Behavioral/Systems/Cognitive

Chronic Cocaine Administration Switches Corticotropin-Releasing Factor$_2$ Receptor-Mediated Depression to Facilitation of Glutamatergic Transmission in the Lateral Septum

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Corticotropin-releasing factor (CRF) and urocortin (Ucn I) are endogenous members among a family of CRF-related peptides that activate two different and synaptically localized G-protein-coupled receptors, CRF_1 and CRF_2. These peptides and their receptors have been implicated in stress responses and stress with cocaine abuse.

In this study, we observed significant alterations in excitatory transmission and CRF-related peptide regulation of excitatory transmission in the lateral septum mediolateral nucleus (LSMLN) after chronic cocaine administration. In brain slice recordings from the LSMLN of control (saline-treated) rats, glutamatergic synaptic transmission was facilitated by activation of CRF_1 receptors with CRF but was depressed after activation of CRF_2 receptors with Ucn I. After acute withdrawal from a chronic cocaine administration regimen, CRF_1 activation remained facilitatory, but CRF_2 activation facilitated rather than depressed LSMLN EPSCs. These alterations in CRF_2 effects occurred through both presynaptic and postsynaptic mechanisms. In saline-treated rats, CRF_1 and CRF_2 coupled predominantly to protein kinase A signaling pathways, whereas after cocaine withdrawal, protein kinase C activity was more prominent and likely contributed to the CRF_2-mediated presynaptic facilitation. Neither CRF nor Ucn I altered monosynaptic $GABA_A$-mediated IPSCs before or after chronic cocaine administration, suggesting that loss of $GABA_A$-mediated inhibition could not account for the facilitation. This switch in polarity of Ucn I-mediated neuromodulation, from a negative to positive regulation of excitatory glutamatergic transmission after chronic cocaine administration, could generate an imbalance in the brain reward circuitry associated with the LSMLN.

Key words: **cocaine; CRF; facilitation of transmission; glutamatergic excitatory transmission; urocortin; CRF_2; CRF_1; $GABA_A$ inhibitory transmission; acute withdrawal**

Introduction

Cocaine abuse continues to be a prominent societal and medical problem. Abuse of cocaine is often precipitated by stress (Piazza and Le Moal, 1998). Moreover, psychological stress can be a consequence of cocaine abuse, especially during its withdrawal (Sarnyai et al., 1995), and stress is cited as a major contributor to relapse (Erb et al., 1996). Corticotropin-releasing factor (CRF) is a primary regulator of the stress response (Dunn and Berridge, 1990), and stress along with CRF has been implicated in drug addiction (Koob, 1996). A second endogenous CRF-related peptide, urocortin (Ucn I), coexists with cocaine and amphetamine-regulated transcript (CART) within neurons in the Edinger–Westphal nucleus. This nucleus and other CNS nuclei, including the lateral septum mediolateral nucleus (LSMLN), along with their associated pathways are non-hypothalamic-pituitary-adrenal (HPA) circuits that use CRF-related peptides/receptors in the regulation of synaptic activities. After acute stress induced by lipolysaccharide, increased levels of Ucn I, CART, and c-*fos*, a marker of neuronal activation, occur within Edinger–Westphal nucleus neurons that terminate within the lateral septum (Kozicz, 2003). These findings indicate that increases in CRF-related peptides and additional bioactive substances may also occur after other stressors, namely, withdrawal from chronic cocaine and be released into the LSMLN.

Ucn I (rat) and CRF (rat/human) [CRF(r/h)] regulate excitatory glutamatergic transmission in the rat LSMLN by depressing and facilitating, respectively, evoked EPSCs and action potential-independent spontaneous synaptic currents [miniature EPSCs

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(mEPSCs)] (Liu et al., 2004). The LSMLN exhibits among the highest CNS levels of CRF_2 receptors, one of the two major CRF-related peptide receptors in the brain (Hauger et al., 2003). Moreover, within the LSMLN, CRF_2 establishes a depressant presynaptic and postsynaptic tone to regulate both EPSCs and mEPSCs, whereas CRF_1 maintains a facilitatory postsynaptic tone (Liu et al., 2004). These tonic effects may represent modulations of metabolically dependent signaling activity associated with synaptic events and/or changes in the frequency/amplitude of spontaneous transmitter release. Phasic effects on EPSCs represented responses to temporary changes in synaptic CRF-related peptide concentrations (Liu et al., 2004). Synchronous activation of CRF_1 and CRF_2 receptors and their associated signaling pathways functioned in tandem with activation of glutamate receptors and their effectors to facilitate/depress EPSCs.

Table 1. Comparison of membrane properties of chronic cocaine versus control/saline neurons from LSMLN

Groups	RMP (mV)	R_m (MΩ)	C_m (pF)
Control/saline	57.5 ± 0.6 (n=58)	214.8 ± 19.0 (n=40)	141.7 ± 7.0 (n=40)
Chronic cocaine	60.9 ± 0.5* (n=40)	224.3 ± 21.5 (n=35)	134.9 ± 10.0 (n=35)

RMP, Resting membrane potential; R_m, membrane resistance; C_m, membrane capacitance. Data are expressed as mean ± SEM. *$p < 0.001$.

