Cocaine and Kindling Alter the Sensitivity of Group II and III Metabotropic Glutamate Receptors in the Central Amygdala

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Neugebauer, Volker, Fatiha Zinebi, Rex Russell, Joel P. Gallagher, and Patricia Shinnick-Gallagher. Cocaine and kindling alter the sensitivity of group II and III metabotropic glutamate receptors in the central amygdala. J Neurophysiol 84: 759–770, 2000. G-protein-coupled metabotropic glutamate receptors (mGluRs) are being implicated in various forms of neuroplasticity and CNS disorders. This study examined whether the sensitivities of mGluR agonists are modulated in a distinct fashion in different models of synaptic plasticity, specifically, kindling and chronic cocaine treatment. The influence of kindling and chronic cocaine exposure in vivo was examined in vitro on the modulation of synaptic transmission by group II and III metabotropic glutamate receptors using whole cell voltage-clamp recordings of central amygdala (CeA) neurons. Synaptic transmission was evoked by electrical stimulation of the basolateral amygdala (BLA) and ventral amygdaloid pathway (VAP) afferents in brain slices from control rats and from rats treated with cocaine or exposed to three to five stage-five kindled seizures. This study shows that after chemical stimulation with chronic cocaine exposure or after electrical stimulation with kindling the receptor sensitivities for mGluR agonists are altered in opposite ways. In slices from control rats, group II agonists, (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (LCCG1) and (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740), depressed neurotransmission more potently at the BLA-CeA than at the VAP-CeA synapse while group III agonist, L(+)-2-amino-4-phosphonobutyrate (LAP4), depressed neurotransmission more potently at the VAP-CeA synapse than at the BLA-CeA. These agonist actions were not seen (were absent) in amygdala neurons from chronic cocaine-treated animals. In contrast, after kindling, concentration response relationships for LCCG1 and LAP4 were shifted to the left, suggesting that sensitivity to these agonists is increased. Except at high concentrations, LCCG1, LY354740, and LAP4 neither induced membrane currents nor changed current-voltage relationships. Loss of mGluR inhibition with chronic cocaine treatment may contribute to counter-adaptive changes including anxiety and depression in cocaine withdrawal. Drugs that restore the inhibitory effects of group II and III mGluRs may be novel tools in the treatment of cocaine dependence. The enhanced sensitivity to group II and III mGluR agonists in kindling is similar to that recorded at the lateral to BLA synapse in the amygdala where they reduce epileptiform bursting. These findings suggest that drugs modifying mGluRs may prove useful in the treatment of cocaine withdrawal or epilepsy.

INTRODUCTION

Neurotransmitter mechanisms may have fundamental similarities but also significant differences in various models of synaptic plasticity. Understanding the synaptic and cellular processes in the forms of synaptic plasticity may provide insight into brain function. Kindling is an established animal model of human epilepsy in which repeated and focal applications of initially subconvulsive electrical stimuli to certain brain areas result in the progressive development of partial and generalized seizures (Goddard et al. 1969; Racine 1972). Cessation of chronic cocaine use produces complex behavioral changes in humans and in animal models (Gawin 1991). Anxity and depression are the major symptoms of the initial phase of cocaine abstinence (Gawin and Ellinwood 1989), and severe anxiety after recurrent binges and during withdrawal has been considered to be one of the most important factors that maintains repetitive cycles of chronic cocaine use.

