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Calpain cleavage of brain glutamic acid decarboxylase 65 is pathological and impairs GABA neurotransmission

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Previously, we have shown that the GABA synthesizing enzyme, L-glutamic acid decarboxylase 65 (GAD65) is cleaved to form its truncated form (tGAD65) which is 2-3 times more active than the full length form (fGAD65). The enzyme responsible for cleavage was later identified as calpain. Calpain is known to cleave its substrates either under a transient physiological stimulus or upon a sustained pathological insult. However, the precise role of calpain cleavage of fGAD65 is poorly understood. In this communication, we examined the cleavage of fGAD65 under diverse pathological conditions including rats under ischemia/reperfusion insult or rat brain synaptosomes or primary neuronal cultures subjected to excessive stimulation with high concentration of KCl. We have shown that the formation of tGAD65 progressively increases with increasing stimulus concentration. More importantly, direct cleavage of synaptic vesicle - associated fGAD65 by calpain was demonstrated and the resulting tGAD65 bearing the active site of the enzyme was detached from the synaptic vesicles. Vesicular GABA transport of the newly synthesized GABA was found to be reduced in calpain treated SVs. Furthermore, we also observed that the levels of tGAD65 in the focal cerebral ischemic rat brain tissue increased corresponding to the elevation of local glutamate as indicated by microdialysis. Based on these observations, we conclude that calpain-mediated cleavage of fGAD65 is pathological, presumably due to decrease in the activity of synaptic vesicle - associated fGAD65 resulting in a decrease in the GABA synthesis - packaging coupling process leading to reduced GABA neurotransmission.

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INTRODUCTION

The brain constantly strikes a balance between the excitatory and inhibitory networks, whose key players are the neurotransmitters L-glutamic acid and γ-amino butyric acid (GABA), respectively. Too much excitation or too little inhibition could tip the balance and is linked to a plethora of diseases such as epilepsy, Parkinson's disease and Huntington's chorea etc. (Wong *et al.* 2003). The decision in maintaining the state of equilibrium is controlled by the activity of the GABA synthesizing enzyme, L-glutamic acid decarboxylase (GAD) which utilizes L-glutamic acid as its substrate. GAD exists in two isoforms, namely, GAD65 and GAD67, where 65 and 67 denote their respective molecular weights in KDa. While 90% of GABA is constitutively generated by GAD67 for non-neurotransmitter purposes, the GABA generated by GAD65 is transient and is channeled exclusively for neurotransmission (Pinal & Tobin 1998). Unlike GAD67, GAD65 is in close proximity to vesicular GABA transporter (VGAT) and such an alignment facilitates efficient synthesis and packaging of the newly synthesized GABA into the synaptic vesicles, ready to be released for neurotransmission (Jin *et al.* 2003). Our studies largely focus on understanding the factors that govern the regulation of the GAD enzyme, since modulation of enzyme activity corresponds to the quanta of GABA synthesized. Because GAD65 is solely involved in neurotransmission, the work presented here delves into understanding how GAD65 is regulated, with special emphasis on the proteolytic cleavage of GAD65 by calpain. Our earlier work has indicated that GAD65 cleavage by calpain occurs both *in vivo* and *in vitro* (Christgau *et al.* 1992; Wei *et al.* 2003; Wei *et al.* 2006). Calpain is known to cleave its substrates either under a physiological stimulus or upon a sustained pathological insult. However, the precise role of calpain cleavage of GAD65 is poorly understood. Therefore, it became imperative to investigate the role of calpain in GAD65 cleavage, thereby understand its implications on GABA neurotransmission.

HYPOTHESIS

Calpain cleavage of full length GAD65 (fGAD65) to form truncated GAD65 (tGAD65) occurs under pathological conditions.

OBJECTIVES

•Under what conditions is the cleavage of fGAD65 favored?

•Is fGAD65 on the synaptic vesicles (SVs) susceptible to cleavage by calpain or is the cleavage site masked by protein-protein interactions?

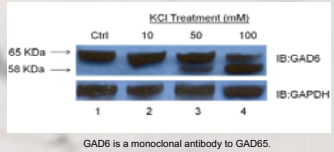
•As indicated by *in vitro* data that tGAD65 is more active than fGAD65, post calpain cleavage is tGAD65 bearing the active site of the enzyme attached or released from the SVs?