Interactions between drugs abused chronically, brain reward mechanisms, and their neuronal circuitry are unclear. The lateral septum exhibits increased neuronal activity or increased c-*fos*/Fos-related antigen expression after exposure to cocaine (Brown et al., 1992; Franklin and Druhan, 2000; Trinh et al., 2003), PCP (Sato et al., 1997), morphine (Liu et al., 1994), and ethanol (Bachtell et al., 2003).

In the present study, we investigated interactions of CRF-related peptide receptors and glutamatergic transmission in the LSMLN, a brain area known to play a role in brain reward circuitry (Sheehan et al., 2004). Our results demonstrate that chronic cocaine administration facilitates excitatory transmission. In addition, the CRF_2-mediated depression of excitatory transmission observed in control animals was switched to facilitation after withdrawal from chronic cocaine administration. The combined facilitatory effects by CRF_2 and CRF_1 strongly enhanced excitatory transmission attributable to the functional conversion of CRF_2. Neither CRF nor Ucn I affected $GABA_A$-mediated inhibition.

Materials and Methods

Animal experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publications number 80-23; revised 1996). In addition, all efforts were made to minimize the number of animals used and to minimize their suffering.

Animals and tissue collection. After 1 week of acclimatization, male rats (Sprague Dawley; 150–200 gm; Holtzmann, Madison, WI) were divided into two groups. Rats received injections of either cocaine (15 mg/kg, i.p., twice per day for 14 d at 9:00 A.M. and 4:00 P.M.) or saline in their home cages. Data collected from saline-treated rats were comparable with our previously published results (Liu et al., 2004).

Rats were decapitated within 17 hr of receiving their last injection of saline or cocaine, and their brains were removed rapidly, immersed in a modified cold (~5°C) artificial CSF (ACSF), and bubbled continuously with 95% O_2 plus 5% CO_2 to maintain proper pH (7.3–7.4). The ACSF composition was as follows (in mM): 117 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, 25 $NaHCO_3$, and 11.5 glucose. The brain was quickly blocked to transverse sections ~5 mm thick with the caudal edge at the level of the optic chiasm. A 400 μm slice from the original block of tissue and containing the LSMLN was cut and transferred to the recording chamber and maintained at 32 ± 2°C.

Whole-cell patch-clamp recording. Whole-cell recordings were obtained with patch electrodes having tip resistances of 3–5 MΩ when filled with an internal solution (in mM): 122 K-gluconate, 5 NaCl, 0.3 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 10 HEPES, 5 Na_2-ATP, and 0.4 Na_3-GTP, adjusted to pH 7.2–7.3 with KOH and to an osmolarity of 280 mmol/kg with sucrose (Yu et al., 2002; Liu et al., 2004). Acceptable recordings only included stable access resistances of <25 MΩ. Recording and stimulating electrodes were positioned using a dissecting microscope (~0.5× magnification). A brain slice was mounted in a recording chamber (RC-27L; Warner Instruments, Hamden, CT) attached to the microscope base and visualized with trans-illumination.

Evoked EPSCs. Actions of CRF-related peptides were examined from individual neurons while monitoring evoked EPSCs. A bipolar electrode (SNE-100; Kopf Instruments, Tujunga, CA) placed in the ventral aspect of the LSMLN activated afferent inputs, whereas a recording electrode was positioned visually (Yu et al., 2002). For evaluation of drug effects on EPSCs, stimulus intensities were adjusted to one-half of threshold for orthodromic spike generation at a frequency of 0.1–0.25 Hz. In all experiments, EPSCs were recorded from brain slices superfused with a mixture of picrotoxin (50 μM), bicuculline methiodide (10 μM), and (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl]-(phenylmethyl)phosphinic acid (CGP55845) (2 μM) added to the ACSF. To record monosynaptic $GABA_A$-mediated IPSCs, the ACSF contained a mixture of DNQX, D-(−)-2-amino-5-phosphonopentanoic acid (D-APV), and CGP55845. In the paired-pulse paradigm, the first $EPSC_1$ and second $EPSC_2$ were elicited at intervals of 35–200 msec. EPSCs were acquired and analyzed using pClamp version 9.1. Recordings were collected with an Axoclamp-2A amplifier: switching frequency, 5–6 kHz (30% duty cycle); gain, 3–8 nA/mV; time constant, 20 msec.

Spontaneous mEPSCs. Action potential-independent mEPSCs were collected for 2 min after a 20 min period (control) following the addition of tetrodotoxin (TTX; 1 μM) to the standard ACSF. A similar period was used after the application of Ucn I. mEPSC activity was analyzed off-line using Synaptosoft software; the miniature events were defined as an amplitude above a preset baseline/noise level (5 pA) and reviewed visually by the investigator before analyses.

Drug application. Drugs were superfused at known concentrations until equilibrium was established (minimum, 10 min). After removal of drug from superfusing media, return of electrophysiological parameters under study to control levels was taken as evidence of recovery. The following drugs were obtained: CRFr/h, rat Ucn I, picrotoxin, bicuculline methiodide, CGP55845, and TTX from Sigma (St. Louis, MO); D-APV and DNQX from Research Biochemicals (Natick, MA); astressin$_2$B from one of the authors (J.R.); and 5-chloro-*N*-(cyclopropylmethyl)-2-methyl-*N*-propyl-*N*′-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride (NBI 27914) from one of the authors (D.E.G.). Peptides and CRF-related peptide antagonists were water soluble and dissolved in the ACSF. Cocaine HCl was provided by National Institute on Drug Abuse.

