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Research report

Amphetamine withdrawal differentially affects hippocampal and peripheral corticosterone levels in response to stress

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ABSTRACT

Amphetamine withdrawal is associated with heightened anxiety-like behavior, which is directly driven by blunted stress-induced glucocorticoid receptor-dependent serotonin release in the ventral hippocampus. This suggests that glucocorticoid availability in the ventral hippocampus during stress may be reduced during amphetamine withdrawal. Therefore, we tested whether amphetamine withdrawal alters either peripheral or hippocampal corticosterone stress responses. Adult male rats received amphetamine (2.5 mg/kg, ip) or saline for 14 days followed by 2 weeks of withdrawal. Contrary to our prediction, microdialysis samples from freely-moving rats revealed that restraint stress-induced corticosterone levels in the ventral hippocampus are enhanced by amphetamine withdrawal relative to controls. In separate groups of rats, plasma corticosterone levels increased immediately after 20 min of restraint and decreased to below stress-naïve levels after 1 h, indicating negative feedback regulation of corticosterone following stress. However, plasma corticosterone responses were similar in amphetamine-withdrawn and control rats. Neither amphetamine nor stress exposure significantly altered protein expression or enzyme activity of the steroidogenic enzymes 11β-hydroxysteroid dehydrogenase (11β-HSD1) or hexose-6-phosphate dehydrogenase (H6PD) in the ventral hippocampus. Our findings demonstrate for the first time that amphetamine withdrawal potentiates stress-induced corticosterone in the ventral hippocampus, which may contribute to increased behavioral stress sensitivity previously observed during amphetamine withdrawal. However, this is not mediated by either changes in plasma corticosterone or hippocampal steroidogenic enzymes. Establishing enhanced ventral hippocampal corticosterone as a direct cause of greater stress sensitivity may identify the glucocorticoid system as a novel target for treating behavioral symptoms of amphetamine withdrawal.

1. Introduction

Amphetamine dependence is a global health problem with a high incidence of relapse and few successful treatment options (Fone and Nutt, 2005; Heal et al., 2013; Pomerleau et al., 2012; Wilens et al., 2008). Amphetamine withdrawal is associated with anxiety and hypersensitivity to stressors in humans (Cleck and Blendy, 2008; Shoptaw et al., 2009) and rodents (Barr et al., 2010; Li et al., 2014; Russig et al., 2006; Tu et al., 2014; Vuong et al., 2010) that can induce relapse in humans (Gossop, 2009) and maintains the cycle of addiction (Koob et al., 2014; Shoptaw et al., 2009).

Stress induces serotonin release in the ventral hippocampus, which has been implicated in reducing anxiety and stress responsiveness (Graeff et al., 1996; Herman et al., 2003; Li et al., 2014; Tu et al., 2014). We have previously found that rats in the second week of withdrawal from repeated amphetamine exposure show heightened behavioral anxiety (Barr et al., 2010; Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010), enhanced behavioral measures of stress-induced arousal (Li et al., 2014), and severely blunted stress-induced serotonin release in the ventral hippocampus (Li et al., 2014), which is known to cause increased behavioral anxiety (Tu et al., 2014). Therefore, it is important to understand the mechanism by which amphetamine withdrawal alters stress-induced serotonin levels in the ventral hippocampus.

Abbreviations: 11-DHC, 11-dehydrocorticosterone; 11β-HSD1, 11β-hydroxysteroid dehydrogenase; 4, -(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); CBG, Corticosteroid binding globulin; H6PD, Hexose-6-phosphate dehydrogenase; SDR, Steroid displacement reagent; SNK, Student-Newman-Keuls

