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Vitamin D Receptor in the Paraventricular Nucleus of the Hypothalamus is Necessary for Beneficial Effects of $1,25D_3$ on Peripheral Glucose Levels

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Introduction

Low vitamin D levels correlate with type 2 diabetes. However, the mechanisms by which vitamin D might regulate glucose metabolism are not well studied. The only known mechanism linking vitamin D to glucose homeostasis was identified in pre-clinical studies showing $1,25D_3$ increases insulin secretion. However, VDR are found within multiple body organs, including the brain, which is very important in the regulation of glucose.

Hypothesis

We hypothesized that central VDR activation links vitamin D to the regulation of glucose.

Methods

- For rat studies, male Long-Evans rats were placed on high fat diet (40%) for 12 weeks.
- Brain cannulations performed at least 1 week prior to studies.
- 0.1 mcg 1,25-hydroxyvitamin D_3 (1,25-OHD3) was given into the third ventricle (i3vt) or paraventricular nucleus 60 minutes prior to the studies. The vehicle was hydroxypropyl-β-cyclodextrin (THPB-EC; CTD, Inc).
- Rats with lentiviral injections had SPWGM-V463 or SPWGM-NC (Viral Vector Core, University of South Carolina) injected unilaterally into the paraventricular nucleus (PVN)
- Antagonist ZK159222 was a generous gift from Bayer Pharma AG (Berlin, Germany).
- For mice studies, $VDR^{f/f}$ mice were used. They received AAV9.CMV.HI.eGFP-Cre.WPRE.SV40 or AAV-CMV-GFP-9 (University of Pennsylvania Vector Core) injected bilaterally into the PVN.
- Study specific methods in the figure legends

Results

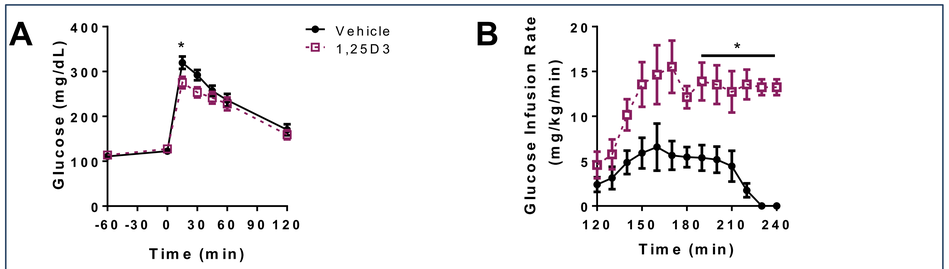

Fig. 1. Hypothalamic $1,25D_3$ lowers plasma glucose by inhibition of hepatic glucose production. A. I3vt $1,25D_3$ improves glucose excursion following an i.p. bolus of dextrose in DIO rats (n = 12 vs 13). B. High-fat fed rats treated with 0.1 µg i3vt $1,25D_3$ at 120 and 180 minutes during a physiologic hyperinsulinemic-euglycemic clamp had increased glucose infusion rate (n = 4-5/group).* $p<0.05$ vs. vehicle.

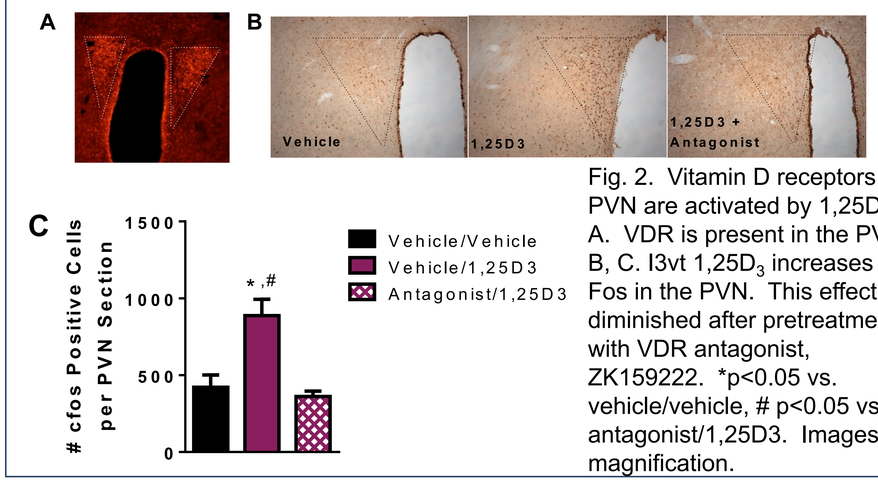

Fig. 2. Vitamin D receptors in PVN are activated by $1,25D_3$. A. VDR is present in the PVN. B, C. I3vt $1,25D_3$ increases c-Fos in the PVN. This effect is diminished after pretreatment with VDR antagonist, ZK159222. *$p<0.05$ vs. vehicle/vehicle, # $p<0.05$ vs. antagonist/1,25D3. Images 10X magnification.