Analysis of data. When comparing chronic cocaine versus control data, a two-tailed, unpaired Student's t test was applied. We carefully compared our results collected with saline-injected rats (controls in this study) with our data reported with noninjected rats in our previous study (Liu et al., 2004); no differences in results were observed. n refers to the number of animals used to collect data; to avoid bias, only one neuron per animal was used for analysis and from which recordings were made. Experiments with drugs were analyzed using a paired protocol (i.e., each neuron served as its own control before and after drug) and analyzed using a paired t test. A level of $p \leq 0.05$ was indicative of statistical significance. All averaged values were given as the mean ± SEM.

Results

Administration of chronic cocaine and its acute withdrawal changed LSMLN neuronal membrane properties

Resting membrane potentials in LSMLN neurons were more hyperpolarized in chronic cocaine-treated rats, whereas no changes were observed in either input resistance or cellular capacitance

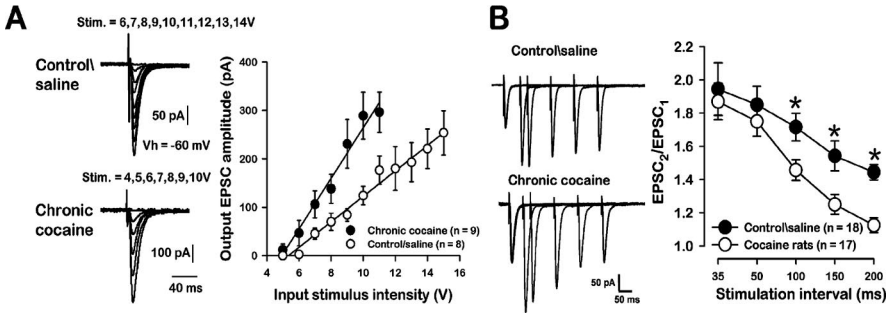

Figure 1. Chronic cocaine administration and its acute withdrawal facilitated LSMLN EPSCs. *A*, Left, EPSCs increased in amplitude with increasing stimulus intensity after chronic cocaine administration. Right, Chronic cocaine administration and its withdrawal shifted the input–output relationships for LSMLN EPSCs to the left. *B*, Left, Pairs of EPSCs were elicited at various interstimulus intervals in LSMLN synapses from control/saline- and chronic cocaine-treated rats. Right, Paired-pulse ratios were decreased after chronic cocaine administration, suggesting a presynaptic increase in probability of transmitter release. The asterisk indicates significant differences from control ratios at 100, 150, and 200 msec intervals ($p < 0.05$).

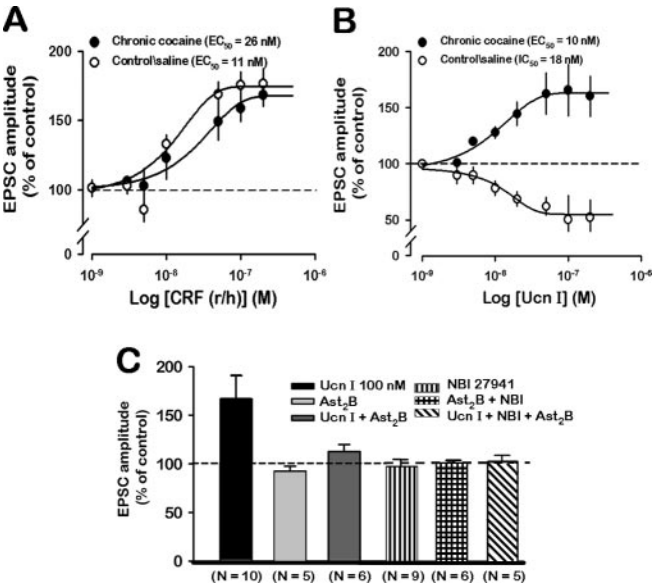

Figure 2. After chronic cocaine administration and its acute withdrawal, Ucn I facilitated LSMLN-EPSCs, which is blocked by the CRF_2 antagonist Ast_2B. *A*, After chronic cocaine administration, CRFr/h still facilitated EPSCs, but with diminished potency. *B*, Ucn I-induced depression of EPSCs in control neurons was switched to facilitation after chronic cocaine treatment. *C*, The CRF_2 antagonist Ast_2B blocked the Ucn I-induced facilitation but did not cause reversal to EPSC depression. CRF_1- or CRF_2-specific antagonists alone did not affect EPSCs.

(Table 1). Similarly, we reported previously that resting membrane potentials were more hyperpolarized and cellular input resistances were reduced in the dorsolateral septal nucleus (DLSN) *in vitro* (from −58.8 to −60.9 mV and from 133 to 122 MΩ), using the same paradigm of a short period of withdrawal (17 hr) from cocaine administered chronically *in vivo* (15 mg/d, i.p., twice daily for 14 d) (Shoji et al., 1997).

