The Neurodevelopmental Effects of Synthetic Glucocorticoid at Different Time Point on Stress and Metabolism Gene Expression in the Developing Hypothalamus.

David L. Chong, David L. Carbone, and Robert J. Handa

Department of Basic Medical Sciences, The University of Arizona College of Medicine-Phoenix, Phoenix, AZ

Abstract/Introduction

The clinical use of synthetic glucocorticoids (sGC) in newborns to enhance respiratory function has been shown to have other undesired effects such as increasing the risk of developing metabolic and neuropsychiatric disorders in adulthood.

In this study, we tested the hypothesis that exposure to the sGC dexamethasone (DEX) at different time points during early development will alter expression profiles of hypothalamic genes in the adult rats.

Rats were treated with DEX at Postnatal Day (PND) 4-6 and the effects of this exposure on gene expression were compared to that from a previous study in which fetuses were exposed to DEX at gestation day (GD) 18-21 by treatment of pregnant dams.

Hypothalamic Genes of Metabolism Measured

- Thyrotropin releasing hormone (TRH) is a key neuropeptide found in the paraventricular n. (PVN) and is responsible for regulating hypothalamic–pituitary–thyroid (HPT) axis function. It ultimately affects T3 and T4 secretion to regulate protein, fat, and carbohydrate metabolism.
- GHIH / Somatostatin is also found in neurons in the PVN and it acts upon the pituitary to inhibit GH release.
- IGF-1 - Insulin-like growth factor 1 (IGF-1), plays an role in childhood growth and anabolic effects in adults

Hypothalamic Genes of Stress Measured

- Oxytocin (OT): aside from its role in birth and lactation, it-decreases sympathetic activity and inhibits the secretion of cortisol. Thereby playing a role of inhibiting the stress axis.

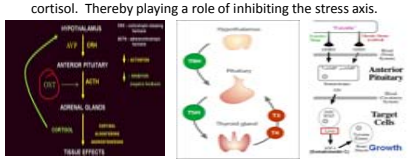

Figure 1: hypothalamic–pituitary thyroid/adrenal/growth axis

Methods

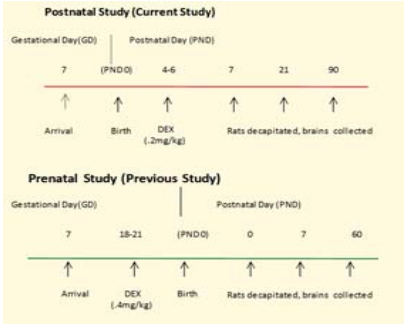

Figure 2: Postnatal vs Prenatal Study design

Male and female neonates were subcutaneously injected with a daily dose of DEX (0.2mg/kg in 100µl safflower oil) from PND 4 through 6, while the control groups received 100µl safflower oil. AT PND 7, 21, 90, The pups were anesthetized, killed, and brains were harvested

These brains were cryosectioned with the PVN and arcuate nucleus (ArcN) harvested individually using a 1mm diameter tissue punch. Quantitative RT PCR was performed to determine the level of gene expression.

In addition, immunohistochemistry was performed to visualize PreproTRH neurons, Western Blot analysis was performed to measure plasma levels of IGF-1.

Results

Postnatal DEX treatment alters TRH mRNA levels in PVN

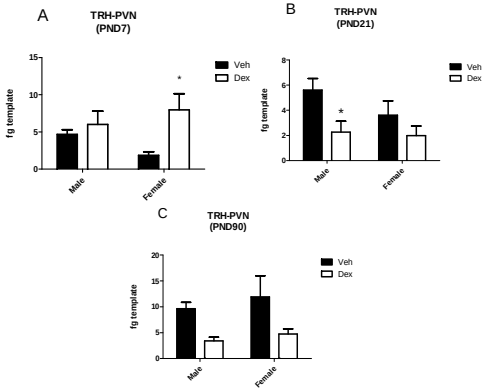

Figure 3: Trh hypothalamic gene expression in response to Postnatal DEX exposure. ppTRH mRNA levels are reported as a ratio of target gene (fg) per pg total cDNA per reaction. Each bar represents the mean ± SEM of no less than 7 animals. Statistical significance (p<0.05) between vehicle and DEX-exposed groups is indicated by asterisk. Panel A shows Trh mRNA levels in PND7 animals. Panel B shows TRH mRNA in PND 21 animals. Panel C shows TRH mRNA levels in PND 90 animals.

Postnatal DEX treatment does not alter TRH Neuron and fibers numbers

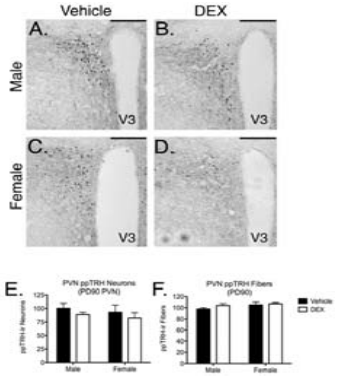

Figure 4: Photomicrographs showing ppTRH-ir neurons and fibers numbers within the PVN of adult male and female rats that were exposed to postnatal DEX. Each bar represents the mean ± SEM of no less than 6 animals per group. Males are shown in (Panels A–B); female (Panels C–D. Bilateral neuron counts (Panel E) or ppTRH-ir fiber counts (Panel F) were taken through the PVN in adult offspring (Panel E).

