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. At 80% confluence, cells were treated with 100 nM 1,25-dihydroxyvitamin D_3 (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 10 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 on either HFD (45% fat) or standard chow for 20 weeks were used. Through a third-ventricle cannula, 0.1 µg 1,25D3 was administered 120 minutes prior to sacrifice. The vehicle was hydroxypropyl-β-cyclodextrin (THPB-EC; CTD, Inc). We used 4 groups: 1) HFD-fed + 1,25D3, 2) HFD-fed + vehicle, 3) chow-fed + 1,25D3, and 4) chow-fed + vehicle. RNA sequencing on whole hypothalamic blocks was performed by the Genomic and RNA Profiling Core at Baylor College of Medicine on Illumina HiSeq 2500 Sequencing System. Genes with >1.25 fold increase were analyzed by Gene Set Enrichment Analysis.
- Sim1-Cre-tdTOMATO mice (at 6-10 weeks of age) were used for electrophysiological recordings. Patch pipettes were filled with intracellular solution (adjusted to pH 7.3) containing (in mM) 128 K gluconate, 10 KCl, 10 HEPES, 0.1 EGTA, 2 MgCl2, 0.3 Na-GTP and 3 Mg-ATP. Current clamp was engaged to test neural firing frequency at the baseline and after puff of 1µM 1,25D3 for 1s. The values for firing frequency were averaged within 2-min bin at the baseline or after 1,25D3 treatment. 50 µM wortmannin was added 10 minutes prior to 1,25D3.

