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Effects of Restraint Stress and Allopregnanolone Inhibition on Amphetamine Locomotor Sensitivity

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INTRODUCTION

The chronic, recurring nature of addiction remains a worldwide problem. Even after apparently successful clinical treatment and long term abstinence, individuals may still relapse many months or years later. Although many individual differences exist among substance abusers, relapse tends to occur during periods of high stress (Sinha et al., 2006). Behavioral training and therapy can help cope during these high stress times, but pharmacological interventions have not been shown to be effective (Ross & Peselow, 2009). Although some therapeutic options decrease relapse rates, more effective treatments for relapse need further consideration.

The effect of stress on use of and relapse to drugs of abuse likely stems from coupled stress and reward circuits in the brain. Stress leads to increased release of stress-related hormones including 3α, 5α tetrahydroprogesterone or, allopregnanolone (Purdy et al., 1991). Allopregnanolone is a neurosteroid tied to several brain circuits involved with stress and reward. Elevated levels of this neurosteriod occur throughout the mammalian brain and periphery after cocaine administration, and rats show enhanced dopamine release in the nucleus accumbens after an injection of finasteride, which inhibits the enzyme (5-α reductase I) responsible for allopregnanolone synthesis (Dazzi et al., 2002). Finally, acutely stressed rats exhibit increased dopamine release in the prefrontal cortex after an injection of finasteride, further indicating allopregnanolone's involvement with brain reward systems (Devoto, 2012). Based on this information, we hypothesized that administration of finasteride would result in increased stress induced amphetamine locomotor sensitization.

METHODS

Subjects: Thirty adult male Long-Evans rats (Harlan) were pair housed on a 12:12 light dark cycle with lights on at 0700 hours. Rats were randomly assigned to one of three pretreatment conditions (saline, 10.0 mg/kg finasteride, or 25.0 mg/kg finasteride) followed by pseudo-random assignment to control or restraint stress condition.

Drugs: Finasteride (Steraloids, Inc., Newport, RI) was sonicated until dissolved in 20% 2-OH-propyl-β-cyclodextrin in a concentration of 10.0 mg/ml or 25.0 mg/ml. D-amphetamine (Sigma) was dissolved in saline in a concentration of 1.0 mg/ml.

Pretreatment: Rats received subcutaneous injections of either: vehicle, 10.0 mg/kg finasteride, or 25.0 mg/kg finasteride both 48 hours and 24 hours before stress procedure.

Acute stress: Twenty-four hours after the last pretreatment injection rats received control or restraint stress procedure (no injections were administered on this day). Starting between 90-120 minutes after lights on, rats in the stress group were placed in restrainer tubes for 60 minutes while rats in the control group remained in their home cages. Experimenters collected blood samples via tail incision for both groups at three time points: before stress, after one hour of stress or control, and 30 minutes after recovery.

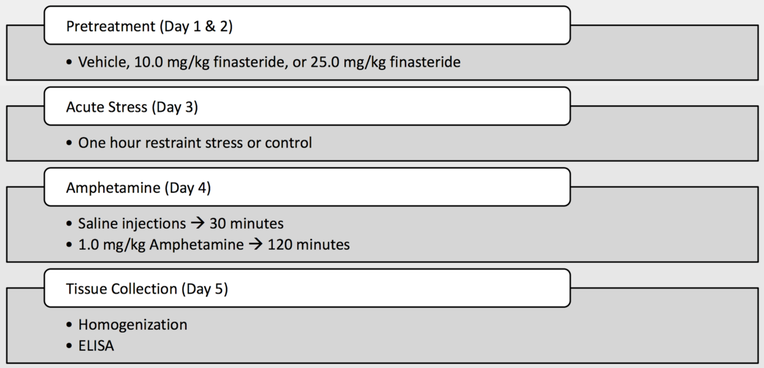
Amphetamine sensitivity: Twenty-four hours after restraint stress, rats were transported to the testing room and allowed to habituate for 30 minutes. All rats received a saline injection (s.c.) and were placed in an open field chamber for 30 minutes. After 30 minutes, rats were injected with 1.0 mg/kg amphetamine and placed back in the chamber for 120 minutes. Distance traveled was collected using a 16 x 16 photobeam tracking system (MotorMonitor), and cameras recorded the session for later scoring of stereotyped behavior. Stereotypy was rated using a point for each present behavior and a zero for each absent behavior.