LSMLN EPSCs were facilitated after chronic cocaine administration and its acute withdrawal

An input–output relationship that measured EPSC amplitude as a function of stimulus intensity was shifted to the left in brain slices from chronic cocaine-treated animals, indicating that less orthodromic input was required to generate larger EPSCs (Fig. 1*A*, ●). When pairs of these facilitated EPSCs were analyzed at different interstimulus intervals, the paired-pulse ratio was reduced at intervals of ≥100 msec (Fig. 1*B*) ($p < 0.05$; 1.71 ± 0.08 vs 1.45 ± 0.06 at 100 msec; 1.54 ± 0.09 vs 1.25 ± 0.06 at 150 msec; 1.44 ± 0.05 vs 1.12 ± 0.05 at 200 msec). This change in the paired-pulse ratio suggested that facilitated EPSCs recorded after the chronic cocaine paradigm resulted from a presynaptic effect to increase probability of release (Fig. 1). To test this hypothesis, we measured the frequency of action potential-independent mEPSCs, a parameter that changes with alterations in release probability. After chronic cocaine administration, however, the mean mEPSC frequency (2.8 mEPSCs/sec) and interevent interval (355 ± 34 msec) were similar to those reported in control rats (data not shown) (Liu et al., 2004, their Fig. 7). In addition, mEPSC amplitudes were not different from the mean values recorded from controls (17 ± 0.5 pA) (Liu et al., 2004). These results suggested that the chronic cocaine paradigm exerted a presynaptic effect on evoked (phasic) transmitter release, while having no apparent effect on action potential-independent (tonic) release of transmitter. The absence of changes in mEPSC amplitude also suggested that the cocaine treatment paradigm did not alter the postsynaptic responsiveness to synaptically released glutamate.

Decreased potency of CRF and reversal of Ucn I action after chronic cocaine administration and its acute withdrawal

After chronic cocaine administration, the actions of CRFr/h and Ucn I on evoked EPSCs were different from those of controls (Fig. 2). After chronic cocaine administration, CRFr/h facilitated EPSCs to a comparable maximal level (~165%) as in neurons from control/saline animals (Fig. 2*A*, ●), but higher concentrations of CRF were required to achieve this plateau. The concentration–effect curve for CRFr/h was shifted rightward after chronic cocaine administration, and the apparent EC_{50} value was more than doubled (from 11 to 26 nM). This loss in potency of CRF occurred despite the lower electrical threshold and increased EPSC amplitude after chronic cocaine administration and its acute withdrawal (Fig. 1).

Surprisingly, the expected depression of LSMLN EPSCs by Ucn I (Fig. 2*B*, ○) (an apparent IC_{50} value of ~18 nM in controls) was eliminated completely after chronic cocaine administration. Rather, Ucn I facilitated EPSC amplitudes with an apparent EC_{50} value of ~10 nM (Fig. 2*B*, ●). After chronic cocaine administration, the selective CRF_2 antagonist Ast_2B reduced significantly the Ucn I (100 nM)-induced facilitation from 163 ± 22.9 to 112.7 ± 7.1% ($n = 6$; $p < 0.01$) (Fig. 2*C*). However, Ast_2B did not restore the Ucn I-induced depression observed in control animals but rather reduced amplitudes to near (95%) baseline levels (Fig. 2*C*). The small degree of EPSC facilitation in the presence of Ucn I plus Ast_2B (~112%) was blocked by NBI 27914 (Fig. 2*C*). Application of neither Ast_2B (92.5 ± 5.1% of control; $n = 6$; $p > 0.05$) nor NBI 27914 (95.6 ± 7.2% of control; $n = 9$; $p > 0.05$) significantly affected evoked EPSCs after chronic cocaine administration. These latter results differed from the opposing actions of Ast_2B to facilitate (≈123% of control) and NBI 27914 to depress (≈83% of control), respectively, evoked EPSCs in controls (Liu et al., 2004) and suggested that this normal facilitatory and depressant tone was eliminated by the chronic cocaine treatment. This loss of the tonic CRF_2-mediated depression and CRF_1-mediated facili-