Contact Information

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Results

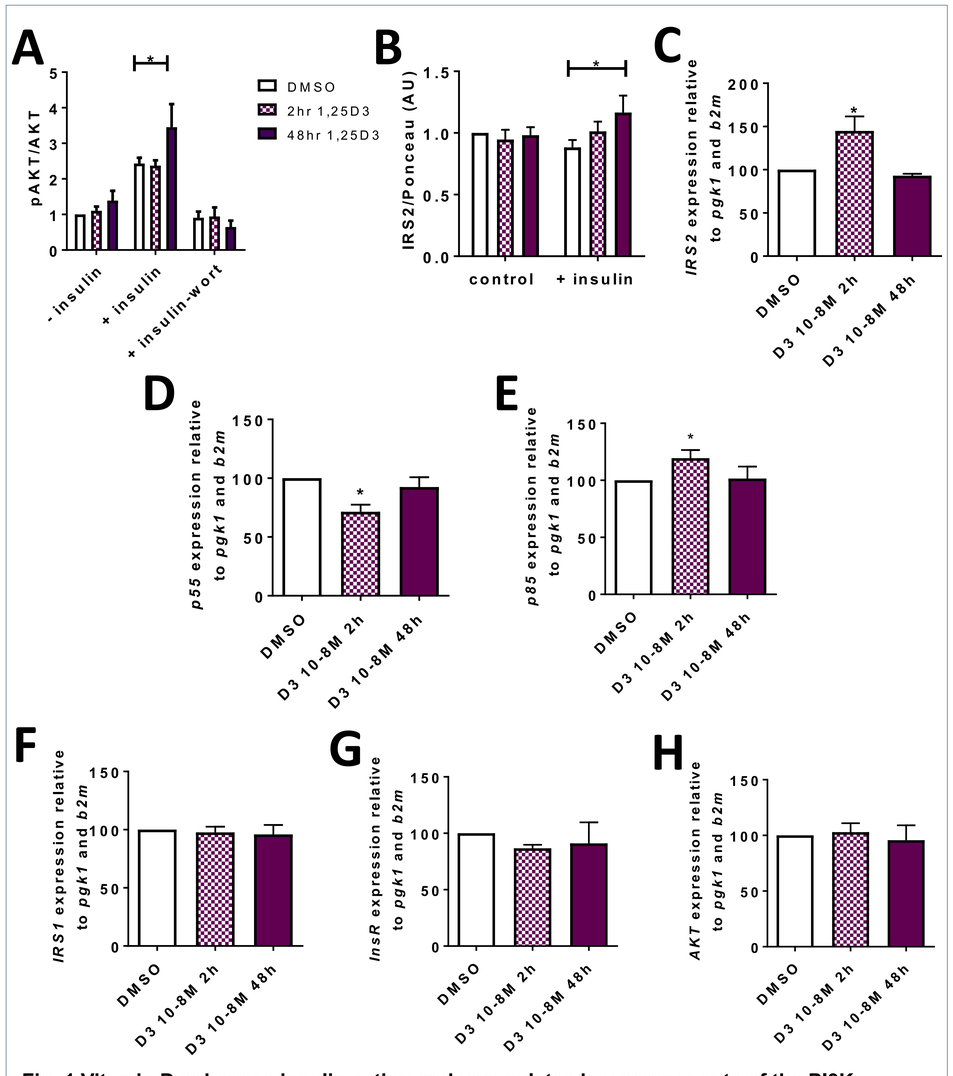

Fig. 1 Vitamin D enhances insulin action and upregulates key components of the PI3K pathway in hypothalamic (GT1-7) cells. A. Active vitamin D (1,25D3) increased insulin-induced phosphorylation of AKT. This was blocked in the presence of a PI3K inhibitor, wortmannin. B. 1,25D3 increased IRS2 protein concentrations in insulin-treated cells. C—H. Transcriptional regulation of key PI3K pathway genes showed vitamin D increased mRNA levels of *IRS2* (C) and *p85* (D), while decreasing levels of *p55* (E), a negative regulator of PI3K. Vitamin D did not affect transcription of *IRS1* (F), *InsR* (Insulin Receptor) (G) or *AKT* (H). * p < 0.05 compared to control.

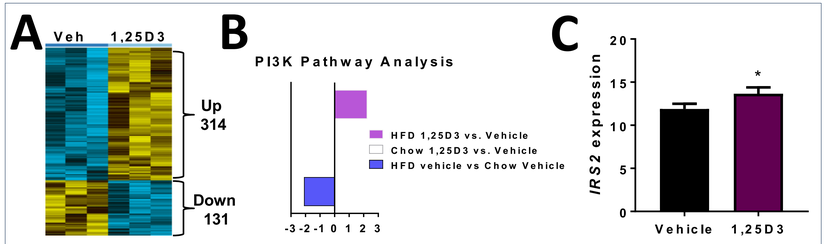

Fig. 2 1,25D3 upregulates the PI3K pathway in RNA-seq Analysis. RNA-seq analysis of rat hypothalamic tissue treated after intracranial 1,25D3 (0.1 µg) administration showed upregulation of 314 genes (A) and overall upregulation of the PI3K pathway (B), with specific upregulation of *IRS2* (C). * p < 0.05

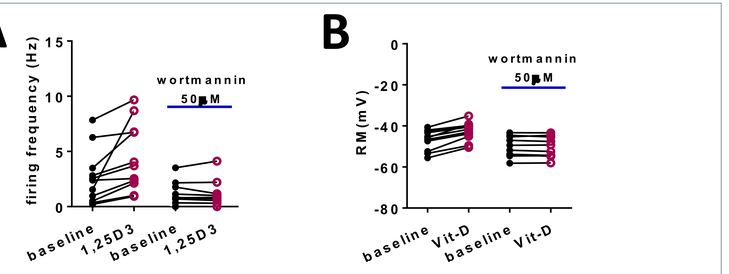

Fig. 3 Vitamin D increases neuronal activity in Sim1 expressing neurons in a PI3K dependent manner. A, B. 1,25D3 increased firing frequency (A) and resting membrane potential (B) in Sim1-expressing cells in the paraventricular nucleus. Both frequency and membrane potential were blocked with the addition of a PI3K inhibitor, wortmannin.

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 hypothalamus 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.**

Acknowledgements

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