Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 415 (2007) 269–273
Neuroscience Letters
www.elsevier.com/locate/neulet

Restraint-induced corticosterone secretion and hypothalamic CRH mRNA expression are augmented during acute withdrawal from chronic cocaine administration

John R. Mantsch *, Sarah Taves, Tayyiba Khan, Eric S. Katz, Tanveer Sajan, Lee C. Tang, William E. Cullinan, Dana R. Ziegler

Department of Biomedical Sciences, Marquette University, Milwaukee, WI 53201, USA

Received 2 November 2006; received in revised form 5 January 2007; accepted 17 January 2007

Abstract

Stress responses during cocaine withdrawal likely contribute to drug relapse and may be intensified as a consequence of prior cocaine use. The present study examined changes in stressor-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis during acute withdrawal from chronic cocaine administration. Adult male Sprague–Dawley rats received daily administration of cocaine (30 mg/kg, i.p.) or saline for 14 days. Twenty-four hours after the last injection, rats in each group were sacrificed under stress-free conditions or following 30 min of immobilization. Plasma corticosterone (CORT) was measured in trunk-blood using radioimmunoassay, corticotropin-releasing hormone (CRH) mRNA levels in the paraventricular nucleus (PVN) of the hypothalamus were measured using in situ hybridization and glucocorticoid receptor (GR) protein expression in the pituitary gland and dissected brain regions was measured using Western blot analysis. Basal CRH mRNA in the PVN was unaltered as a result of prior cocaine administration. However, a significant increase in CRH mRNA was observed 90 min following the termination of restraint in cocaine withdrawn, but not saline-treated, rats. Basal CORT was also unaffected by prior cocaine administration, but the CORT response measured immediately after restraint was significantly augmented in cocaine-withdrawn rats. Differences in GR protein expression in number of regions implicated in negative feedback regulation of HPA function, including the hypothalamus, were not observed. These findings indicate that the HPA response to stressors is intensified during early withdrawal from cocaine administration and may be independent of changes in GR-mediated negative feedback.

Keywords: Cocaine; Stress; Glucocorticoid receptor; CRF; HPA axis; Corticosterone

A growing body of evidence suggests that stress plays an important role in cocaine addiction [11,28]. In addition to findings that stress promotes cocaine-seeking behavior, it has been reported that stress-related behavioral responses emerge and/or are exaggerated as a consequence of prior cocaine exposure, suggesting that the relationship between stress and cocaine abuse represents a self-perpetuating cycle within which stress serves as both a precipitating factor for and a consequence of drug use.

The hypothalamic–pituitary–adrenal (HPA) axis is a critical mediator of physiological responses that enable organisms to adapt during times of stress. Such responses likely include changes in behavior that involve the same neurocircuitry responsible for illicit drug use and addiction. Accordingly, it has been reported that although stressor-induced glucocorticoid secretion is not necessary for acute stressor-induced cocaine-seeking behavior [9], glucocorticoids play an important role in the effects of repeated stress on the addiction process as substrates through which chronic stressors increase cocaine self-administration [20] and facilitators of addiction-related neuroplasticity [18].

Like stressors, cocaine stimulates the HPA axis through a mechanism dependent on the release of the peptide corticotropin-releasing hormone (CRH) from the terminals of parvocellular neurons originating in the paraventricular nucleus (PVN) of the hypothalamus [23,24]. Repeated cocaine administration has been reported to produce long-term alterations in basal HPA function [19,26,30,32] that can also be observed in human addicts as a dysregulation of circadian HPA activity [7]. Less is known about how stressor-induced HPA activation is

* Corresponding author at: Department of Biomedical Sciences, Marquette University, Schroeder Health Complex, P.O. Box 1881, Milwaukee, WI 53201-1881, USA. Tel.: +1 414 288 2036; fax: +1 414 288 6564.
E-mail address: john.mantsch@marquette.edu (J.R. Mantsch).

0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.01.036

altered as a consequence of prior cocaine use. Although there are reports that restraint-induced corticosterone (CORT) secretion is unchanged following repeated psychomotor stimulant drug administration [17], others have found that HPA responses to stressors [2] and cocaine [27] are augmented, while still others have reported that individual differences in stressor-induced CORT are eliminated following chronic cocaine administration, with the CORT response increasing in rats that were initially low CORT responders and decreasing in high responder rats [26].

Clinical studies have found that the HPA response to stressors is augmented in recovering cocaine abusers with a history of high frequency drug use [10] and that stressor-induced cortisol and ACTH responses predict propensity towards drug relapse [29]. Thus, intensification of stressor-induced HPA function following chronic cocaine administration could contribute to the addiction process by promoting further drug use.

The goal of this study was to examine the activity of the HPA axis under basal conditions and in response to a stressor, restraint, after 24 h of withdrawal from 14 days of cocaine administration (30 mg/kg, i.p. daily). HPA function was assessed through determination of plasma CORT concentrations and CRH mRNA levels in the PVN measured using in situ hybridization. Additionally, since glucocorticoid receptors (GR) are known to play an inhibitor feedback role in HPA function [8], we also examined GR expression in pituitary and a number of brain regions using Western blot analysis.