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One mechanism by which amphetamine withdrawal could alter stress-related serotonin function in the ventral hippocampus is via glucocorticoid actions in this region. Stress-induced serotonin release in the ventral hippocampus is mediated by corticosterone activation of local glucocorticoid receptors (Barr and Forster, 2011; Li et al., 2014). Amphetamine withdrawal causes a reduction in ventral hippocampus glucocorticoid receptor expression (Barr and Forster, 2011), which may partly explain the corresponding dampening of stress-evoked serotonin release observed in withdrawn rats (Li et al., 2014). However, glucocorticoid receptor expression is not totally abolished following amphetamine withdrawal, but only reduced by ~30% compared to controls, while mineralocorticoid receptor expression remains unaltered. This raises the possibility that other mechanisms regulating glucocorticoid availability during stress are affected by amphetamine withdrawal to account for blunted glucocorticoid receptor-dependent increases in serotonin release. For instance, stress-induced corticosterone levels in the dorsal hippocampus have been found to increase up to 200% from baseline in drug-naïve rats (Droste et al., 2008; Keeney et al., 2006). Therefore, it is conceivable that stress-induced levels of corticosterone in the ventral hippocampus are reduced or absent during amphetamine withdrawal, lowering corticosterone availability and contributing to the lack of glucocorticoid receptor-dependent stress-induced serotonin release in this region (Li et al., 2014) and to the resultant heightened anxiety states (Tu et al., 2014). This possibility was addressed by the current study, using the same amphetamine treatment and withdrawal regime previously shown to produce heightened behavioral anxiety (Barr et al., 2010; Reinbold et al., 2014; Tu et al., 2014; Vuong et al., 2010), enhanced stress-induced behavioral arousal (Li et al., 2014), and blunted stress-induced serotonin release in the ventral hippocampus (Li et al., 2014).

Corticosterone is secreted primarily from the adrenal cortex in the periphery and readily crosses the blood brain barrier to act on target tissues, including the hippocampus (Pura and Kreze, 2005; Robel and Baulieu, 1994). Amphetamine withdrawal does not alter basal plasma corticosterone levels relative to saline controls at either 24 h or 4 weeks of withdrawal (Barr et al., 2010), but it is unknown whether stress-induced plasma corticosterone responses are affected. Therefore, we also tested whether amphetamine withdrawal is associated with reduced stress-induced corticosterone levels in the plasma to explain any alterations in ventral hippocampus concentrations.

Glucocorticoid activation of target tissues can also be regulated at the cellular level by extra-adrenal synthesis (Harris et al., 2001) by enzymes such as 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which reduces inert 11-dehydrocorticosterone (11-DHC) to active corticosterone (Harris et al., 2001; Taves et al., 2011). Of the intracellular enzymes that regulate steroidogenesis locally in central tissues including the hippocampus (Harris et al., 2001; Seckl, 1997; Taves et al., 2011), 11β-HSD1 appears to play a major role in stress-induced alterations of hypothalamic-pituitary-adrenal output regulation (Atanasov et al., 2004; Edwards et al., 1988; Ergang et al., 2014; Harris et al., 2001; Muller et al., 2006; Odermatt and Kratschmar, 2012; Vodicka et al., 2014; Wyrwoll et al., 2011). For example, 11β-HSD1 mRNA expression was increased in the ventral CA1 hippocampus following a variable 3-day stress protocol (Ergang et al., 2014) and following a resident intruder paradigm of repeated social stress (Vodicka et al., 2014), suggesting that stress may exert its effects on ventral hippocampus corticosterone availability by altering local 11β-HSD1 activity. Another enzyme, hexose 6 phosphate dehydrogenase (H6PD), directly interacts with 11β-HSD1 in central tissue to stabilize 11β-HSD1 reductase activity (White et al., 2007), regulating 11β-HSD1-imposed glucocorticoid activation in peripheral and central tissues (Hewitt et al., 2005; White et al., 2007). Increased H6PD production contributes to 11β-HSD1 up-regulation of glucocorticoids in liver tissues (Wang et al., 2011) but the role of H6PD expression in central glucocorticoid activation is largely unknown (Wang et al., 2011). Therefore, we also tested the hypothesis that amphetamine exposure alters expression and/or activity of either 11β-HSD1 or H6PD in the ventral hippocampus to alter stress-induced levels of corticosterone during amphetamine withdrawal.

