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SHORT COMMUNICATION

Serotonin 2 receptor modulation of hyperthermia, corticosterone, and hippocampal serotonin depletions following serial exposure to chronic stress and methamphetamine

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Summary Chronic stress precipitates drug seeking behavior and alters the effects of drugs of abuse. Although it is known that chronic stress potentiates acute neurochemical and hyperthermic responses to the drug of abuse methamphetamine, no studies have investigated if and how chronic stress alters other physiological responses to methamphetamine. Therefore the objective of these studies was to determine if 10 days of chronic unpredictable stress modulates corticosterone (CORT) responses to methamphetamine and furthermore how chronic stress may modulate methamphetamine-induced increases in hyperthermia and CORT. As chronic stress potentiates hyperthermic responses to serotonin 2 (5-HT2) stimulation and 5-HT2 receptors are important in mediating both hyperthermic and CORT responses, we also investigated if 5-HT2 antagonism would block hyperthermia and CORT secretion by the serial exposure to stress and methamphetamine (stress/methamphetamine). The results of these studies illustrate that stress potentiates methamphetamine-induced increases in body temperature and CORT secretion and that these increases are blocked by the 5-HT2 antagonist ketanserin. Furthermore, the combination of stress and methamphetamine depletes 5-HT content in the hippocampus 7 days after methamphetamine administration which is blocked by the 5-HT2 antagonist ketanserin. Overall, these results indicate a pharmacological mechanism for the depletion of hippocampal 5-HT by the serial exposure to stress and methamphetamine and further illustrate the deleterious interactions between chronic stress and methamphetamine use.

1. Introduction

Chronic stress potentiates behavioral, neurochemical, and physiological responses to drug challenges and novel stressors.

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Specifically, chronic stress potentiates hyperthermic responses to serotonin 2 (5-HT2) receptor agonism (Matuszewich and Yamamoto, 2003). Chronic stress also potentiates the acute hyperthermic and CORT responses to a novel stressor (Bhatnagar et al., 2006) as well as the acute neurochemical and hyperthermic responses to the drug of abuse, methamphetamine (Tata et al., 2007). The acute neurochemical responses to methamphetamine are characterized by the release of dopamine and 5-HT (Kuczenski et al., 1995) and acute increases in 5-HT and downstream activation of 5-HT2 receptors have been implicated in the acute hyperthermic and CORT responses to the amphetamine derivative 3,4-methylenedioxymethamphetamine (Nash et al., 1988). Although the acute hyperthermic responses to methamphetamine are known to be increased following chronic stress, it is unknown how chronic stress modulates the CORT response to methamphetamine and the putative role of the 5-HT2 receptor in mediating hyperthermic and CORT responses to methamphetamine in chronically stressed rats. We hypothesize that chronic stress will potentiate CORT responses to methamphetamine and that potentiated hyperthermic and CORT responses will be blocked by the 5-$HT2_{A/C}$ antagonist, ketanserin.

Since CORT and hyperthermia mediate the long-term toxicity of amphetamine derivatives to dopamine and 5-HT terminals in the brain (Johnson et al., 1989; Bowyer et al., 1994) and chronic stress potentiates toxicity to dopamine terminals in the striatum (Tata et al., 2007), we also wanted to investigate the effect of chronic stress and methamphetamine (stress/methamphetamine) on 5-HT content in the hippocampus given the link between 5-HT abnormalities and behavioral deficits in human methamphetamine abusers and the importance of the hippocampus in learning and memory (Thompson et al., 2004; Sekine et al., 2006). To this end, we hypothesized that the serial exposure to chronic stress and methamphetamine would deplete 5-HT in the hippocampus which would be blocked by the 5-$HT2_{A/C}$ antagonist, ketanserin.

2. Methods and materials

2.1. Chronic unpredictable stress

Male Sprague–Dawley rats (175–200 g) were purchased from Harlan Sprague–Dawley and housed 2–3 per cage in a temperature (21–23 °C) and humidity controlled room with a 12-h light/dark cycle (lights on at 07:00 h off at 19:00 h). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available.

Rats were either handled daily or exposed to 10 days of chronic unpredictable stress: Day 1 10:00 h 50 min cold room (4 °C), 13:00 h 60 min cage agitation; Day 2 10:00 h 60 min restraint stress, 18:00 h lights on overnight (12 h); Day 3 10:00 h 3 h lights off, 15:00 h 50 min cold room (4 °C); Day 4 11:00 50 min cage agitation, 18:00 h food and water deprivation overnight (12 h); Day 5 09:00 h 60 min restraint stress, 18:00 h lights on overnight; Day 6 15:00 h 15 min cold room individual housing, 16:00 h individual housing overnight; Day 7 10:00 h 60 min restraint stress, 18:00 h food and water deprivation overnight; Day 8 11:00 h 30 min cage agitation, 15:00 h individual housing overnight; Day 9 09:00 h 15 min cold room, 18:00 h lights on overnight; Day 10 10:00 h 3 h lights off, 13:00 h 20 min cage agitation.

2.2. Drug administration

Methamphetamine hydrochloride (M8750) and ketanserin tartrate salt (S006) were purchased from Sigma–Aldrich and administered on Day 11, the day following the last stressor. In studies measuring plasma CORT levels, a single injection of methamphetmine (7.5 mg/kg) or saline (1 mL/kg) was administered at 07:00 h and rats killed 1 h later. For temperature and tissue content studies, rats received four injections of saline (1 mL/kg) or methamphetamine (7.5 mg/kg) every 2 h. In the ketanserin studies, rats were administered the 5-HT2 antagonist ketanserin (0.5, 1, and 3 mg/kg) 30 min prior to each saline (1 mL/kg) or methamphetamine (7.5 mg/kg) injection.

2.3. Plasma corticosterone

Rats were killed via rapid decapitation and trunk blood collected 1 h after methamphetamine or saline administration. Samples were centrifuged at 800 × *g* for 15 min at 4 °C, plasma removed and re-centrifuged at 800 × *g* for 7 min. The supernatant was analyzed for CORT using an EIA kit (Immunodiagnostic Systems).

2.4. Temperature measurements

Rectal temperatures were measured 1 h after each methamphetamine or saline injection via a Thermalert rectal probe thermometer. Baseline temperatures were taken prior to all injections.

2.5. 5-HT tissue content

Rats were killed 7 days after methamphetamine or saline injections via rapid decapitation and whole hippocampi dissected as previously described (Tata et al., 2007). This time point was chosen based on the known time-course of high-dose methamphetamine (10 mg/kg q 2 h × 4) to produce decreases in neurotransmitter content (Yamamoto and Bankson, 2005). Tissue was sonicated in 1200 μL 0.25 M perchloric acid and centrifuged at 14,000 × *g* for 20 min at 4 °C. The supernatant was analyzed for 5-HT using high-performance liquid chromatography with electrochemical detection as previously described (Breier et al., 2006). 5-HT was separated on a C18 column (100 × 2.0 mm, 3 μm particle size, Phenomenex, Torrance) and eluted with a mobile phase containing 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sodium sulfate, and 3% methanol (pH 3.8). Compounds were detected with a LC-4C amperometric detector (BAS Bioanalytical) and data recorded using EZ Chrom Software (Scientific Software). The pellet was resuspended in 1200 μL 1 M NaOH and protein determined using the method of Bradford.

2.6. Statistical analysis

CORT and 5-HT data were analyzed using a one-way ANOVA followed by Tukey's *post hoc* test. Temperature data were