Enhancing Neurosteroidogenesis Attenuates HIV-1 Tat Mediated Stress-Related Outcomes

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Neurosteroidogenesis Reduces HIV-1 Tat Stress Effects

Abstract

Human immunodeficiency virus (HIV) is associated with comorbid affective, stress-sensitive neuropsychiatric and neuroendocrine complications that afflict ~50% of infected individuals, but the mechanisms are not known. One factor that may contribute to hypothalamic-pituitary-adrenal (HPA) stress axis dysfunction is the neurotoxic HIV-1 regulatory protein, trans-activator of transcription (Tat). We previously demonstrated that HIV-1 Tat promotes anxiety-like behavior in mice concurrent with an elevation of basal corticosterone (seen in males and females) and adrenal insufficiency (seen only in males). The HPA axis is tightly regulated by GABAergic signaling, therefore impairments in GABAergic signaling may contribute to neuroendocrine dysfunction, conferring vulnerability to stress-sensitive disorders. Given that neurosteroids are potent allosteric modulators of $GABA_A$ receptor, enhancing neurosteroidogenesis may ameliorate Tat-mediated HPA dysfunction. Adult male transgenic mice that expressed Tat_{1-86} protein [Tat(+)] or not [Tat(-)] were administered i.c.v. vehicle, the neurosteroid allopregnanolone (AlloP; 100nM), or the neurosteroid-enhancing compound FGIN-1-27 (5µg/µL). Mice were exposed to swim stress (or not) and behaviorally-tested in an open field. qRT-PCR was performed to assess expression of steroidogenic enzymes in the hypothalamus. AlloP reduced the latency to enter the brightly-illuminated center of an open field concurrent with normalizing Tat-mediated downregulation of the 3α-HSD-synthesizing enzyme. These data provide proof-of-principle that enhancing neurosteroidogenesis in the central nervous system can influence the HPA axis and related affective dysfunction. Reinstatement of central neurosteroid content may restore HPA function and reduce vulnerability to psychiatric disorders.

Hypothesis

1. In vivo, neurosteroids will attenuate anxiety-like behavior.
2. Neurosteroids will normalize Tat-mediated dysregulation of neurosteroid-synthesizing enzymes.

Methods

Animal Subjects: Transgenic mice were bred in the vivarium at the University of Mississippi (University, MS). Tat(+) mice expressed a Tat 1-86 protein that became transcriptionally-active in the presence of doxycycline (induced via doxycycline injection, 30mg/kg/d for 5d). Tat(-) mice expressed the transcription factor necessary to activate transgene induction, but did not express the transgene itself. Anxiogenic effects of Tat induction have been previously-observed using these mice1-3 . Mice were administered vehicle or AlloP or FGIN-1-27 directly to the lateral ventricle via an ALZET osmotic minipump.

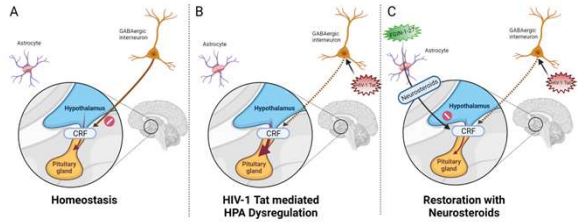
Behavioral Assessment: Mice were behaviorally-tested in open field and light dark transition task. All tests were completed within 8 days of doxycycline induction and occurred 2-3 h into the dark phase of the light cycle. Data were encoded by an ANY-maze behavioral tracking system (Stoelting Co., Wood Dale, IL).

Forced Swim Stimulus: The Porsolt forced swim test was used to activate the HPA stress axis. In brief, mice were placed in room temperature water (~22 ° C) and allowed to swim for 15 min followed by injection with saline and behavior assessment.

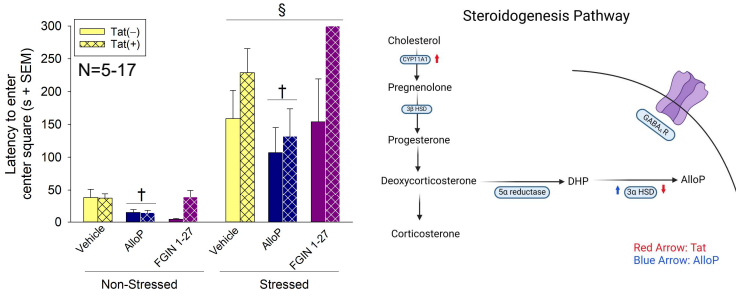
Quantitative Real Time Polymerase chain reaction: A panel of neurosteroidogenic enzyme gene expression levels were quantified from the hypothalamic tissues by qRT-PCR as previously described (3). Briefly, isolated tissues were homogenized in TRizol reagent and total RNA concentration was determined by Nanodrop spectrophotometer.

Results

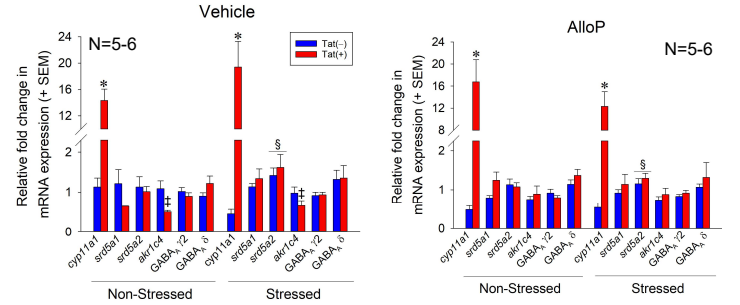
Working Hypothesis



Neurosteroids influence anxiety-like behavior



AlloP restored Tat-mediated dysregulated neurosteroidogenesis



Summary

- AlloP attenuated anxiety-like phenotype.
- Tat(+) mice exhibited upregulation of *cyp11a1* expression and downregulation of *akr1c4* expression
- AlloP restored *akr1c4* expression levels
- Irrespective of Tat or AlloP exposure, stress upregulated expression of *srd5a2*

Conclusion

Prior work has shown HIV Tat to influence HPA axis responding concurrent with increased anxiety-like behavior[1-3]. Tat expression promotes hypercortisolemia[1-3]. AlloP reduces anxiety-like behavior. Neuroendocrine modulators may be useful adjuncts to HIV therapeutics for their capacity to restore HPA function or affective homeostasis.

References

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Acknowledgments

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