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PERSISTENT INCREASES IN RAT HYPOTHALAMIC POMC GENE EXPRESSION FOLLOWING CHRONIC WITHDRAWAL FROM CHRONIC ''BINGE'' PATTERN ESCALATING-DOSE, BUT NOT STEADY-DOSE, COCAINE

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Abstract—Recent research suggests an involvement of pro-opiomelanocortin (POMC) gene products (e.g., beta-endorphin) in modulating cocaine-induced reward and addiction-like behaviors in rodents. In this study, we investigated whether chronic ''binge'' cocaine and its withdrawal altered POMC gene expression in the brain of rats. Male Fischer rats were treated with two different chronic (14-day) ''binge'' pattern cocaine administration regimens (three injections at 1-h intervals, i.p.): steady-dose (45 mg/kg/day) and escalating-dose (90 mg/kg on the last day). Although there was no POMC mRNA alteration after chronic steady-dose cocaine, a significant decrease in POMC mRNA levels in the hypothalamus was found after chronic escalating-dose cocaine. In contrast, after acute (1-day) withdrawal from chronic ''binge'' escalating-dose regimen, but not steady-dose regimen, there were increased hypothalamic POMC mRNA levels that persisted into 14 days of protracted withdrawal. To study the role of the endogenous opioid systems in the cocaine withdrawal effects, we administered a single naloxone injection (1 mg/kg) that caused elevated POMC mRNA levels observed 24 h later in cocaine naïve rats, but it did not lead to further increases in cocaine-withdrawn rats. Our results suggest that during withdrawal from chronic ''binge'' escalating-dose cocaine: (1) there was a persistent increase in hypothalamic POMC gene expression; and (2) hyposensitivity of the POMC gene expression to naloxone indicates altered opioidergic tone at or above the hypothalamic level.

Key words: chronic ''binge'' cocaine, cocaine withdrawal, steady-dose, escalating-dose, POMC, hypothalamus.

INTRODUCTION

Opioid receptor antagonists (especially the mu opioid receptor selective antagonists) have been found to reduce both the cocaine reinforcement using the self-administration model (Carroll et al., 1986; Ramsey and van Ree, 1991) and the rewarding action of cocaine using the conditioned place preference (CPP) model in rodents (Suzuki et al., 1992; Gerrits et al., 1995; Houdi et al., 1998). These early findings raise the possibility that cocaine may trigger the release of endogenous opioid peptides (e.g., beta-endorphin) and further suggest that these opioid neuropeptides play a functional role in the cocaine-induced behavior. Of interest, a recent study has found that the cocaine-induced CPP is reduced in beta-endorphin-deficient mice, indicating a reduced rewarding effect of cocaine with less endogenous beta-endorphin (Marquez et al., 2008). Together, these studies suggest a modulatory role for the endogenous opioid peptide beta-endorphin in cocaine reward or reinforcement.

Pro-opiomelanocortin (POMC), a large peptide precursor, produces several biologically active neuropeptides, including beta-endorphin, adrenocorticotropic hormone (ACTH) and melanocortins. The presence of POMC neurons or cells was originally found to be mainly restricted to the rodent arcuate nucleus in the hypothalamus, nucleus of the solitary tract and pituitary (Mansour et al., 1995; Mercer et al., 2013). The opioid peptide beta-endorphin is distributed in the hypothalamus, and the dopaminergic mesocorticolimbic regions, probably from hypothalamic POMC neuronal projections, although it is still a topic of debate. Since activation of the mu opioid receptor by beta-endorphin is rewarding and modulates dopamine release in the nucleus accumbens (Spanagel et al., 1991), beta-endorphin may be involved in the motivational behavior and reinforcing effect of several drugs of abuse (Koob and Kreek, 2007; Roth-Deri et al., 2008). For instance, central administration of beta-endorphin via intra-cerebro-ventricular injection has been found to induce CPP in rats (Amalric et al., 1987). In line with above findings, we have recently found that POMC gene expression in the hypothalamus is increased by cocaine in the setting of drug-induced place conditioning (Zhou et al., 2012).

Since the early 1990's, many laboratories (including our laboratory) have investigated the effect on opioid peptides and their receptors of drugs of abuse.

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Abbreviations: ACTH, adrenocorticotropic hormone; CPP, conditioned place preference; CRF, corticotrophin releasing factor; EDTA, ethylenediaminetetraacetic acid; POMC, pro-opiomelanocortin.

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Alterations of preproenkephalin, mu opioid receptor, preprodynorphin and kappa opioid receptor gene expression in mesolimbic brain areas of mice or rats after chronic cocaine exposure or across long-term withdrawal have been broadly studied (see reviews [Kreek et al., 2009; Le Merrer et al., 2009]). However, it is unclear if POMC gene expression in specific brain regions is altered by cocaine, particularly after chronic exposure and withdrawal.

