Acute and Chronic Corticotropin-Releasing Factor 1 Receptor Blockade Inhibits Cocaine-Induced Dopamine Release: Correlation with Dopamine Neuron Activity

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ABSTRACT
Corticotropin-releasing factor (CRF) is a neuropeptide associated with the integration of the physiological and behavioral responses to stress. Recently, CRF₁ receptor antagonists have been shown to decrease cocaine self-administration and inhibit stress-induced reinstatement of cocaine-seeking behavior. The exact mechanisms underlying this effect are not clear. Based on the large amount of literature demonstrating an association between dopaminergic neurotransmission and reward-related behavior, the aim of the present study was to examine the effects of acute versus chronic CRF₁ receptor blockade on mesencephalic dopamine (DA) neuron activity (determined by in vivo extracellular recordings) and extracellular DA levels in the nucleus accumbens (Acb) (using in vivo microdialysis). In addition, the effect of CRF₁ receptor antagonism on cocaine-induced DA overflow in the Acb was examined and correlated with DA neuron activity in the ventral tegmental area (VTA).

Acute (but not chronic) CRF₁ receptor blockade by CRA-0450 [1-[8-(2,4-dichlorophenyl)-2-methylquinolin-4-yl]-1,2,3,6-tetrahydropyridine-4-carboxamide benzenesulfonate] was found to significantly increase DA neuron population activity without affecting burst firing, average firing rate, or Acb DA levels. In addition, both acute and chronic CRF₁ receptor antagonism significantly reduced cocaine-stimulated DA overflow in the Acb, and this reduction was correlated with an attenuated cocaine-induced inhibition of DA population activity. Taken as a whole, these data demonstrate that, although DA neuron population activity exhibits tolerance to chronic CRF₁ receptor antagonism (by CRA-0450), tolerance does not develop to the selective inhibition of cocaine-induced DA release (in the Acb) and, as such, may be beneficial in the treatment of cocaine addiction.

Corticotropin-releasing factor (CRF) is a peptide neurotransmitter found in high abundance throughout the neuraxis, where its actions include control of the secretion of adrenocorticotropic hormone (ACTH) from the pituitary in response to stressful stimuli (Chalmers et al., 1996; Carrasco and Van de Kar, 2003; Rivier et al., 2003). Recent research into the neurobiological actions of CRF has implicated this peptide in a variety of biological actions including anxiety (Takahashi, 2001), depression (Reul and Holsboer, 2002), cardiovascular control (Briscoe et al., 2000), appetite regulation (Hope et al., 2000), and central reward processing (Sarnyai et al., 2001). The CRF system has also been shown to play a role in the actions of drugs of abuse, in particular cocaine (Goeders and Guerin, 2000; Lu et al., 2001; Sarnyai et al., 2001). This was originally suggested following the demonstration that acute cocaine administration induces elevated plasma ACTH and cortisol levels via an action dependent on central CRF receptor activation (Rivier and Lee, 1994), whereas other reports have shown that cocaine administration significantly alters extrahypothalamic markers of central CRF neurotransmission (Sarnyai et al., 1993; Zhou et al., 1996; Gardi et al., 1997). These findings have led to the suggestion that the central CRF system may play a role in cocaine self-administration (Sarnyai et al., 2001); this is further supported by a recent study reporting that CRF₁ receptor blockade (by CP-154,526) can decrease cocaine self-administration without affecting responding for food (Goeders and Guerin, 2000). In addition to decreasing cocaine self-administration, CRF receptor antagonists reduce stress-induced reinstatement of drug-seeking behavior and attention...
ulate cocaine withdrawal-induced anxiety (Erb et al., 1998; Shaham et al., 1998; Basso et al., 1999; Erb and Stewart, 1999).

Although there is a vast literature examining the central processes underlying the actions of CRF in altered behavioral states such as anxiety and stress (for review, see Bale and Vale, 2004), there have been fewer reports on how CRF affects central reward processing. Nonetheless, CRF and CRF receptors are distributed throughout brain regions associated with the control of emotive processing and hormone regulation, as well as throughout regions centrally involved in the actions of abused drugs such as the ventral mesencephalon, amygdala, bed nucleus of the stria terminalis, and prefrontal cortex (Behan et al., 1996). Moreover, immunohistochemical investigations have demonstrated that CRF1 receptors are localized on a proportion of neurons throughout the ventral tegmental area (VTA) that may synthesize DA and project to forebrain regions such as the nucleus accumbens (Acb) (Sauvage and Steckler, 2001). These findings suggest that CRF may be able to modulate DAergic neurotransmission throughout the rat mesolimbic system. For these reasons, the present study used a potent and selective CRF1 receptor antagonist, CRA-0450 (Chaki et al., 2004), to examine the effects of acute versus chronic CRF1 receptor blockade on mesoencephalic DA neuron activity (determined by in vivo extracellular recordings) and DA overflow in the Acb (using in vivo microdialysis). In addition, the effect of CRF1 receptor antagonism on cocaine-induced DA overflow in the Acb was examined and correlated with DA neuron activity in the VTA.

