Activity-dependent cleavage of brain glutamic acid decarboxylase 65 by calpain

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摘要

Abstract

Previously, we reported that l-glutamic acid decarboxylase isoform 65 (GAD65) could be cleaved in vitro to release a stable truncated form which lacks amino acid 1-69 from the N-terminus, GAD65(Delta1-69). However, whether such a truncated form is also present under certain physiological conditions remains elusive. In the present study, we showed that, upon sustained neuronal stimulation, GAD65 could be cleaved into a truncated form in a rat synaptosomal preparation. This truncated form had similar electrophoretic mobility to purified recombinant human GAD65(Delta1-69). Furthermore, we demonstrated that this conversion was calcium dependent. Calcium-chelating reagents such as EDTA and 1,2-bis-(o-aminphenoxy)-ethane-N,N,N',N'-tetra-acetic acid tetra-acetoxy-methyl ester prevented the cleavage of GAD65. In addition, our data suggested that calpain, a calcium-dependent cysteine protease, is activated upon neuronal stimulation and could be responsible for the conversion of full-length GAD65 to truncated GAD65 in the brain. Moreover, calpain inhibitors such as calpain inhibitor I or calpastatin could block the cleavage. Results of our in vitro cleavage assay using purified calpain and immunopurified rat GAD65 also supported the idea that GAD65 could be directly cleaved by calpain