Postnatal DEX treatment alters oxytocin mRNA levels in PVN

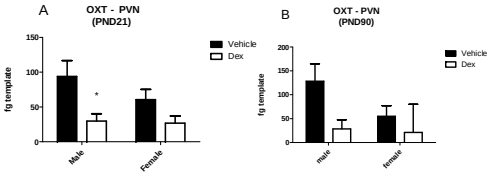

Figure 5: Oxytocin mRNA expression in PND 21 (panel A) and PND 90 (panel B) animals following postnatal DEX exposure. Gene expression is reported as a ratio of target gene (fg) to pg total cDNA per reaction. Data are represented as the mean ± SEM of no less than 7 animals. Statistical significance (p<0.05) between vehicle and DEX-exposed groups is indicated by an asterisk.

Postnatal DEX exposure alters Ghih mRNA expression

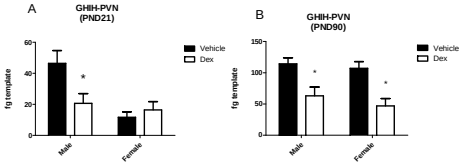

Figure 6: Growth Hormone Inhibiting Hormone (Ghih) gene expression in response to Postnatal DEX exposure. Gene expression level is reported as a ratio of target gene (fg) to total cDNA per RT-PCR reaction (pg). Each bar represents the mean ± SEM of no less than 7 animals. Statistically significant differences (p<0.05) between vehicle and DEX-exposed groups is indicated by asterisk. Panel A shows Ghih mRNA levels in PVN of PND21 animals. Panel B shows GHIH mRNA levels in PVN of PND 90

Comparison Post DEX vs. Pre DEX

	Post-DEX		PRE-DEX	
Trh	PND7	D	PND 7	V
	PND21			
	PND 90	V	PND 60	V
TRH IHC - Neurons	PND 90	O	PND 60	V
TRH IHC - Fibers	PND 90	O	PND 60	V

Figure 7: Comparison of Pre-DEX vs. Post-DEX in regards to gene expression (Trh, Ghih, Oxt), TRH IHC – Neuron, TRH IHC-Fibers, and Plasma IGF-1 levels. Thyrotropin releasing hormone (*Trh*) expression was decreased in the adult animals when DEX was administered either prenatally or postnatally. Subsequent examination of brain sections by immunohistochemistry (IHC) showed decreases in fiber and neuron counts that were only seen in the offspring treated with DEX prenatally. Ghih and Oxt levels decreased in post-DEX animal at PND 21 and 90, while no effect was seen in the pre-DEX treated animals.. No changes were fond in the Plasma IGF-1 levels in Post-DEX and Pre-DEX animals.

	Post-DEX		PRE-DEX	
Ghih	PND7	O		
	PND21	V		
	PND 90	V	PND 60	O
Plasma IGF-1	PND 90	O	PND 60	O
Oxt	PND7	O	PND 7	O
	PND21	V		
	PND 90	V	PND 60	O

Legend

1) V - Denotes higher level in Vehicle when compared to Dex
2) D - Denotes higher level in Dex when compared to Vehicle
3) O - No Treatment effect Seen

Summary and Conclusions

- Different critical windows exist for sGC treatment to affect expression of genes in the hypothalamus. *Trh*, TRH neurons, *Oxt* levels all seem to be affected differently by DEX treatment at different times.
- Trh expression was decreased in the adult animals when DEX was administered either prenatally or postnatally.
- TRH neuron and fiber density in the PVN showed decreases that were only seen in the offspring treated with DEX prenatally.
- Ghih and Oxt levels decreased by postnatal DEX treatment when examined at PND 21 and 90, while no effect was seen in the pre-DEX treated animals.
- Collectively, these data demonstrate that permanent programming effects of sGCs on hypothalamic gene expression are dependent upon the timing of the exposure (Gestational vs Post Gestational)

Future Directions

- One possible mechanism that should be further explored is the role of epigenetic marks on DNA in the persistence of these effects into adulthood. Studies measuring methylation of CpG islands in *Trh and Oxt* promoters regions might help us understand the mode of action. It would also be interesting to see if the changes in gene expression correlated with changes in neuron proliferation or death.
- IHC of neuron population of OXT in the PVN may show changes in neuron population.
- The exact critical window of each gene should be narrowed. Repeating this experiment with additional exposure points such as GD 21 to birth, and PND 1 to PND 3 can help determine the exact critical window of vulnerability for each of the genes measured in this paper.