Vitamin D Enhances Insulin Sensitivity in Neurons

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Introduction
We previously published vitamin D acts in the paraventricular nucleus of the hypothalamus to improve glucose tolerance. Additionally, we showed that the vitamin D receptor (VDR) in the brain is required for normal glucose tolerance in high-fat diet (HFD)-fed mice. However, the molecular mechanisms in the brain by which vitamin D or VDR action might alter glucose homeostasis is unknown. Interestingly, insulin in the brain acts through the phosphoinositide-3 kinase (PI3K) pathway to exert similar actions on glucose regulation. Vitamin D increases insulin sensitivity in other cell lines, such as muscle and adipose cells. We had preliminary data showing vitamin D could increase insulin-induced phosphorylation of Akt, a downstream protein in the PI3K pathway in nerve cells. Thus, we sought to determine if vitamin D might alter insulin action in nerve cells through the PI3K pathway.

Hypothesis
We hypothesized that vitamin D requires PI3K to enhance insulin action in hypothalamic neuronal cells.

Methods
• Hypothalamic cell line GT1-7 cells were cultured in D-MEM supplemented with 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin and grown at 37°C in 95% humidified air with 5% CO2.
• 80% confluent cells were treated with 100 nM 1,25-dihydroxyvitamin D₃ (1,25D₃) for either 2 or 48 hours. For experiments involving insulin, 100 nM insulin was added 20 minutes prior to cell collection. To block PI3K, wortmannin, 200 nM, was added 15 minutes prior to insulin treatment. Cell culture experiment results are displayed as the mean of 3 biological replicates, each consisting of 3 experimental replicates.
• For RNA-seq experiments, male Long-Evans rats were anesthetized with isoflurane and sacrificed by decapitation then hypothalami were collected. Hypothalamic cell line GT1

Results

Conclusions
• Vitamin D (1,25D₃) enhances insulin-induced phosphorylation of AKT through a PI3K-dependent manner.
• Vitamin D alters transcription of key genes in the PI3K pathway, increased IRS2 and p85 and decreased p55.
• In vivo, RNA seq analysis correlates with the cell culture findings and reveals upregulation of both the PI3K pathway and specifically IRS2 in rat hypothalamic tissue.
• Vitamin D has rapid actions to depolarize neurons, which is also dependent upon active PI3K.
• Overall, these findings suggest that vitamin D enhances insulin sensitivity but is dependent upon a functional PI3K pathway. This has clinical implications given that the PI3K pathway is likely altered in an obese, insulin-resistant individual.

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