Materials and Methods

All experiments were performed in accordance with the guidelines outlined in 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 University of Pittsburgh.

Survival Surgery. Chronic i.c.v. drug/vehicle administration (CRA-0450, 0.3 μg/0.5 μl/h for 14 days; vehicle, 0.5 μl/h for 14 days) was performed using Alzet micro-osmotic minipumps (model 2002) in conjunction with Alzet brain infusion kits (type I). Briefly, male Sprague-Dawley rats (250–400g) were anesthetized with a mixture of ketamine (80 mg/kg i.p.) and xylazine (12 mg/kg i.p.) and placed in a stereotaxic apparatus. A burr hole was drilled overlying the third ventricle (bregma: AP, −0.8 mm; ML, +1.5 mm), and an L-shaped (28-gauge) cannula was lowered into the third ventricle (5 mm ventral of skull surface) and fixed in place with dental cement covering the bregma. The electrode signal was amplified, filtered, and discriminated from noise using a combination amplification and window discrimination unit (WDR-420, Fintronic USA, Inc., Foster City, CA), and data were acquired, stored and analyzed using custom-designed computer software (Neuroscope).

Extracellular microelectrodes were lowered into the VTA (−6.5 to −9.0 mm ventral of brain surface) using a hydraulic microdrive, and the activity of the population of DA neurons was determined by recording from spontaneously active DA neurons encountered while making five to nine vertical passes, separated by 200 μm, throughout the VTA. Spontaneously active DA neurons were identified using previously established electrophysiological criteria (Grace and Bunney, 1983), and once isolated, their activity was recorded for 2 to 3 min. Three parameters of activity were measured: population activity (defined as the number of spontaneously active DA neurons recorded per electrode track), basal firing rate, and the proportion of action potentials occurring in bursts (defined as the occurrence of two spikes with an interspike interval of <30 ms, and the termination of the burst defined as the occurrence of an interspike interval of >160 ms; Grace and Bunney, 1984). At the cessation of the experiment, the recording site was marked via iontophoretic ejection of Pontamine sky blue dye from the tip of the recording electrode (−30-μA constant current, 20–30 min).

Microdialysis. Concentric microdialysis probes with 2-mm exposed membrane (CMA/12, 20-kDa permeability, 0.5-mm outer diameter) were implanted in the contralateral Acb (AP, +1.6; ML, −1.2; DV, −7.5 mm from bregma) and perfused at a rate of 2 μl/min with artificial cerebrospinal fluid (124 mM NaCl, 2.2 mM KCl, 1.3 mM KH2PO4, 1.3 mM MgSO4, 20 mM NaHCO3, and 2.0 mM CaCl2). After 60 to 90 min of equilibration, samples were collected at 20-min intervals into an equal volume (4 μl) of HPLC mobile phase (modified MD-TM 70-1332, pH 4.69, 50 mg/l sodium octyl sulfate) to minimize transmitter degradation. Samples were immediately injected into an HPLC system via an ESA model 540 autosampler (ESA Biosciences, Inc., Chelmsford, MA) and separated on an ESA MD-150/RP-C18 analytical column (150 × 3.2 mm) perfused with HPLC mobile phase (0.6 ml/min). DA was detected by oxidation using an ESA Couloum II detector equipped with a guard cell (+375 mV) and an ESA 5014 dual electrode analytical cell (E1, −175 mV; E2, +185 mV). Chromatographic data were acquired and analyzed using an ESA 501 data system. The HPLC system was calibrated at the start of each experiment using external DA standards. For acute experiments, three basal samples were collected before the administration of CRA-0450 (5 μg/5 μl i.c.v.) or vehicle (5 μl i.c.v.), and drug effects were measured for 40 min after which cocaine was injected (10 mg/kg i.p.) and data recorded for a further 140 min. For chronic experiments, four baseline samples were collected before administration of cocaine (10 mg/kg i.p.) and subsequent analysis for 140 min.

