Topographic Phosphorylation of Neuronal Cytoskeleton is Regulated by Kinases and Phosphatases

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Introduction

Cyclin dependent kinase-5 (Cdk5) is a critical post-mitotically active kinase restricted to neurons by its activator protein, p35(1). Topographic regulation of phosphorylation of neuronal cytoskeletal elements, axonal transport, neurite outgrowth, and cell survival by Cdk5 has been demonstrated(1, 2, 3). Aberrant hyperactivity due to neuronal insult has been shown to lead to pathologic states(4). P5, a modified truncated 24-aa peptide, derived from the Cdk5 activator p35, has been shown to penetrate the blood-brain barrier and selectively inhibit abnormal Cdk5 hyperactivity, significantly rescuing Alzheimer's Disease (AD) pathology (up to 70–80%) in model mice(5). It has also been shown that Cdk5 plays a central role in glioblastoma/glioma tumorigenesis(6, 7). Utilizing P5, we aimed to demonstrate a balanced topographic regulation of neuronal cytoskeleton by kinases and phosphatases known to cross-talk(8) that would explain the observed compartmentalization of neuronal cytoskeletal phosphorylation.

Methods

Homogenization and differential centrifugation of extruded axoplasm and giant fibrous lobe (GFL) cell body compartments cleanly separated in loligo pealei model system allowed evaluation of endogenous phosphatase and kinase for comparative study of the effect of concentration variation of P5 on activity of protein phosphatase 2A (PP2A). A colorimetric molybdate-based free-phosphate detection system was employed to evaluate the effect of variation in P5 concentration on phosphatase activity in the axon and cell body compartments.

Results

The GFL compartment demonstrated elevated baseline PP2A activity compared to the axonal compartment. Dose-dependent inhibition of PP2A activity was seen in both compartments with higher concentrations of P5 showing two-fold inhibition in the GFL compartment compared to the axonal compartment.

Conclusions

P5 peptide is known to specifically inhibit hyperactivated Cdk5 in situ, which is critical to development of neuropathology. The preliminary study presented shows that P5 also inhibits phosphatase activity at higher concentrations. This elucidates balanced topographic regulation of the neuronal cytoskeleton in compartment specific fashion.

Learning Objectives

By conclusion of this session, participants should be able to:

1) Understand the dynamic nature of phosphorylation in topographic regulation of the cell body and axon

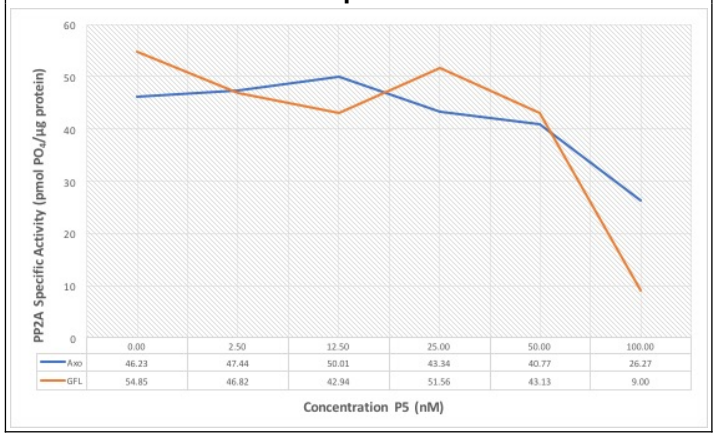
2) Appreciate the importance of the loligo pealei model system in evaluation of cell body and axonal compartments in neuropathology which is not cleanly afforded by mammalian systems.

3) Understand the role of Cdk5 in neuropathology, both neurodegeneration and tumorigenesis, and the inhibitory effect of the P5 peptide on aberrant hyperactivity of Cdk5 in situ

4) Understand the homeostatic topographic regulation of neuronal cytoskeletal elements by kinases and phosphatases in compartment specific fashion and the relationship of this to physiologic regulation and neuropathologic development.

5) Identify the P5 peptide as a specific means of further study of this

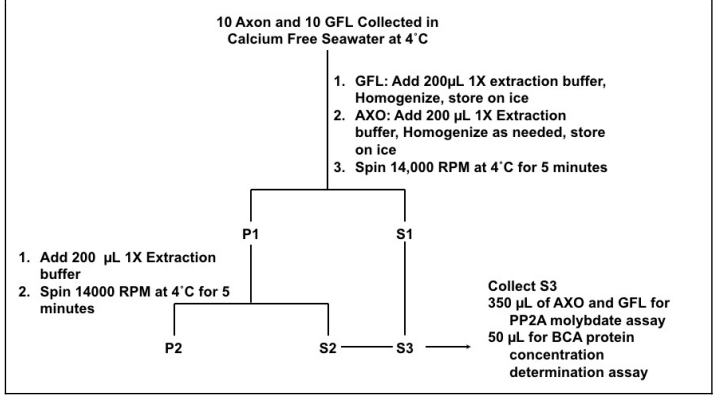
Axonal and GFL Compartment PP2A Inhibition by P5 Peptide



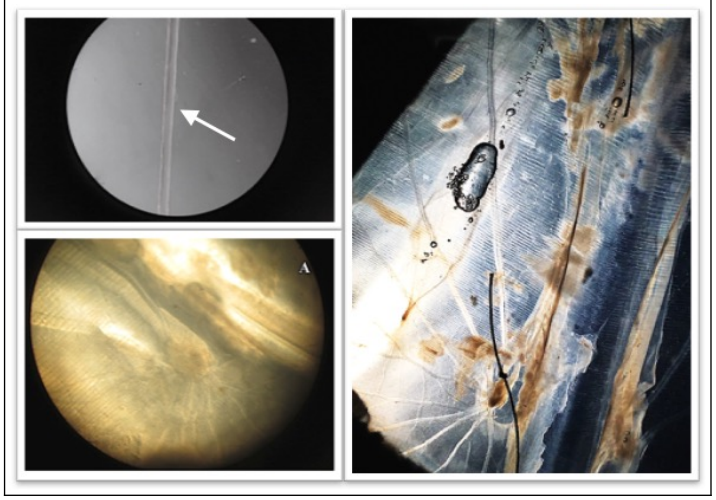
	0.00	2.50	12.50	25.00	50.00	100.00
Axo	46.23	47.44	50.01	43.34	40.77	26.27
GFL	54.85	46.82	42.94	51.56	43.13	9.00

Chart 1. Specific activity of endogenous pp2a activity in the axonal and GFL compartments exposed to a range of concentration of P5 peptide, known to inhibit cdk5 at lower concentrations. Concentrations of P5 were 0.0, 2.5, 12.5, 25, 50, and 100 nM. Axonal compartment demonstrated lower baseline pp2a activity than the GFL compartment in the absence of the peptide. The lowest three concentrations of P5 showed an inflection point in the axonal compartment, suggesting an increase in the free phosphate at a range known to inhibit cdk5, likely due to dissociation of phsophate from cdk5/p35. The GFL compartment showed this inflection point at a P5 peptide two-fold higher concentration. The axon showed a slow steady decrease in activity of pp2a as P5 concentration increased. The GFL demonstrated a sharper decrease in activity down to a final 83.59% decrease in activity compared to 43.18% decrease in the axon.

Homogenization and Differential Centrifugation Protocol



Clean Separation of Axon and GFL Compartments



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