Previous experiments have suggested that the amygdala is involved in both temporal lobe epilepsy and in cocaine mechanisms in the brain. The central nucleus of the amygdala (CeA) in particular is a highly epileptogenic brain area and, of the amygdaloid nuclei, responds most rapidly to a kindling stimulus (Le Gal La Salle 1981). The amygdala is also involved in the learning of stimulus-reward associations (Aggleton 1992; Gallagher and Holland 1994), the acquisition of drug-seeking behavior associated with cocaine self-administration (McGregor and Roberts 1993; Wilson et al. 1994), and the behavioral sensitization to cocaine (Kalivas and Alesdatter 1993; Richter et al. 1995). Amygdala lesions (Post et al. 1987) or MK-801, a glutamate N-methyl-D-aspartate (NMDA) antagonist, injected into the amygdala prevented behavioral sensitization to cocaine (Kalivas and Alesdatter 1993). The central nucleus of the amygdala is involved in the reinforcing efficacy of self-administered cocaine (Caine et al. 1995; McGregor and Roberts 1993) and in reward-related behavior affected by psychostimulants (Robledo et al. 1996). Amygdala sensitivity to electrical (Neugebauer et al. 1997a; Shinnick-Gallagher et al. 1998) and cocaine-induced kindling (Post et al. 1987) and similarities between kindling and behavioral sensitization suggest the amygdala might be especially vulnerable to repeated cocaine exposure.

G-protein-coupled metabotropic glutamate receptors (mGluRs) play important roles in neuroplasticity and nervous system disorders (Anwyl 1999; Conn and Pin 1997; Knöpfel et al. 1995; Pin and Duvoisin 1995), but their role in epilepsy and cocaine depen-
dence has not been studied in detail. Previous studies in our laboratory in the basolateral amygdala (Holmes et al. 1996; Keel et al. 2000; Neugebauer et al. 1997a,b) and others in the hippocampal dentate (Klapstein et al. 1999) have shown that mGluR responses are altered in kindling. Recent data also suggest, based on changes in mRNA, that mGluRs may play a role in the biochemical and behavioral effects of cocaine (Ghasemzadeh et al. 1999). Eight mGluR subtypes have been cloned and are classified into groups I–III based on their sequence homology, agonist pharmacology, and coupling to intracellular effector systems (Conn and Pin 1997; Knöpfel et al. 1995; Pin and Duvoisin 1995; Schoepp et al. 1999). Activation of mGluRs can produce excitatory or inhibitory effects on neurotransmission depending on the receptor type, synapse, and brain area (Choi and Lovinger 1996; Conn and Pin 1997; Davis and Laroche 1996; Gereau and Conn 1997a) and since cocaine itself can induce kindling (Post et al. 1987), we hypothesized that, in rats treated chronically in vivo with cocaine, the sensitivity to mGluR agonists in vitro would be enhanced particularly in the CeA where cocaine effects had been reported previously (Caine et al. 1995; McGregor and Roberts 1993; Robledo et al. 1996). To ensure that the effects of kindling were similar at different amygdala synapses, we examined the effect of kindling in CeA neurons. Here we report that kindling enhances the sensitivity of Group II and III mGluR agonists in CeA neurons but disprove our hypothesis and instead show that at the same synapses mGluR agonists are rendered ineffective after cocaine treatment.

METHODS

Cocaine treatment regimen

Adult male Sprague-Dawley rats (70–90 g) are housed three per cage with free access to food and water. Rats are either untreated or receive twice-daily (9:00 and 16:00) injections of saline (0.9%) or cocaine HCl (15 mg/kg ip; NIDA, Rockville, MD) for 14 consecutive days. No difference in weight gain of the animals injected with saline or cocaine is measured over this 14-day period (Gallagher and Simms, unpublished observations). The animals are monitored behaviorally to ensure that they are responding to cocaine. Previous studies from this group have analyzed behavioral activity and individual neuronal electrophysiology during and following 14 days of chronic cocaine treatment (Shoji et al. 1997; Simms and Gallagher 1996). Chronic cocaine treatment results in sensitization of locomotor activity and stereotyped behavior (e.g., gnawing, rearing, fast repetitive head and/or foreleg movement), which are rated on a six-point behavioral scale (Gifford and Johnson 1992) 15 min after cocaine injection. Twice-daily injections of cocaine for 2 wk produce behavioral sensitization (Cunningham 1988; Shoji et al. 1997) and electrophysiological changes in specific brain areas (locus ceruleus, Harris and Williams 1992; ventral tegmentum, Bonci and Williams 1996; septum, Shoji et al. 1997, 1998; nucleus accumbens, Manzoni et al. 1998, Zhang et al. 1998) other than the amygdala. In our studies with the amygdala slice, the electrophysiological experiments are conducted on the day following the last injection of cocaine (15 mg/kg ip; twice daily; 14 days) or saline.

