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Hypocretin Neurotransmission Within the Central Amygdala Mediates Escalated Cocaine Self-administration and Stress-Induced Reinstatement in Rats

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ABSTRACT

BACKGROUND: Cocaine addiction is characterized by patterns of compulsive drug-taking, including preoccupation with obtaining cocaine and loss of control over drug intake. The lateral hypothalamic hypocretin/orexin (HCRT) system has been implicated in drug-taking and the reinstatement of drug-seeking. Evidence suggests that HCRT may drive drug-seeking through activation of specific brain regions implicated in stress system dysfunction, including the central amygdala (CeA). The role of HCRT in the persistence of compulsive-like cocaine-taking has yet to be fully elucidated.

METHODS: Systemic and intra-CeA microinfusions of the HCRT-receptor 1 antagonist, SB-334867, were administered to rats allowed either short (1 hour; ShA) or long (6 hours; LgA) access to cocaine self-administration. Animals were tested for fixed and progressive ratio responding for cocaine and stress-induced reinstatement of drug-seeking. In addition, using electrophysiological techniques on in vitro slices, we investigated gamma-aminobutyric acidergic (GABAergic) neurotransmission in the medial CeA and the sensitivity of GABAergic synapses to modulation of the HCRT system in ShA or LgA rats.

RESULTS: We found systemic administration of SB-334867 (0, 7.5, 15, 30 mg/kg) dose dependently decreased cocaine intake specifically in LgA rats but not in ShA rats. Microinjections of SB-334867 (20 nmol) bilaterally into the CeA significantly reduced cocaine intake in LgA rats. We also observed a significant attenuation of yohimbine-induced reinstatement of cocaine-seeking after intra-CeA SB-334867 (10 nmol) administration. Finally, electrophysiological data indicated enhanced GABAergic neurotransmission within the medial CeA in LgA rats, which was blocked with SB-334867 (10 μmol/L).

CONCLUSIONS: These findings suggest that HCRT neurotransmission within the CeA is implicated in compulsive-like cocaine-seeking.

Keywords: Central amygdala, Cocaine, Drug dependence, Hypocretin/orexin, Intravenous self-administration, Reinstatement

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Psychostimulants, including cocaine, are widely abused drugs that exert robust reinforcing and arousal-enhancing effects, contributing to their use and abuse. Cocaine addiction is a disorder in which both humans and animals ultimately transition from nondependent, episodic drug use to compulsive drug-taking. Compulsive-like behavior in humans (1) and rodents (2,3) is characterized by excessive drug-seeking/taking patterns that are difficult to terminate once responding has been initiated. Compulsive-like behavior in rats is operationally defined as the emergence of escalated drug intake, increased motivation to obtain the drug (i.e., elevated progressive ratio [PR] breakpoints), responding despite punishment, reward system deficits during abstinence, and increased likelihood of relapse (2–6). Animal models of extended access (≥6 hours) to cocaine self-administration mimic compulsive-like behavioral patterns, maintaining face and construct validity for the transition to drug dependence (7,8). The escalated cocaine intake and increased PR breakpoints that are observed in long access (LgA; 6 hours) animals is in contrast to lower, stable intake observed in animals allowed short access (ShA; 1 hour) to cocaine (7,9). It is hypothesized that escalated cocaine-taking is partially mediated by dysregulation of brain motivational and stress systems, particularly within extended amygdala subregions, including the central amygdala (CeA), the bed nucleus of the stria terminalis, and the medial and caudal nucleus accumbens (7). Escalated cocaine intake is also associated with a significant blunting of the dopamine response to cocaine and an increase in intracranial self-stimulation reward thresholds that imply reduced rewarding effects of drugs over time (4,10). Thus,

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under conditions of extended cocaine access, it is posited that brain stress systems are recruited and sensitized with concomitant reward system deficits and that the drug is taken to alleviate negative emotional states associated with these dynamic allostatic changes (11).