Tissue Collection: Hippocampal tissue was collected 24 hours following amphetamine treatment and stored in -80C. Tissue samples were weighed and recorded then transferred into buffer mixture of 1:2 methanol and chloroform (respectively); two beads were placed inside. Tissue was homogenized for two minutes at 30Hz, repeated twice. Following homogenization, tissue was centrifuged for five minutes at 10,000 rpm. Supernatant was then transferred into a new eppendorf tube and samples were returned to -80 freezer.

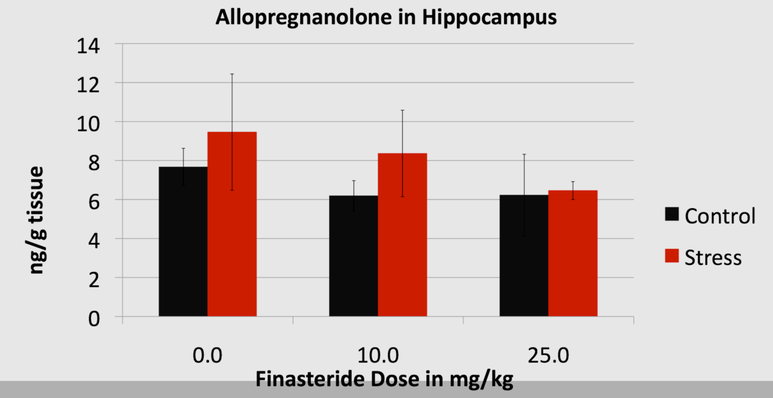
Allopregnanolone ELISA: ELISA kit was supplied by Ucsn Life Science, Inc. 96-well strip plate was pre-coated with allopregnanolone antibody. Five wells were prepared for standard points, one well for blank. All samples, including tissue were run in duplicates. 50 ul of standard, blank, and samples were pipetted into appropriate wells. Detection Reagent A was added to each well prior to one hour incubation. Following incubation, wells were aspirated and washed. Detection Reagent B was added followed by 30 minute incubation. Aspiration/wash process was repeated. Substrate solution was added to each well followed by 15 minute incubation. Stop solution was added and plate was read through micro-plate reader at 450 nm to analyze absorbance.

Corticosterone ELISA: Plasma samples were collected at baseline, during stressor, and after stressor for a total of three samples per animal; albeit only nine out of 31 animals provided sufficient plasma on all three samples to qualify for the assay. ELISA kit was supplied by Enzo, Life Sciences. 96-well strip plate was precoated with corticosterone antibody. 100 ul of assay buffer 15 were pipetted in NSB and Bo wells. 100 ul of standard and samples were pipetted into their respective wells. Additional 50 ul of assay buffer were pipetted into NSB wells. Blue conjugate was pipetted into all wells, except TA and Blank wells. Yellow antibody was pipetted into each well, except blank, TA, and NSB wells. The plate was incubated for 2 hours at room temperature on a plate shaker. Following incubation, wells were washed/aspirated. Blue conjugate was added to TA wells. PNpp substrate solution was added to all wells, followed by one hour incubation. Stop solution was added to all wells and plate was read through microplate reader at 405 nm to analyze absorbance.

