Examining the Therapeutical Effects of Lanthonine Ketimine (LK) in Neuronal Connectivity

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Abstract

Collapsin response mediator proteins (CRMPs), are cytoskeletal adaptor molecules involved in a variety of normal cellular functions including alteration of cell shape and cell communication. CRMP2s have also been associated with pathological disorders and neurological diseases. For instance, CRMP2 protein collects in cytoskeletal tangles in Alzheimer's disease, which may contribute to neural degeneration in this disorder. In other examples, differences in CRMP2 expression have been documented in some subsets of patients suffering paranoid schizophrenia. Lastly, the anticonvulsive drug lacosamide (VimPat) was found to act by binding to CRMP2, which unmasked the pharmacological importance of CRMP2-binding in epilepsy.

Thus, based on these observations, we hypothesize that CRMP2 plays a central role in neuronal connectivity and may represent a critical junction linking neural brain function with neural pathologies. Moreover, we reasoned that if we target CRMP2 therapeutically, we may reverse or slow-down onsets of many neurodegenerative disorders. To this end, we began a study focused on the in vivo effects of lanthionine ketimine (LK), a natural brain metabolite and neurotrophic agent that was recently reported to function, in part, through binding to CRMP2 (i.e. as a functional CRMP2 agonist). We have begun researching CRMP2 biology and potential for therapeutic manipulation using the nematode C. elegans, which expresses UNC-33, a homolog of mammalian CRMP2. In our work, C. elegans were grown in the presence of the cell permeable LK-ester (LKE) and synaptic connections were examined both structurally and functionally. Fluorescent imaging analysis and functional assays demonstrated that LKE affects neuronal morphology and physiology by rescuing mutant nematodes expressing partially functional UNC-33 isoforms.

These data provide evidence for in vivo function of LKE and reveal new opportunities for therapy development when CRMP2 functionality is compromised.

Background

1. Lanthionine ketimine (LK)

LK is a brain-endogenous cyclic thioether derived from amino acid condensation and ketimine metabolites.

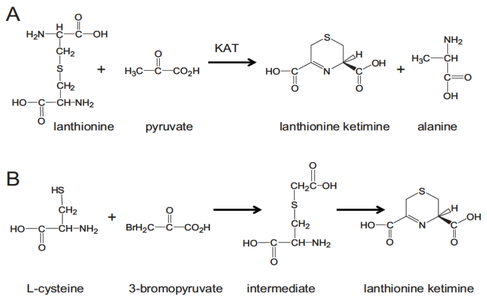

Figure 1: Pathways involved in LK synthesis

A: Structures of lanthionine and lanthionine ketimine. **B:** Chemical synthesis of lanthionine ketimine from L-cysteine and 3-bromopyruvate. Figure obtained from Hensley et al., J Neurosci. 2010 Feb 24;30(8):2979-88.

2. Chromatography Column

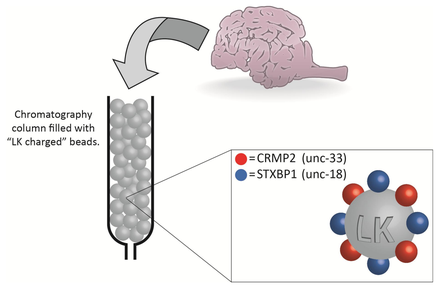

Figure 2 Chromatography column containing LK charged beads. Proteins from bovine cerebral cortex were run through a LK-charged chromatography column and CRMP2/UNC-33 and STXBP1/UNC-18 proteins were identified as LK interacting partners.

Picture generated by Jonas Holloway

Background Continued

3. LKE shown to reduce frequency of early terminating commissures of *unc-33 (e204)* nematodes in cholinergic neurons

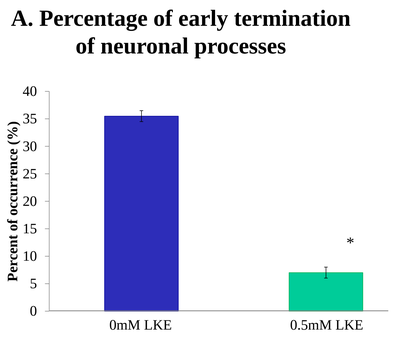

Figure 3. 96 hr LKE treatment of *unc-33* mutant nematodes promotes neuronal elongation.
A: *unc-33* hypomorph mutant (*e204)* overexpressing YFP in cholinergic neurons grown in 0mM LKE show significant termination of early commissures. *unc-33* hypomorph mutants overexpressing YFP and grown in 0.5mM LKE exhibited significant rescue of premature axonal termination.