To extend our research, we here report a set of experiments addressing the research question: are POMC mRNA levels in the hypothalamus or amygdala (where POMC expression is relatively abundant [Zhou et al., 2010]) altered after chronic cocaine administration or its withdrawal? As recent studies showed an involvement of different amygdalar neuropeptides (e.g., dynorphin, vasopressin and corticotrophin releasing factor (CRF)) after drug withdrawal (e.g., Zhou and Kreek, 2014), the amygdala was included for the POMC mRNA measurement in the present study. On the basis of evidence implicating POMC-derived beta-endorphin in the rewarding property of cocaine, we predicted that POMC gene expression would be altered in animals after chronic exposure or during withdrawal.

EXPERIMENTAL PROCEDURES

Experiment 1. Withdrawal from chronic (14-day) escalating-dose ''binge'' pattern cocaine administration and interactions with naloxone in rats

Animals. Male Fischer 344 rats (190–220 g; Charles River Labs, Kingston, NY, USA) were housed individually in a stress-minimized facility with free access to food and water. In order to minimize stress, noise and animal handling unrelated to the experimental protocol were kept to a bare minimum. Prior to the beginning of the experiment, animals were adapted to a standard 12-h light/dark cycle (lights on from 9:00 h to 21:00 h) for 7 days. The protocol was approved by the Rockefeller University Animal Care and Use Committee.

Before cocaine or saline administration, all rats were handled and received three daily intraperitoneal (i.p.) injections of saline (3 × 1 ml/kg/day) at 9:30, 10:30 and 11:30 h for 7 days in order to minimize injection-induced stress when the experiment began on day 8 (a method validated in earlier studies, see [e.g., Zhou et al., 2004]). Fischer rats were selected because this inbred strain self-administers cocaine at a high level after cocaine self-administration behavior is established, with high anxiety phenotype (Kosten and Ambrosio, 2002).

Procedure of escalating-dose ''binge'' pattern cocaine administration and withdrawal with naloxone pretreatment. The ''binge'' pattern regimen of drug administration (cocaine or saline) consisted of i.p. injections three times daily with two 1-h intervals, beginning 30 min after the light cycle (9:30, 10:30, and 11:30) in the home cage (Branch et al., 1992). This dosing schedule was selected to mimic the pattern often observed in human cocaine abusers with relation to the circadian rhythm of rest and activity during the day, and with respect to repeated administrations over several hours. For animals treated with cocaine, the drug doses were increased after every three days, with the same volume of saline (3 ml/kg/day). The volume of injection was kept constant through the entire experiment and only the concentration of cocaine was escalated. Therefore, the cocaine-treated rats received initial cocaine dosing at 45 (3 × 15) mg/kg/day on days 1–3, 60 (3 × 20) mg/kg/day on days 4–6, 75 (3 × 25) mg/kg/day on days 7–9, and then 90 (3 × 30) mg/kg/day on days 10–14. As previously reported, this dosing schedule models the cocaine dose range self-administered by rats given long access (6–10 h) to the drug (Koob and Kreek, 2007). For animals treated with saline, the volume was 3 ml/kg/day through all experimental days.

The experiment paradigm contained three phases: chronic 14-day escalating-dose ''binge'' pattern cocaine exposure, 1-day acute withdrawal and 14-day chronic withdrawal (Fig. 1A).

In Experiment 1.1 chronic (14 days) escalating-dose ''binge'' cocaine, rats received saline (n = 8) or escalating-dose ''binge'' cocaine (from 45 up to 90 mg/kg/day) (n = 8) injections for 14 days and were then sacrificed at 12:00 h on day 14, 30 min after the last saline or ''binge'' cocaine injection. During exposure to 90 mg/kg/day dose of cocaine, two animals died with seizures, resulting in an n = 6 for the cocaine-treated group.

In Experiment 1.2 acute (1 day) cocaine withdrawal with naloxone, rats received saline or ''binge'' cocaine (from 45 up to 90 mg/kg/day) injections for 14 days and

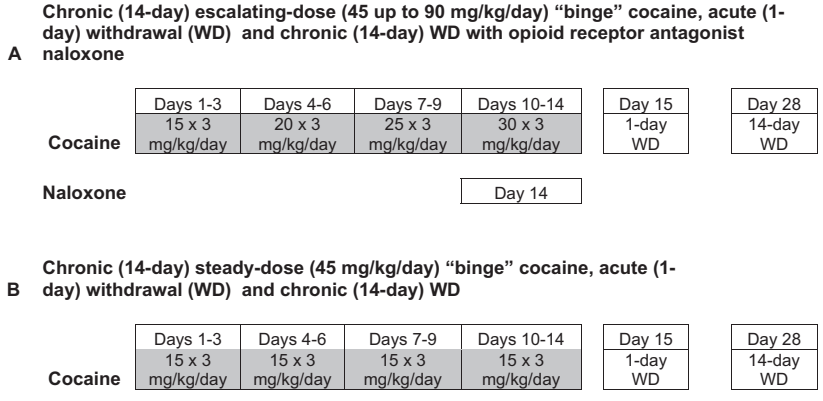

Fig. 1. Timelines for cocaine administration regimens with antagonist applications.