Histology. At the cessation of the experiment, rats were killed by an overdose of anesthetic, decapitated, and their brains removed, fixed for at least 48 h [8% (w/v) paraformaldehyde in phosphate buffer], and cryoprotected [25% (w/v) sucrose in phosphate buffer] until saturated. Brains were then sectioned (60-μm-thick coronal sections), mounted onto gelatin-chrom alum-coated slides, and stained with cresyl violet for histochemical verification of electrode dye markers, probe, and cannula sites with reference to a stereotaxic atlas (Paxinos and Watson, 1986).

Analysis. Electrophysiological analysis of DA neuron activity was performed using custom-designed computer software (Neuroscope), and microdialysis samples were analyzed against DA standards of known concentration using the ESA501 software package (ESA Biosciences, Inc.). All data are represented as the mean ± S.E.M. Differences in electrophysiological recording parameters or DA content between CRA-0450 vehicle- and cocaine/saline-treated rats were ex-
amined using a two-way analysis of variance (ANOVA) followed by a Dunnett’s post hoc test. All statistics were calculated using the SigmaStat software program (SPSS Inc., Chicago, IL).

**Drug Administration.** CRA-0450 was administered directly into the lateral ventricle for both acute and chronic studies. Central administration was preferred over a peripheral route for a number of reasons: to curtail the known peripheral effects of CRF₁ receptor blockade and to minimize effects on peripheral ACTH and cortisol secretion and to maintain a continuous, steady-state level of drug in the brain that is beneficial in determining its potential for inducing tolerance.

The doses used for i.c.v. administration were based on brain levels of CRA-0450 reported after oral administration of an effective dose of compound (Chaki et al., 2004). These levels were in the order of 2500 ng/g or ~4 to 5 μg/brain. Moreover, chronic infusions of 0.3 μg/h were employed to result in a daily dose of ~7 μg/day, slightly higher than that administered acutely to compensate for degradation/clearance of the drug.

**Materials.** CRA-0450 (Chaki et al., 2004) was a gift from Taisho Pharmaceuticals (Saitama, Japan) and was dissolved by sonication in 2% (v/v) Tween 80 in dH₂O. Cocaine hydrochloride was dissolved in saline and purchased from Sigma-Aldrich (St. Louis, MO), whereas all other chemicals and reagents were of either analytical or laboratory grade and purchased from various suppliers.

**Results**

**Effect of CRA-0450 Administration on DA Neuron Activity.** These data were collected from 430 neurons recorded in 51 rats. Rats that received either acute or chronic vehicle infusions (n = 14 rats; 125 neurons) exhibited an average of 1.06 ± 0.09 spontaneously active DA neurons per electrode track (cells/track), with an average firing rate of 4.25 ± 0.18 Hz and 26.7 ± 2.4% of action potentials occurring in bursts (Figs. 1–3), consistent with previous findings (Floresco et al., 2003). Acute i.c.v. infusion of the CRF₁ receptor antagonist, CRA-0450 (n = 6 rats; 73 neurons) resulted in a significant increase in DA neuron population activity (acute vehicle, 1.04 ± 0.28 cells/track; acute CRA, 1.86 ± 0.21 cells/track; p < 0.05) (Fig. 1A). Interestingly, acute CRA-0450 administration alone had no significant effect on either average firing rate (acute vehicle, 4.31 ± 0.28 Hz; acute CRA, 4.06 ± 0.20 Hz) or the percentage of action potentials fired in bursts (acute vehicle, 25.0 ± 3.4%; acute CRA, 20.0 ± 2.8%) (Figs. 2A and 3A). In contrast, chronic CRA-0450 administration (n = 7 rats; 59 neurons) did not significantly influence any of the basal electrophysiological properties measured (Figs. 1B, 2B, and 3B).

Acute cocaine administration (10 mg/kg i.p.) resulted in a significant decrease in DA neuron population activity (saline, 1.04 ± 0.28 cells/track; cocaine, 0.43 ± 0.06 cells/track; p < 0.05) and burst firing (saline, 25.0 ± 3.4%; cocaine, 13.0 ± 3.5%; p < 0.05) to a similar degree in both acute (n = 6; 19 neurons) and chronic (n = 6; 23 neurons) vehicle-treated rats (Fig. 1). In addition, a statistically significant decrease in firing rate was observed in acute vehicle and CRA-0450 treated rats (Fig. 2A); however, the small magnitude of this effect suggests that it is unlikely to be physiologically relevant and was not observed in chronically treated animals (Fig. 2B). On the other hand, both acute (n = 6; 72 neurons) and chronic (n = 6; 59 neurons) CRF₁ receptor blockade completely inhibited the effect of cocaine on population activity (Fig. 1), without significantly affecting burst firing (Fig. 3), suggesting a dissociation between the regulation of these two parameters.