•What are the functional consequences of fGAD65 cleavage with respect to synthesis and uptake into SVs?

•If our hypothesis is true, does the formation of tGAD65 readily occur in an *in vivo* pathological condition such as in a rat middle cerebral artery occlusion (MCAO) model?

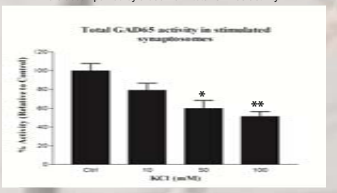
KEY DATA

1. Progressive accumulation of tGAD65 with increasing excitotoxic stimulus.

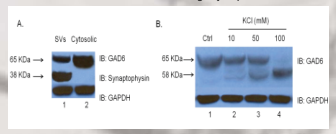


GAD6 is a monoclonal antibody to GAD65.

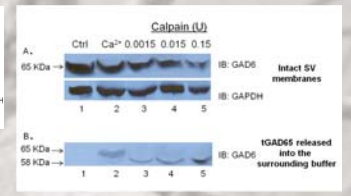
2. Conditions that led to progressive formation of tGAD65 were accompanied by a decline in total GAD65 activity.



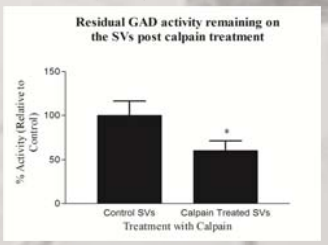
3. fGAD65 on SVs was not masked by associated partners but was amenable to cleavage by calpain.



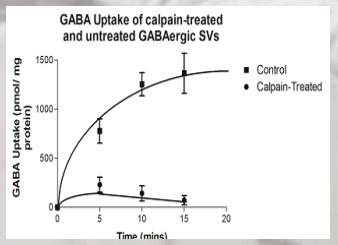
4. tGAD65 formed due to calpain cleavage detached from the SVs and was liberated into the surrounding cytosol.



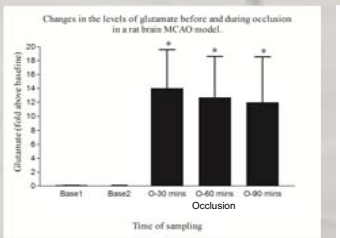
5. Total GAD activity remaining on the SVs post calpain treatment.



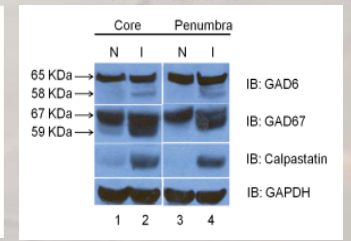
6. GABA uptake of calpain-treated and untreated GABAergic SVs.



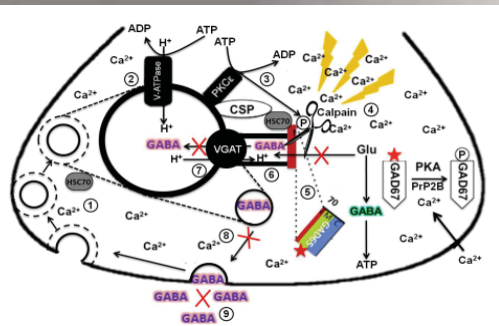
7. *In vivo* glutamate released during MCAO.



8. tGAD65 readily formed in MCAO ischemic tissue and not the matched controls..



PROPOSED MODEL



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CONCLUSIONS

•Formation of tGAD65 progressively occurs with increase in excitotoxic stimulus indicating that the formation of tGAD65 occurs under pathological conditions.

•fGAD65 on the SVs was amenable to cleavage by calpain.

•Post calpain cleavage, tGAD65 was no longer attached to the SVs but was liberated into the surrounding cytosolic fraction.

•Total GAD activity and total GABA uptake into the SVs rapidly declined after calpain-mediated cleavage of fGAD65.

•tGAD65 fragments were detected in the core and the penumbra of the ipsilateral but not contralateral regions of a rat MCAO model serving as a proof-of-concept to our hypothesis that formation of tGAD65 occurs under pathological conditions.

ACKNOWLEDGEMENTS

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