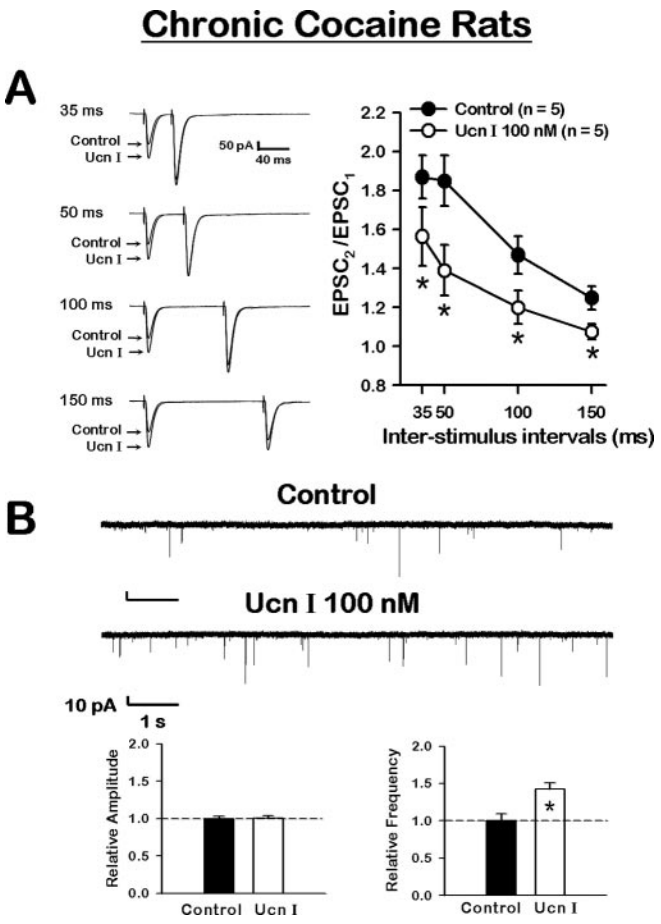

Figure 3. The Ucn I switch from depression to facilitation of EPSCs after chronic cocaine administration was attributable to changes at presynaptic and postsynaptic sites *A*, Left, Pairs of enhanced EPSCs were further facilitated by Ucn I at all four paired-pulse intervals tested. Right, Summary of paired-pulse data depicting a downward shift after Ucn I (○). The asterisk indicates significant differences from control ratios at all intervals ($p < 0.05$). *B*, Top, Spontaneously released mEPSCs were increased in frequency after the addition of Ucn I. Bottom, Summary of data showed that Ucn I had no effect on mEPSC amplitude but significantly increased mEPSC frequency. The asterisk indicates significant difference from control ($p < 0.05$).

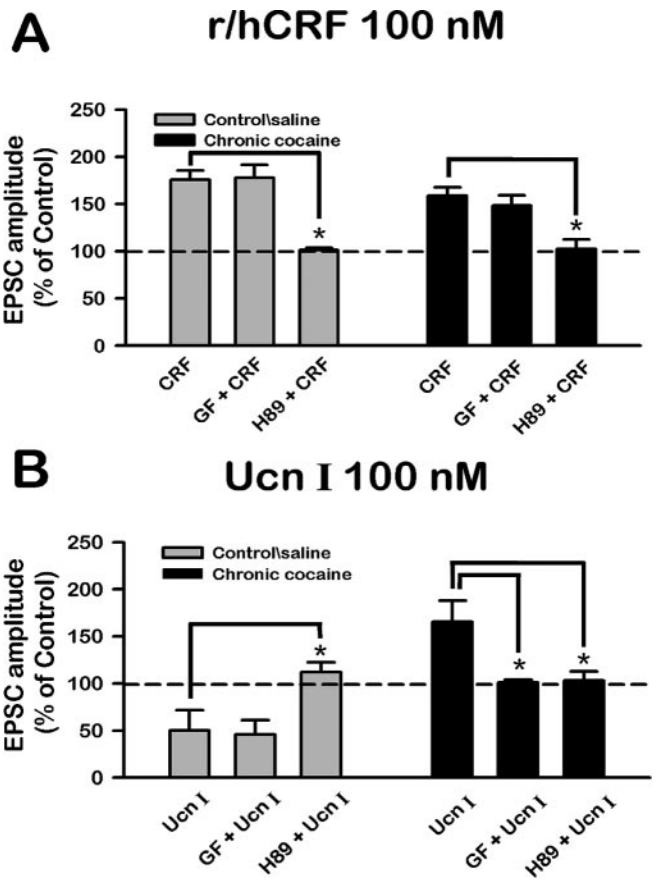

Figure 4. CRF_1 and CRF_2 coupling to PKA and PKC was altered differentially after chronic cocaine administration and its acute withdrawal. *A*, PKA inhibition by H-89 blocks CRF facilitation in neurons from control/saline rats (▨) and after chronic cocaine administration (■). PKC inhibition with GF 109203X did not affect CRF_1 facilitation or CRF_2 depression of EPSCs in neurons from control/saline animals but also did not alter postsynaptic facilitation mediated through CRF_1 in chronic cocaine animals. *B*, After chronic cocaine administration, PKC inhibition blocked the switched presynaptic facilitation mediated by CRF_2. The asterisk indicates significant differences from control CRF or Ucn I values before and after chronic cocaine administration ($p < 0.05$).

tation may have also contributed to the switch of phasic results obtained with CRF and Ucn I after chronic cocaine administration and its acute withdrawal. Additionally, the loss of postsynaptic tonic actions of Ucn I may contribute to its inability to enhance mEPSC amplitude after chronic cocaine, despite the switch from depression to facilitation of phasic evoked EPSCs.

When paired-pulse experiments were conducted after chronic cocaine administration and its acute withdrawal, the addition of Ucn I (100 nM) further shifted the EPSC2/EPSC1 ratio (Figs. 1*B*, 3*A*,*B*) downward. However, unlike controls in which Ucn I depressed both the frequency and amplitude of mEPSCs by acting at inhibitory presynaptic and postsynaptic CRF_2 receptors (Liu et al., 2004, their Fig. 8), after chronic cocaine Ucn I increased mEPSC frequency (interevent interval: 355.3 ± 33.9 msec vs 250.8 ± 21.6 msec), while having no effect on mEPSC amplitude (17.1 ± 0.5 nA vs 17.3 ± 0.05 nA). Thus, the actions of Ucn I switched from (1) depression of EPSCs (Fig. 2*B*, control), (2) depression of mEPSC frequency (Liu et al., 2004, their Fig. 7), and (3) depression of mEPSC amplitude (Liu et al., 2004, their Fig. 7). But, Ucn I after chronic cocaine administration switched to (1) facilitation of EPSCs by increasing the probability of evoked release (Fig. 3*A*), (2) facilitation of the frequency of spontaneous (tonic) mEPSCs (Fig. 3*B*), while (3) having no effect on mEPSC amplitude (Fig. 3*B*).