Results

4. LKE reduces frequency of early terminating commissures of *unc-33 (e204)* nematodes

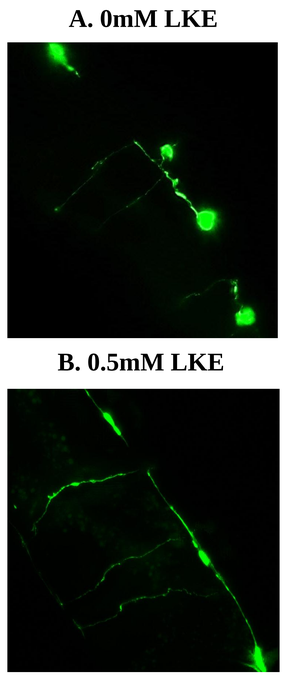

Figure 5. *unc-33 (e204)* nematodes over-expressing GFP in neurons and grown in 0.5 mM LKE exhibited a lower frequency of premature terminating commisures. A: *unc-33 (e204)* nematodes over-expressing GFP in GABAergic neurons grown in 0mM LKE.
B: *unc-33 (e204)* nematodes over-expressing GFP in GABAergic neurons grown in 0.5mM LKE. **C:** Quantification of the frequency of early terminating commissures in 20 individuals per treatment showed that LKE results in a significant decrease in premature termination.
* p<0.001

Results

5. LKE has a positive affect on ventral nerve cord development of *unc-33 (e204)* nematodes over-expressing GFP

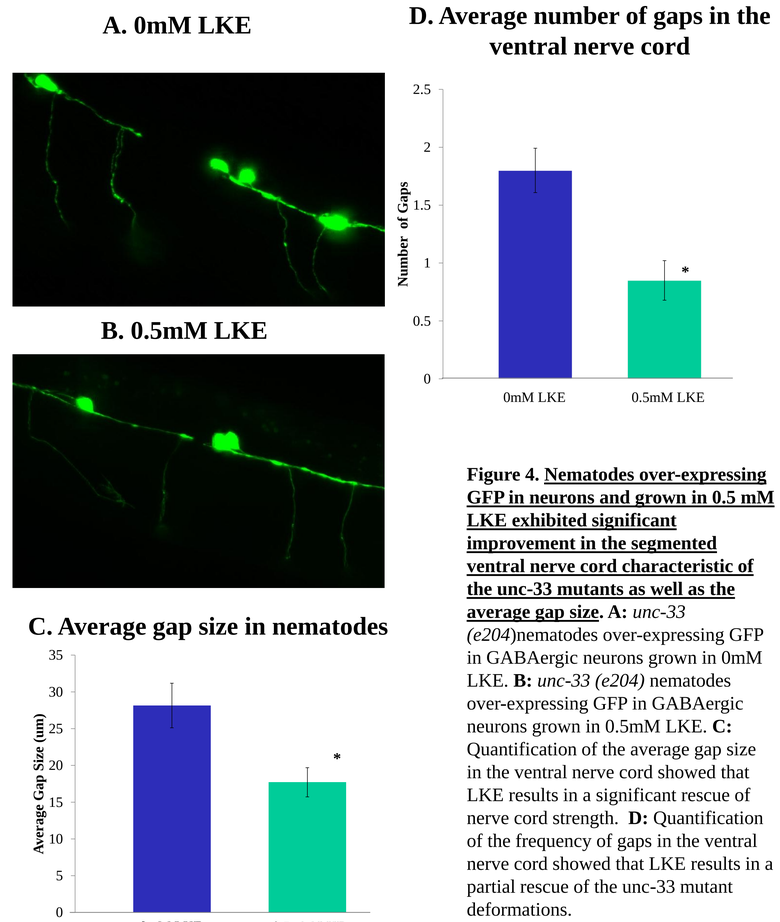

Figure 4. Nematodes over-expressing GFP in neurons and grown in 0.5 mM LKE exhibited significant improvement in the segmented ventral nerve cord characteristic of the unc-33 mutants as well as the average gap size. A: *unc-33 (e204)*nematodes over-expressing GFP in GABAergic neurons grown in 0mM LKE. **B:** *unc-33 (e204)* nematodes over-expressing GFP in GABAergic neurons grown in 0.5mM LKE. **C:** Quantification of the average gap size in the ventral nerve cord showed that LKE results in a significant rescue of nerve cord strength. **D:** Quantification of the frequency of gaps in the ventral nerve cord showed that LKE results in a partial rescue of the unc-33 mutant deformations.
* p<0.001

Conclusion

- **This research corroborates previous findings using a cholinergic fluorescent marker.**
- **In brief, we demonstrated that LKE partially rescued *unc-33(e204)* mutant synaptic phenotype.**
- **More specifically, LKE treatment significantly decreased the number of gaps observed at the ventral nerve cord. The size of the gaps, as well as the proportion of early terminated commissures.**

Acknowledgements

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