Kindling

Rats (70–90 g) are anesthetized with 35 mg/kg pentobarbital sodium and 145 mg/kg chloral hydrate (Equithesin) and implanted (n = 14 kindled, n = 6 not stimulated) with tripolar electrodes (Plastics One, Roanoke, VA) into the right BLA as previously described (Holmes et al. 1996; Neugebauer et al. 1997a,b; Rainnie et al. 1992). Using the coordinates from Paxinos and Watson (1986), the tips of the two leads are positioned 2.0 mm posterior and 4.5 mm lateral to Bregma at a depth of 7.3 mm from the dura surface; the third lead served as a ground for monitoring and/or recording afterdischarges (ADs). Electrodes are fixed to the skull with dental acrylic (Plastics One). The kindling stimulation of the BLA is initiated after a postimplantation recovery period of 5 days. The stimulation consisted of a 2-s train of 60-Hz monophasic square waves, each 2 ms in duration, administered twice daily at least 8 h apart. Kindling stimulation is applied 50–100 µA above the AD threshold (200–400 µA). Behavioral seizure severity is rated according to the ranking scale of Racine (1972). The time to the fully kindled state is within a range of 6–14 days [mean: 9.7 ± 0.6 (SE) days, n = 14 animals]. Three to 7 (mean: 4.7 ± 0.4) days after three consecutive stage-five fully kindled seizures are evoked, animals (180–263 g) are killed and brain slices prepared for the electrophysiological experiments. Control slices are obtained from both unoperated (n = 18) and unstimulated-implanted (n = 6) rats.

Amygdala slice preparation

Brain slices containing the central nucleus of the amygdala (CeA) are obtained as previously described (Neugebauer et al. 1997a). Rats are decapitated, the brains quickly dissected out and blocked in cold (4°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 117 NaCl, 4.7 KCl, 1.2 NaH2PO4, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, and 11 glucose. ACSF is oxygenated and equilibrated to pH 7.4 with a mixture of 95% O2–5% CO2. Coronal brain slices (500 µm) are prepared using a Vibroslice (Campden Instruments, London, UK). After incubation in ACSF at room temperature (21°C) for ≥1 h, a single brain slice is transferred to the recording chamber and submerged in ACSF (31 ± 1°C), which superfuses the slice at ~2 ml/min.

Whole cell patch-clamp recording

“Blind” whole cell recordings (Blanton et al. 1989) are obtained from CeA neurons using patch electrodes made from 1.5 mm borosilicate glass capillaries (1.5 mm OD, 1.12 mm ID; Drummond, Broomall, PA) pulled on a Flaming-Brown micropipette puller (P-80/PC, Sutter Instrument, Novato, CA). Recording electrodes are positioned in the central medial and lateral capsular nuclei under visual control. The boundaries of the CeA are discerned under light microscopy; each slice is matched with the corresponding level in Paxinos and Watson (1986). The internal solution of the recording electrodes (3- to 5-MΩ tip resistance) contains (in mM) 122 K-gluconate, 5 NaCl, 0.3 CaCl2, 2 MgCl2, 1 EGTA, 10 HEPES, 5 Na2-ATP, and 0.4 Na3-GTP; pH is adjusted to 7.2–7.3 with KOH and the osmolality to 280 mmol/kg with sucrose. After tight (>2 GΩ) seals are formed and the whole cell configuration is obtained, neurons are included in the sample if the resting membrane potential is more negative than −55 mV and action potentials overshooting 0 mV are evoked by direct cathodal stimulation. Voltage and current signals are low-pass filtered at 1 kHz with a 4-pole Bessel filter (Warner Instrument, Hamden, CT), digitized at 5 kHz (Digidata 1200 interface, Axon Instruments, Foster City, CA), and stored on a computer (4DX2–66V, Gateway 2000). Data are also continuously recorded on a pen chart recorder (Gould 2400, Gould Instruments, Valley View, OH). Evoked potential and evoked current data are acquired and analyzed using pCLAMP6 software (Axon Instruments, Valley View, OH).
Instruments). Discontinuous single-electrode voltage-clamp (d-SEVC) recordings are acquired using an Axoclamp-2A amplifier (Axon Instruments) with a switching frequency of 5–6 kHz (30% duty cycle); gain of 3–8 nA/mV, time constant 20 ms. Phase shift and anti-alias filter are optimized. The headstage voltage is monitored continuously on an oscilloscope (Tektronix, Pittsfield, MA) to ensure precise performance of the amplifier.