2. Results

2.1. Experiment 1 – amphetamine withdrawal enhances stress-induced corticosterone levels in the ventral hippocampus

2.1.1. Microdialysis probe placements and baseline corticosterone levels in the ventral hippocampus

Representative placements of probe membrane surfaces for the ventral hippocampus are drawn to scale and illustrated in Fig. 1A. Probe placements were similar between saline and amphetamine pretreated rats, and baseline levels of corticosterone also did not differ between saline (1.83 ± 0.07 ng/mL) and amphetamine (1.65 ± 0.22 ng/mL) pretreatment ($t_{(11)}=0.717$, $P=0.488$). Data from rats where the probe missed the ventral hippocampus were excluded from the subsequent analyses.

2.1.2. Stress-induced corticosterone in the ventral hippocampus

Amphetamine-pretreated rats undergoing withdrawal exhibited increased restraint-induced corticosterone in the ventral hippocampus (Fig. 1B) with a maximal post-stress average of 3.69 ± 0.74 ng/mL at 20 min post-stress. Two-way repeated measure ANOVA revealed significant effects of time ($F_{(9,\ 96)}=4.175$, $P<0.001$) and an interaction between treatment and time ($F_{(9,\ 96)}=2.005$, $P=0.047$) on corticosterone levels. There was no effect of stress over time in saline rats ($F_{(9,\ 43)}=0.878$, $P=0.552$) with maximal levels of corticosterone of 2.63 ± 0.39 ng/mL measured 20 min post-stress. However, an effect of stress over time was observed for amphetamine-pretreated rats ($F_{(9,\ 53)}=4.267$, $P<0.001$) that was apparent at 20 min post-stress as compared to pre-stress levels (Holm-Sidak $P=0.002$). Ventral hippocampus corticosterone was significantly higher in amphetamine pretreated rats as compared to saline controls immediately following restraint stress (SNK, $P=0.010$) and 20 min later (SNK, $P=0.006$) (Fig. 1B).

2.2. Experiment 2 – mechanisms mediating enhanced corticosterone in the ventral hippocampus during amphetamine withdrawal

2.2.1. Amphetamine withdrawal does not alter stress-induced corticosterone levels in the plasma

A significant effect of stress was observed on total ($F_{(2,\ 62)}=88.427$, $P<0.001$; Fig. 2A), free ($F_{(2,\ 61)}=189.847$, $P<0.001$; Fig. 2B), and bound ($F_{(2,\ 60)}=11.702$, $P<0.001$; Fig. 2C) plasma corticosterone levels. However, no significant effects of amphetamine or saline pretreatment were observed on any measure of plasma corticosterone (total: $F_{(1,\ 62)}=0.030$, $P=0.862$; free: $F_{(1,\ 61)}=0.322$, $P=0.572$; bound: $F_{(1,\ 60)}=0.376$, $P=0.542$). There was no significant interaction present between pretreatment and stress in any of the three measures (total: $F_{(2,\ 62)}=0.218$, $P=0.805$; free: $F_{(2,\ 61)}=0.028$, $P=0.972$; bound: $F_{(2,\ 60)}=0.574$, $P=0.566$) (Fig. 2).

Post-hoc analysis demonstrated that 20 min of restraint stress resulted in higher total (SNK, $P<0.001$; Fig. 2A), free (SNK, $P<0.001$; Fig. 2B), and bound (SNK, $P=0.014$; Fig. 2C) plasma corticosterone levels immediately following restraint relative to stress-naïve levels, and relative to all measures of plasma corticosterone 1 h following restraint (total: SNK, $P<0.001$; free: SNK, $P<0.001$; bound: SNK, $P<0.001$; Fig. 2). All measures of plasma corticosterone levels 1 h following restraint were also decreased