**DA Microdialysis.** As demonstrated in Fig. 4, the systemic administration of cocaine (10 mg/kg i.p.) induced a robust increase (>150%; p < 0.05) in Acb DA release in both acute and chronic vehicle-treated rats, consistent with previous reports (Lu et al., 2003). In addition, neither acute (5 μg/5 μl i.c.v.) nor chronic (0.3 μg/h/14 days i.c.v.) CRA-0450 administration had any significant effect on basal extracellular DA levels determined by microdialysis (Fig. 4). However, both acute and chronic CRF₁ receptor blockade significantly attenuated (by >50%; p < 0.05) the cocaine-induced increase in Acb DA release (Fig. 4).

**Discussion**

In the present study, a combined neurochemical/neurophysiological analysis was used to assess the effects of acute versus chronic CRF₁ receptor blockade on the activity of the midbrain DA system. As such, acute (but not chronic) CRA-0450 administration induced a selective increase in DA neuron population activity, suggestive of a tonic inhibitory role for CRF on the activity of VTA DA neurons. In addition, we report that both acute and chronic CRF₁ receptor blockade potently inhibit cocaine-induced DA overflow in the Acb, which is correlated with a selective attenuation of the effects
of cocaine on DA neuron population activity. As such, these data add further weight to the suggestion that the CRF system plays a role in central reward processing.

**Effect of CRF1 Receptor Antagonism on the Midbrain DA System.** The present study demonstrates that the CRF1 antagonist CRA-0450 selectively increases DA neuron population activity in the VTA, without significantly altering average burst firing or firing rate. This dissociation between the regulation of DA neuron activity states has been investigated previously and demonstrated to be associated with distinct afferent inputs to the VTA (Floresco et al., 2003). As such, it has been reported that spontaneous activity in DA neurons is associated with spontaneous membrane depolarizations and subsequent modulation via GABAergic transmission to/within the VTA (Grace, 1987). Therefore, given the results of the present study, it is likely that CRF exerts a tonic modulatory control on population activity either via a direct effect on the DA neuron or via modulation of GABAergic transmission to/within the ventral mesencephalon. Given the relatively few reports on the effects of extrahypothalamic CRF throughout the central nervous system, the exact neurochemical processes associated with the CRF1 receptor antagonist-induced increase in DA population activity are unclear. However, it has been demonstrated previously that the VTA possesses a high degree of CRF receptor immuno-reactivity suggested to be localized to DAergic neurons (Sauvage and Steckler, 2001). In addition, the effects of CRF receptor activation on DA neuron activity have been recently investigated using in vitro intracellular recordings (Ungless et al., 2003). These studies reported the presence of a CRF2 receptor-mediated potentiation of N-methyl-D-aspartate excitatory postsynaptic potentials, whereas modulation of CRF1 receptor activity was without effect. One possible reason for the apparent disparity with the current results is that a large proportion of the afferent inputs to the VTA are severed during slice preparation for in vitro recordings. Moreover, DA neurons recorded from slice preparations display significantly different firing patterns and membrane characteristics compared with those observed in vivo. Thus, DA neurons recorded in vitro display a significantly higher degree of spontaneous activity and an extremely regular firing rate (Grace, 1987; Grace and Onn, 1989). As such, the modulation of population activity by CRA-0450 observed in the present study may not be observed in the slice preparation due to the high degree of spontaneous activity present in that preparation secondary to severing of afferent processes. In addition, the effect of CRA-0450 on population activity observed in the present study may result from an altered modulation of GABAergic afferents to the VTA, which again would not be observed in the in vitro preparation.