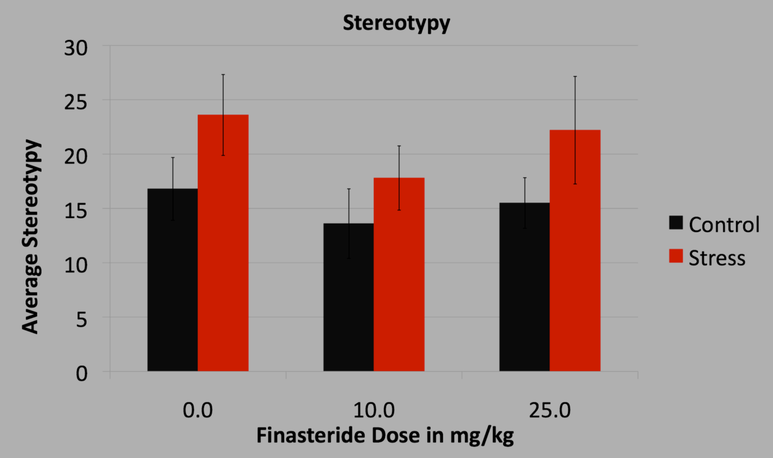
METHODS



RESULTS

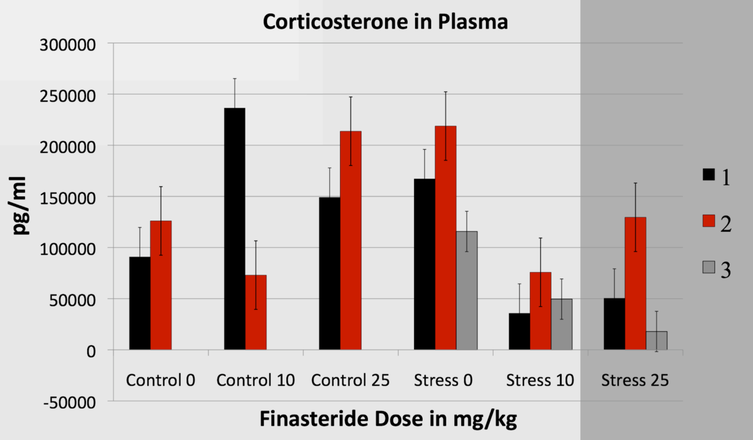


Allopregnanolone concentrations following finasteride pre-treatment. A 2 x 3 (condition (control vs. stress) x dose (vehicle, 10.0 mg/kg, 25.0 mg/kg)) between subjects ANOVA revealed no significant effect of dose ($F_{1,,28}$= 0.612, p > 0.05) , and no significant effect of condition ($F_{1,28}$ = 0.554, p > 0.05). Bars represent standard error.



Stereotypies during amphetamine treatment. A 2 x 3 (condition (control vs. stress) x dose (vehicle, 10.0 mg/kg, 25.0 mg/kg)) between subjects ANOVA revealed a significant effect of condition ($F_{1,28}$= 4.936, p < 0.05) , and no significant effect of dose ($F_{1,28}$ = 1.046, p > 0.05). Bars represent standard error.

RESULTS



Corticosterone concentrations during restraint stress. Time 1= baseline, time 2= one hour after stress or control, time 3= 30 minutes after recovery. Mixed model ANOVA with between subject factors of condition (control vs. stress) and dose (vehicle, 10.0 mg/kg, 25.0 mg/kg) . Within subjects of time (time 1, time 2, time 3). No significant effect of condition ($F_{1,30}$= 1.066, p > 0.05) , no significant effect of dose ($F_{1,29}$ = 2.070, p > 0.05), no significant effect of time ($F_{1, 29}$= .670 > 0.05). Bars represent standard error.

CONCLUSIONS

- One hour of acute restraint stress decreased overall locomotor activity in an open field.
- Finasteride did not lower allopregnanolone levels, regardless of dose.
- Pritchard et al. (unpublished) found similar results using a chronic stressor. Following 5 days of restraint stress rats exhibited locomotor sensitization in response to an acute injection of 1.0 mg/kg amphetamine. However, no effect of finasteride on amphetamine sensitivity was observed after this chronic stressor.
- Stereotypy was rated and effect of stress, not dose was found. Thus, rats that were stressed all demonstrated higher levels of stereotypy than control rats.
- Corticosterone levels did not elevate in response to stress.
- Limitations in our study may have been due to our timing of finasteride pretreatment, stressor type, or duration of stressor. In addition, more thorough lipid extraction will be taken into consideration for future research.

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