Forty-eight adult male Sprague–Dawley rats (Harlan Laboratories, St. Louis, MO), approximately 90 days old were housed in pairs in a temperature- and humidity-controlled, AAALAC-accredited facility under a 12 h light/12 h dark cycle (lights on at 7:00 a.m.) and had access to food and water at all times. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Publication No. 80-23; rev. 1996).

After a 1 week habituation period during which they received daily saline injections, rats were assigned to cocaine or saline treatment groups according to the i.p. injection regimen that they received for the next 14 days. Cocaine rats received daily i.p. injections of cocaine (30 mg/kg; NIDA Drug Supply Program) while saline rats received injections of 0.9% NaCl solution. Rats received injections at 10:00 a.m.

All rats were sacrificed 24 h after the final injection (at 10:00 a.m.) by decapitation under one of the three conditions: (1) immediately after removal from the homecage for baseline CORT and CRH mRNA measurement; (2) immediately following 30 min of restraint for measurement of stressor-induced increases in plasma CORT and regional glucocorticoid receptor protein expression; (3) 90 min after the termination of restraint for measurement of stressor-induced CRH mRNA expression and recovery of CORT levels following stress. During the 90-min period prior to sacrifice, rats in this group were returned to their home cages. Restraint stress consisted of a 30-min period during which rats were placed into acrylic restraining devices (21.6 cm length × 6.4 cm diameter). Following decapitation, trunk-blood was collected for plasma CORT measurement and brain was rapidly excised and frozen in a −30 °C isopentane solution. Pituitary gland was also removed and frozen on dry ice. Brain and pituitary were transferred to a −80 °C freezer for storage.

Plasma CORT was measured using a commercial radioimmunoassay kit (MP Biochemicals, Irvine, CA). Blood for CORT determination was collected into tubes containing heparin, stored on ice, and centrifuged to allow separation of plasma, which was frozen at −80 °C.

Analysis of hybridization signal involved sampling across the rostral-caudal extent of the medial parvocellular PVN and measurements for individual rats were averaged to obtain one mean value. Data from the rostral-most level of the PVN was excluded due to an inadequate number of sections. ^{35}S-labeled anti-sense probes for CRH were generated from linearized cDNA encoding a 760 bp segment spanning from exon 2 to the 3′ untranslated region. The specificity of this probe has been previously characterized [12]. Linearized DNA template was transcribed to anti-sense riboprobe with T7 polymerase, followed by isolation of probe by high salt/ethanol precipitation. Slides with 16 μm cryosectioned coronal brain slices containing the PVN [21] were subjected to standard pre-hybridization treatment (post-fixation in 4% paraformaldehyde; glycine treatment; acetylation; dehydration/delipidation in ethanols/chloroform; air-drying). Probe was diluted in commercial hybridization buffer (Amresco Solon, OH) with 100 mM DTT. Each slide was hybridized with 50 μl of medium containing 10^6 cpm probe/50 μl), coverslipped, and incubated overnight at 55 °C in chambers humidified with 50% formamide. Following incubation, slides were post-treated (rinses in 2× SSC; 200 μg/ml RNAse digestion of unbound probe; SSC rinses; 55 °C hot bath in 0.2× SSC; dehydration in ethanols; air drying) and exposed to Kodak BioMAX MR autoradiographic film for 1 week. Densitometric analysis was performed using Scion Image 1.59 software. Data are presented as corrected gray level (gray level of sampled area minus gray level of non-specifically labeled background region (white matter) in the same section).

Frozen brains were thawed on ice to permit dissection of medial prefrontal cortex, dorsomedial hypothalamus (including PVN), amygdala, dorsal hippocampus, and ventral subiculum. Brains were placed into a stainless steel brain block cooled to −20 °C and slices (1–2 mm) were cut using a straight edge razor blade. Coronal slices from which tissues were dissected were defined according to coordinates from Paxinos and Watson [21]. Samples were homogenized by sonication on ice in a Tris buffer (50 mM Tris, pH 7.2) containing 10% (w/v) sucrose, 6 mM $MgCl_2$ and protease inhibitors (1 mM EDTA, 1 mM PMSF, 3 mM benzamidine, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 μg/ml aprotinin, 5 μg/ml bestatin, 2 μg/ml E64). GR protein was measured in samples of whole cell tissue homogenate using polyacrylamide gel electrophoresis [15] followed by Western blot analysis with the polyclonal antibody, GR M-20 (Santa Cruz Biotechnology, Santa Cruz, CA). Following protein determination using the bicinchoninic acid assay, samples were diluted in buffer (pH 6.8; final concentration 0.25 M Tris–HCl, 4 M urea, 10% glycerol, 1% SDS, 5% beta-mercaptoethanol) and loaded onto a 7.5% polyacrylamide gel (30 μg protein/lane). Gel electrophoresis was performed using a Bio-Rad Mini-protean three system at 200 V for 20 min followed by 35–40 min at