energy metabolism, nitrogen metabolism, pH homeostasis, and protein folding. The following five proteins, all of which are rich in neurons, accumulated exceptional levels of isoaspartate: collapsin response mediator protein 2 (CRMP2/ULIP2/DRP-2), dynamin 1, synapsin I, synapsin II, and tubulin. Several of the proteins identified here are prone to age-dependent oxidation in vivo, and many have been identified as autoimmune antigens, of particular interest because isoaspartate can greatly enhance the antigenicity of self-peptides. We propose that the PIMT-KO phenotype results from the cumulative effect of isoaspartate-related damage to a number of the neuron-rich proteins detected in this study. Further study of the isoaspartate-prone proteins identified here may help elucidate the molecular basis of one or more developmental and/or age-related neurological diseases.

Key words: brain, repair