Although acute CRF receptor blockade significantly increased DA neuron population activity, DA levels in the Acb were not significantly affected by this treatment. Given the previous literature demonstrating the robustness of the co-
firing, in the direction of normalizing DA levels. Consistent with this, the present study demonstrates that vehicle-treated rats display a robust increase in Acb DA release after systemic cocaine administration, and this is correlated with a significant decrease in both population activity and average burst firing. Moreover, both acute and chronic CRF₁ receptor antagonism potently inhibited the effect of cocaine on DA overflow in the Acb, and this was correlated with a reversal of the cocaine-induced decrease in DA neuron population activity. Interestingly CRF₁ receptor blockade did not attenuate the effect of cocaine on burst firing, consistent with the dissociation between the control of population activity and burst firing reported previously (Floresco et al., 2003). Since it has been demonstrated that cocaine not only blocks DA transporters but also 5-HT and noradrenaline transporters (Ritz et al., 1990; Kuhar et al., 1991), it is possible that the persistent effects of cocaine on DA neuron burst firing are associated with a lack of effect of CRF on non-DAergic monoamine release, particularly 5-HT and noradrenaline. Indeed, previous studies have demonstrated a potent inhibitory effect of serotonin on the activity of pedunculopontine tegmental nucleus neurons (Leonard and Linäs, 1994), which have been shown to regulate burst firing in VTA DA neurons (Floresco et al., 2003). This suggests that an increase in 5-HT throughout this region, such as that purportedly induced by cocaine, may lead to a decreased burst firing in the VTA secondary to pedunculopontine tegmental nucleus inhibition. In addition, it has been demonstrated that systemic 5-HT transport blockade (by fluoxetine) decreases DA neuron activity in the VTA (Prisco and Esposito, 1995). As such, it is plausible that the persistent effect of cocaine on burst firing observed in the present study may be associated with an increase in non-DAergic monoamine transmission not affected by CRF₁ receptor blockade.

There is significant evidence demonstrating that the principal effects of cocaine on DAergic transmission result from a pharmacological blockade of the DA transporters (Ritz et al., 1987). The demonstration that acute CRA-0450 administration can inhibit cocaine-induced DA release likely suggests an interaction with the DA transporter. However, this is clearly not a direct pharmacological action since it has been demonstrated that CRA-0450 displays little affinity for any of the monoamine transporters throughout the rat brain (Chaki et al., 2004). In addition, previous studies have demonstrated an inhibition of cocaine-induced behavioral measures and DA release with the acute administration of structurally distinct CRF receptor antagonists (Lu et al., 2003), demonstrating that this effect is attributable to CRF₁ receptor blockade and not a nonspecific action of the drug. Remarkably, acute CRF₁ receptor inhibition did not significantly affect basal DA release, consistent with previous observations (Lu et al., 2003), suggesting that CRA-0450 administration leads to a CRF₁ receptor-specific decreased efficacy of cocaine without influencing normal transporter function. Unfortunately, given the relatively small literature regarding CRF/DA interactions, the exact mechanisms underlying the effect of CRF₁ receptor blockade on cocaine-induced DA release and associated changes in neurophysiology are yet to be elucidated.

An important consideration is the recent report that CRA-0450 may also inhibit σ₁ receptors throughout the central nervous system (Chaki et al., 2004); however, it is unlikely
that this is associated with the results obtained in the present study for a number of reasons. Previous studies have demonstrated α₁ receptor inhibition to have no significant effect on DA neuron activity (Ceci et al., 1988), and the inhibitory effects of CRA-0450 on cocaine-induced DA release and subsequent changes in population activity reported in the current study are consistent with previous reports demonstrating a reduction by CRF₁ receptor antagonists of cocaine-induced behavioral changes and DA release (Lu et al., 2003). Therefore, it is likely that the neurochemical/neurophysiological changes observed in the present study are associated with CRF₁ receptor blockade and not with any purported activity at the α₁ receptor.

Conclusions

Taken as a whole, these data provide the first extensive neurochemical/neurophysiological analysis of the actions of acute and chronic CRF₁ receptor blockade throughout the VTA. More specifically, these data show that acute CRA-0450 administration significantly and selectively increases DA neuron population activity throughout the VTA, suggestive of a tonic CRF-mediated inhibition of DA neuron activity. Furthermore, we report that both acute and chronic CRF₁ receptor blockade significantly attenuates cocaine-induced DA overflow in the Acb, which is correlated with a reversal of the cocaine-induced inhibition of DA neuron population activity. As such, these data add further weight to the suggestion that the CRF system plays a role in central reward and subsequent changes in population activity reported in the current study are consistent with previous reports demonstrating a reduction by CRF₁ receptor antagonists of cocaine-induced behavioral changes and DA release (Lu et al., 2003). Therefore, it is likely that the neurochemical/neurophysiological changes observed in the present study are associated with CRF₁ receptor blockade and not with any purported activity at the α₁ receptor.

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