These data suggest that chronic cocaine administration switched the normal balance maintaining tonic depression of excitatory transmission via inhibitory presynaptic and postsynaptic-CRF_2 receptors, and tonic facilitation via facilitatory postsynaptic CRF_1-mediated actions, to a solely facilitatory tone through facilitatory pre-CRF_2- and post-CRF_1-mediated synaptic actions.

Protein kinase A and protein kinase C mediate actions of CRF and Ucn I in the regulation of LSMLN-EPSCs

We next determined whether protein kinase A (PKA) or protein kinase C (PKC) signaling were responsible for CRF-related peptide effects within the LSMLN from control and chronic cocaine rats. H-89 (10 μM), a PKA antagonist, or a selective PKC antagonist, GF 109203X (4 μM; bisindolylmaleimide I), was applied by bath superfusion for a minimum of 15 min before testing the effects of CRF or Ucn I on evoked EPSCs in the LSMLN slice *in vitro* (Fig. 4). In brain slices from control rats (gray bars), PKA inhibition (Fig. 4*A*) blocked the facilitation of EPSCs caused by CRF, while reversing the control Ucn I-mediated depression of EPSCs to a slight facilitation (Fig. 4*B*). In contrast, inhibition of

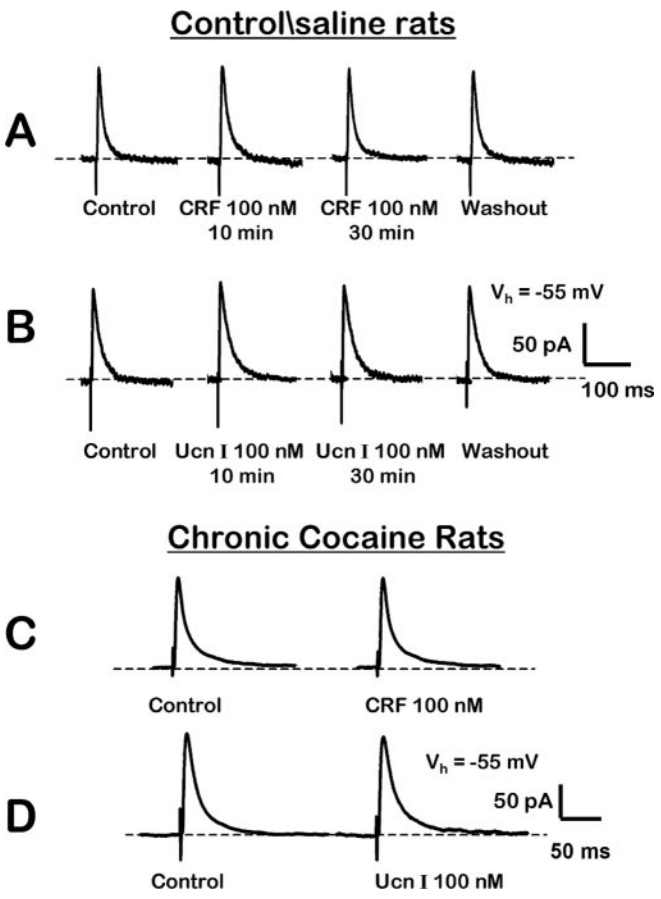

Figure 5. Neither CRF nor Ucn I affected $GABA_A$-IPSCs. *A*, *B*, Control $GABA_A$-IPSCs recorded in neurons from saline/control rats before, during, and after superfusion with CRF and Ucn I. *C*, *D*, $GABA_A$-IPSCs recorded in neurons from rats treated chronically with cocaine and acutely withdrawn before, during, and after superfusion with CRF and Ucn I.

PKC did not affect CRF-induced facilitation (Fig. 4*A*) or Ucn I-induced depression (Fig. 4*B*) of EPSCs.

After chronic cocaine administration (black bars), both CRF and Ucn I facilitated ESPCs (Fig. 4). Inhibition of PKC did not change the post-cocaine facilitation by CRF (Fig. 4*A*) but did block the post-cocaine facilitation by Ucn I (Fig. 4*B*). In contrast, PKA inhibition reduced the post-cocaine facilitation by CRF and Ucn I to control levels (100%) (Fig. 4*A*,*B*), a result identical to that observed with CRF or Ucn I before cocaine treatment.