Over the past decade, the lateral hypothalamus, largely via the hypocretin/orexin (HCRT) neuropeptide system, has been implicated in both stress and reward-seeking across many drug classes, including cocaine (12,13). Despite limited cell numbers in the lateral hypothalamus, HCRT neurons project widely throughout the brain, targeting two G-protein-coupled receptors referred to as HCRT-receptor 1 and 2 (HCRT-R1 and -R2, respectively). These receptors have varying affinities for HCRT peptide ligands such that HCRT-1 peptide binds to both receptors, whereas HCRT-2 peptide binds selectively to HCRT-R2. HCRT neuronal projections include reciprocal connections to the extended amygdala, including the CeA, and other basal forebrain regions (14–16) implicated in the stress surfeit component of addiction. To date, HCRT-R1 signaling has been shown to mediate reinforcement of all major drug classes, including psychostimulants, nicotine, alcohol, and opioids. However, HCRT-R1 antagonism has yielded no or limited effects on low-effort, fixed ratio (FR1) responding for cocaine self-administration (17–19), particularly in animals allowed ShA to the drug. Nonetheless, HCRT-R1 antagonism readily blocks stress-induced reinstatement of cocaine-seeking, whereas central HCRT-1 administration reinstates previously extinguished drug-seeking (20–22). The participation of HCRT in stress-related drug-seeking is not surprising, given that HCRT neurotransmission has been implicated in stress and high-arousal conditions in both humans and animals (12,23,24). These observations suggest that HCRT not only plays a significant role in maintaining the hyperaroused state needed for cocaine-seeking, but also contributes to highly motivated compulsive-like cocaine-seeking. Consistent with this hypothesis, HCRT-R1 antagonists attenuate effortful, compulsive-like responding under a PR schedule (18,25) and at higher FR schedules [e.g., FR5 (16,26)]. The present experiments sought to test the hypothesis that HCRT modulates compulsive-like cocaine-taking associated with LgA conditions. We hypothesized that HCRT stress systems are recruited during compulsive-like drug-taking and that blocking HCRT neurotransmission within the CeA would reduce compulsive-like cocaine-taking and attenuate stress-induced reinstatement of cocaine-seeking in LgA rats.

Previous studies demonstrate that extended access to cocaine self-administration alters plasticity at gamma-aminobutyric acidergic (GABAergic) synapses within the medial CeA (27). We further hypothesized that dysregulated HCRT activity in the CeA contributes to the development or maintenance of negative emotional states associated with cocaine withdrawal via enhancement of GABAergic transmission. There is little evidence regarding the interaction between HCRT and GABAergic neurotransmission in the CeA of animals allowed extended cocaine access. Thus, we used in vitro electrophysiological techniques to measure spontaneous GABAergic transmission and the effects of HCRT stimulation and antagonism in CeA neurons from ShA and LgA rats compared with cocaine-naïve control rats. We hypothesized that LgA rats would exhibit increased spontaneous GABAergic transmission in the CeA and that this increased GABAergic transmission would be blocked by HCRT-receptor antagonism.

Combined, the results from our behavioral and electrophysiological studies demonstrate that HCRT within the CeA is dysregulated after LgA to cocaine and support a role for HCRT in the neuroplasticity associated with cocaine addiction.

METHODS AND MATERIALS

Animals

Adult male Wistar rats ($N = 56$; Charles River, Raleigh, NC), weighing 225–275 g at the beginning of the experiments, were group-housed in a temperature-controlled (22°C) vivarium on a 12-hour light/dark cycle (lights off at 8:00 PM) with ad libitum access to food and water. Behavioral testing occurred once per day during the dark/active cycle. Animals were acclimated to the facility for at least 7 days before surgery. All procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute or the National Institute on Drug Abuse Intramural Research Program.

Surgery

Rats were anesthetized with isoflurane (1.5%–2.5%) and prepared with chronic indwelling jugular vein catheters as previously described (5). Catheters were flushed daily with heparinized sterile saline (0.2 mL; 30 USP units/mL). After self-administration training, a subset of rats underwent stereotaxic surgery under isoflurane anesthesia and were implanted bilaterally with 23-gauge cannulae (Plastics One, Roanoke, VA) aimed at the CeA (flathead; posterior, −2.4 mm; mediolateral, ±4.2 mm; ventral, −6.0 mm relevant to bregma). Cannulae were cemented into position using acrylic resin (Lang Dental Manufacturing Co., Wheeling, IL). A stainless steel stylet with a threaded plastic connector (Plastics One) was inserted into each cannula. Rats recovered for 5–7 days before behavioral testing.

Self-administration

Intravenous self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT) as previously described (28,29). After the acquisition of cocaine self-administration, rats were given either ShA or LgA of daily access to FR1 cocaine self-administration for 14 escalation sessions (Supplemental Figure S1). After escalation, testing occurred under either FR1 or PR schedules of reinforcement (Supplemental Methods).

Electrophysiological Recordings

Coronal slices (300 μm) were prepared as previously described (30) in an ice-cold high-sucrose solution using a vibrating microtome. Whole-cell voltage-clamp recordings were made with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 2–5 kHz, digitized (Digidata 1440A; Molecular Devices), and stored using pClamp 10 software (Molecular Devices). The intracellular solution used