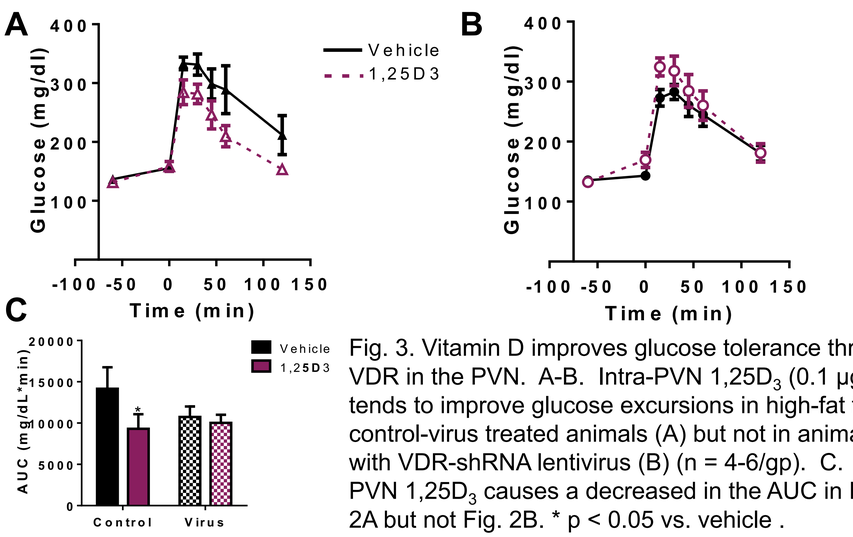

Fig. 3. Vitamin D improves glucose tolerance through VDR in the PVN. A-B. Intra-PVN $1,25D_3$ (0.1 µg) tends to improve glucose excursions in high-fat fed control-virus treated animals (A) but not in animals with VDR-shRNA lentivirus (B) (n = 4-6/gp). C. Intra-PVN $1,25D_3$ causes a decreased in the AUC in Fig. 2A but not Fig. 2B. * $p < 0.05$ vs. vehicle .

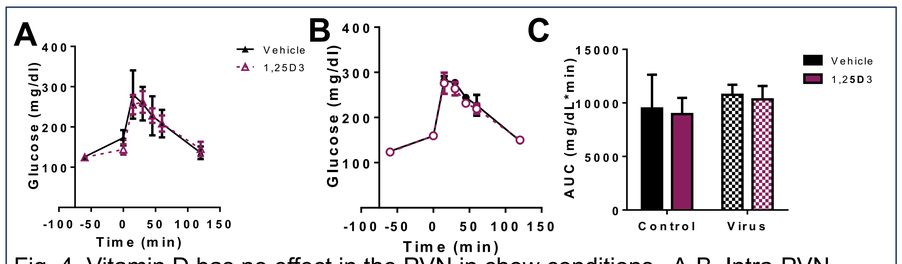

Fig. 4. Vitamin D has no effect in the PVN in chow conditions. A-B. Intra-PVN $1,25D_3$ has no effect on glucose tolerance in control-virus (A) or VDR-shRNA lentivirus-treated animals (B) on a chow diet (n=2/gp). C. AUC of Fig. 3A,B

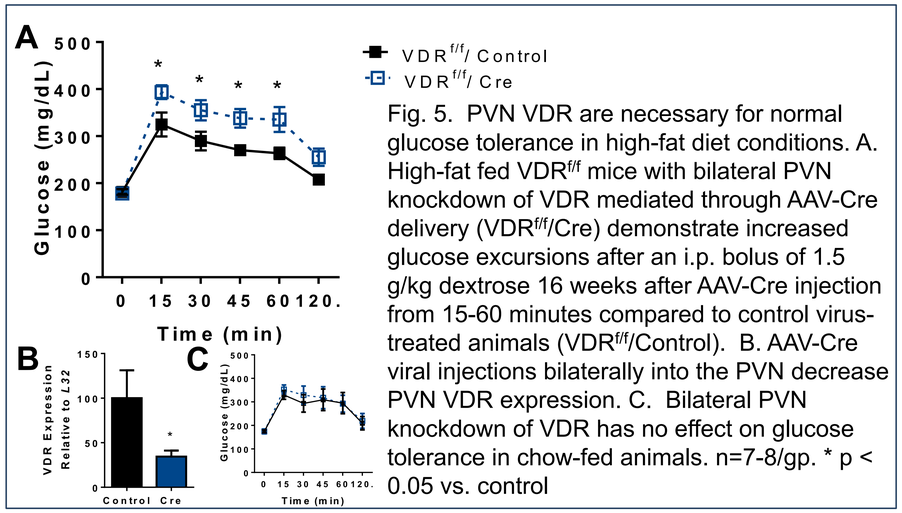

Fig. 5. PVN VDR are necessary for normal glucose tolerance in high-fat diet conditions. A. High-fat fed $VDR^{f/f}$ mice with bilateral PVN knockdown of VDR mediated through AAV-Cre delivery ($VDR^{f/f}$/Cre) demonstrate increased glucose excursions after an i.p. bolus of 1.5 g/kg dextrose 16 weeks after AAV-Cre injection from 15-60 minutes compared to control virus-treated animals ($VDR^{f/f}$/Control). B. AAV-Cre viral injections bilaterally into the PVN decrease PVN VDR expression. C. Bilateral PVN knockdown of VDR has no effect on glucose tolerance in chow-fed animals. n=7-8/gp. * $p < 0.05$ vs. control

Conclusions

- VDRs in the PVN are required for both exogenous and endogenous 1,253 mediated improvements in glucose regulation
- VDR activation is crucial for glucoregulation under a high-fat fed state
- Since vitamin D has limited transport into the brain, these results offer a possible explanation for the conflicting studies showing associations of vitamin D deficiency with diabetes but equivocal effects of supplementation on diabetes control.

Acknowledgements

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