CRF and Ucn I do not affect $GABA_A$-mediated IPSCs in LSMLN neurons from control/saline or those from chronic cocaine administration and its acute withdrawal

A possible explanation for our inability to observe depression of EPSCs by Ucn I is a loss of GABAergic inhibition prominent at this synapse (Liu et al., 2004) (Fig. 2). Because LSMLN synaptic transmission consists of a balance of excitatory and inhibitory synaptic events, we determined whether the CRF-related peptides affected inhibitory transmission. Monosynaptic $GABA_A$-mediated IPSCs were isolated pharmacologically with DNQX (20 μM), D-APV (50 μM), and CGP55845 (2 μM) to block AMPA/kainate, NMDA, and $GABA_B$ receptors, respectively. Under these recording conditions, neither CRF nor Ucn I (5–200 nM) affected $GABA_A$-mediated IPSCs recorded from brains of saline-injected rats (Fig. 5*A*,*B*) [CRF (100 nM): 101.0 ± 2.5%, $n = 6$; Ucn I (100 nM): 102.0 ± 2.9%, $n = 7$)]. Likewise, after chronic cocaine administration, neither CRF nor Ucn I affected $GABA_A$-mediated IPSCs (Fig. 5*C*,*D*) [CRF (100 nM): 99.2 ± 2.1%, $n = 5$; Ucn I (100 nM); 99.6 ± 3.2, $n = 5$)]. Thus, the loss of EPSC depression by Ucn I (Fig. 2*B*) could not be accounted for by diminished $GABA_A$-mediated synaptic inhibition within the LSMLN.

Discussion

This study demonstrated that a paradigm consisting of chronic administration of cocaine and its withdrawal resulted in changes in excitatory glutamatergic transmission and changes in the ability of CRF-related peptides to regulate excitatory transmission.

We consider the chronic cocaine administration and acute withdrawal paradigm we use in rats both rewarding and a significant stressor. Many laboratories use different stressors and paradigms involving administration or self-administration of abused substances, such that a "standardized definition of stressor" has not been established. As a result, making comparison of results from different paradigms is difficult. We have used this same chronic administration and acute withdrawal paradigm previously and have obtained consistent repeatable electrophysiological results while recording from different limbic nuclei [LSMLN (present study), DLSN (Shoji et al., 1997), and central nucleus of the amygdala (Neugebauer et al., 2001)]. We conclude that this paradigm is an appropriate method to focus on cellular processes involved in expression of this chronic administration of cocaine and acute withdrawal phenomenon.

The paradigm consisting of chronic cocaine administration *in vivo* and its acute withdrawal resulted in enhanced excitatory synaptic transmission in the LSMLN *in vitro*. Enhanced EPSCs occurred concurrently with more negative (hyperpolarized) postsynaptic membrane potentials (Table 1). The leftward shift of the input–output relationship (Fig. 1*A*) after chronic cocaine administration may also be related to the lowered intracranial self-stimulation threshold reported after acute cocaine administration (Kenny et al., 2003).

The reduction in the paired-pulse ratios without a change in mEPSC frequency or amplitude suggested that chronic cocaine administration resulted in a differential effect on evoked (phasic) release of glutamate but perhaps not on its spontaneous (tonic) release. These results are consistent with a presynaptic mechanism underlying the EPSC facilitation after chronic cocaine administration and its acute withdrawal. Because CRF and possibly other CRF-related peptides are stored and released with glutamate and other transmitters (Kozicz, 2003; Sauvage and Steckler, 2001; Valentino et al., 2001), as the probability of release of one transmitter is increased, corelease and enhanced release of these neuropeptides could also occur with the opportunity for multiple postsynaptic mechanisms to induce synaptic facilitation. Typically, neuropeptides require a stronger afferent input (stimulus) or increased stimulus frequency before being released compared with non-neuropeptides (Hökfelt, 1991). Also, unlike release of amino acid neurotransmitters that require higher elevations of calcium ions concentrated near the active zone of synapses, neuropeptide release is triggered by small elevations of ionized calcium within the bulk cytoplasm (Hökfelt et al., 2000).

A differential effect on evoked glutamate release (decreased paired-pulse facilitation) versus action potential-independent glutamate release (no change in mEPSC frequency or amplitude) following the chronic cocaine paradigm may be a more generalized phenomenon and apply to other paradigms, drug treatments, or synapses. Furthermore, there may be different mechanisms underlying release processes involving excitatory versus inhibitory transmitters versus neuropeptides (see above). Although extracellular calcium was demonstrated to be an absolute

requirement for evoked release of transmitter (acetylcholine), spontaneous release of this excitatory transmitter at the skeletal neuromuscular junction occurs independent of extracellular calcium (Katz and Miledi, 1968). Vaughan et al. (2001) demonstrated that an endogenous peptide inhibited evoked $GABA_A$-mediated IPSCs, while having no effect on the rate or amplitude of miniature IPSCs (mIPSCs). Yet, after elevation of external potassium, to activate voltage-dependent calcium channels, application of the peptide reduced the frequency of mIPSCs, an action blocked by cadmium.

Wu (2004) reiterates that different processes may be involved in spontaneous and evoked release, including different pools of vesicles and stored transmitters. For instance, Sakaba and Neher (2003) conclude that G-protein-coupled (e.g., $GABA_B$) receptor activation directly modulates synaptic vesicle priming to retard release of evoked EPSCs mediated by glutamate. Harvey and Stephens (2004) suggest that $GABA_B$ receptor activation, with subsequent inhibition of spontaneous GABA release onto cerebellar Purkinje cells, occurs downstream of calcium ion influx and may be mediated in part by an inhibition of a vesicular release mechanism.

Perhaps, among its multiple actions, chronic cocaine administration facilitates presynaptic calcium entry or "primes vesicular release" necessary for evoked release, but not for spontaneous release, by switching the "cross talk" between the PKA and PKC pathways (Kubota et al., 2003). Possibly, one pathway may regulate evoked release, whereas the other regulates spontaneous release.

After the chronic cocaine paradigm, the effects of CRF-related peptides were also different (Fig. 2). Higher concentrations of CRF were required to facilitate EPSCs, despite EPSCs already being facilitated after the chronic cocaine regimen (Fig. 1). Ucn I no longer depressed but instead facilitated evoked EPSCs. In addition to changes in evoked EPSCs, tonic facilitation rather than depression by Ucn I of spontaneous mEPSC frequency emerged, while having no effect on mEPSC amplitude.

The effects of CRF_2 and CRF_1 antagonists also changed after chronic cocaine administration. Ast_2B no longer resulted in facilitation of EPSCs (Fig. 2*C*), as we demonstrated in controls (Liu et al., 2004). Also, application of the selective CRF_1 antagonist NBI 27914 no longer resulted in significant tonic, CRF_2-mediated depression of EPSCs (Fig. 2*C*). These data suggested that endogenous CRF_1 and CRF_2 receptor tone, mediated either by constitutive activity of their respective receptors (Seifert and Wenzel-Seifert, 2002) and/or by enhanced release of (Kozicz, 2003) or elevation in (Rothman et al., 2002) circulating CRF-related peptides, was possibly minimized after chronic cocaine administration. The net effect of this chronic cocaine paradigm was to switch the presynaptic CRF_2 and postsynaptic CRF_1 receptors to function only in a facilitatory mode.

PKC is a candidate-signaling mechanism that could underlie this switch in regulatory activity. PKC activity could have been masked by stronger PKA signaling under control conditions (Fig. 4). After the chronic cocaine paradigm, inhibition of PKA continued to affect facilitation induced by either CRF_1 or CRF_2, whereas PKC inhibition was only effective in blocking the increased facilitation mediated through CRF_2, not the facilitation by CRF_1. These results suggest that PKA may be linked to both CRF_1 and CRF_2, whereas PKC may be preferably linked to a facilitatory CRF_2 within the LSMLN synapse. These data may also imply that the CRF_2 coupled to PKC signaling was most prominent within the nerve terminal, whereas PKA signaling was equivalent within both the nerve terminal and postsynaptic neuron. Based on previous studies from our laboratory (Shoji et al., 1997) and others (Nestler et al., 1990, 1993), we suggested that chronic cocaine administration decreased levels of a specific G-protein subunit, $G_{o\alpha}$, coupled to a presynaptic $GABA_B$ receptor within the DLSN. Uncoupling of a G-protein subunit or posttranslational editing with generation of splice variants (Kilpatrick et al., 1999) may uncover a different functional CRF-related peptide receptor that exhibits a different pharmacology. Furthermore, because CRF_1 and CRF_2 couple to multiple G-proteins with subsequent stimulation or inhibition of various intracellular signaling cascades (e.g., PKA, PKC, and PLC) (Grammatopoulos et al., 2001), the uncoupling of one receptor could shift the balance from one signaling system to another and thereby alter the regulation of basal tone and/or phasic activity of a ligand. Because CRF_1 and CRF_2 receptors localize to the same postsynaptic cell in LSMLN synapses and each produce opposite effects when activated, desensitization or upregulation of one receptor (e.g., because of a chronic stressor) could result in a "switch" in the balance maintained between signaling pathways within that cell. Recently, involvement of cAMP rather than mechanisms distal to its formation (e.g., upregulation of PKA) has been proposed to explain chronic morphine sensitization of the brain norepinephrine system to CRF and stress (Xu et al., 2004). A similar situation may occur with PKC formation in the LSMLN after chronic cocaine administration.

Could facilitation of EPSCs after chronic cocaine administration and its acute withdrawal result from an increased level of endogenous CRF (Sarnyai et al., 2001) or Ucn I? Increased circulating CRF-related peptides may be attributable to an allostatic "stress reaction" associated with chronic cocaine administration and/or as a result of acute withdrawal from chronic cocaine administration. Elevated plasma levels of CRFr/h are recorded in physically stressed rats (Stout et al., 2002).

Currently, molecular mechanisms underlying the plastic changes that affect the regulatory roles of CRF-related peptides after chronic cocaine administration and its acute withdrawal within the LSMLN or possibly other CNS synapses are not known. Our data support non-HPA-mediated actions of CRF-related peptides and are in agreement with data from Müller et al. (2003), who after site- and time-dependent knock-out of CRF_1, concluded that limbic CRF_1 mediated anxiety-related behavior and adaptation to stress.

Our data also showed that glutamatergic transmission is facilitated by CRF-related peptides, whereas $GABA_A$-mediated transmission is not. If an imbalance of CRF or other endogenous CRF-related peptides (e.g., Ucn I, Ucn II, or Ucn III) occurred in the LSMLN (which may result from a variety of factors that include chronic cocaine administration and its acute withdrawal), then circuitry linked to the LSMLN would also be affected by this imbalance (Liu et al., 2004). It is possible that altered regulation of glutamatergic transmission and its LSMLN-dependent circuitry may contribute to the reinstatement of cocaine abuse or precipitate relapse.

Altered regulation of excitatory transmission by CRF-related peptides may also contribute to the development and maintenance of other mental disorders associated with cocaine abuse and stress (e.g., anxiety and depression) (Sheehan et al., 2004). We suggest that CRF-related peptide ligands may provide a rational therapy for these mental health disorders.

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