Synaptic stimulation

The CeA represents the major output nucleus of the amygdala and processes information from other amygdala nuclei and from widespread brain areas. We studied two synapses in the CeA: the BLA-CeA and ventral amygdaloid pathway (VAP)-CeA synapse. Excitatory postsynaptic currents (EPSCs) are elicited with concentric bipolar stimulating electrodes (SNE-100, 22 kΩ resistance, Kopf Instruments) placed on two synaptic pathways to the CeA nucleus. One of the afferent synapses to the CeA, the VAP-CeA synapse, provides afferent inputs from brain stem areas (cf. Alheid et al. 1995; Bernard et al. 1993; Harrigan et al. 1994; T. S. Gray, personal communication), and the synapse between the BLA-CeA provides information about intramygdala communication flow. For stimulation of the VAP, the electrode is positioned under microscopic control on the fibers dorsomedial to the CeA and ventral to but outside of the caudate-putamen (T. S. Gray, personal communication). Electrical stimuli (150-μs square-wave pulses) are delivered at frequencies <0.25 Hz. Thresholds for EPSCs and spiking are defined as the respective intensity, which evoked a response in ≥5 of 10 trials with mean amplitude determined from the 10 trial stimulations. Input-output relations are obtained by increasing the stimulus intensity in 1-V steps. For evaluation of a drug effect on synaptically evoked responses, the stimulus intensity is adjusted to 75–80% of the intensity required for orthodromic spike generation.

Drugs

The following drugs were used: (2S,1’S,2’S)-2-(carboxycyclopropyl)glycine (LCCG1), L(+)-2-amino-4-phosphonobutyrate (LAP4), 7-(hydroxyimino)cyclopropa[b] chromen-1α-carboxylate ethyl ester (CPCCOEt); 2-methyl-6-(2-phenylethynyl)pyridine (SIB-1893); picROTOX (PTX); (2S)-3-[(15)-1-(3,4-dichlorophenyl) ethyl] amino-2-hydroxypropyl phenylmethyl phosphinic acid (CGP 55845); all purchased from Tocris Cookson, Bristol, UK. (+)2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) was a generous gift from Eli Lilly and Company. Drugs were applied by gravity-driven superfusion in the ACSF. Solution flow into the recording chamber (1 ml volume) was controlled with a three-way stopcock. Applications were ≥10 min (usually 12–14 min) in duration to establish equilibrium in the tissue.

Data analysis and statistics

Averaged values are given as the means ± SE. Differences between neurons from fully kindled animals and animals chronically treated with cocaine and control neurons in membrane potential, input resistance, slope conductance, EPSC threshold, and spike threshold are evaluated for statistical significance using a one-way ANOVA followed by a post hoc Dunnett’s multiple comparison test. Input-output relationships and concentration-response relationship of the agonists’ effects on synaptic transmission are compared between control neurons and neurons either from animals chronically treated with cocaine or from kindled animals using the two-way ANOVAs. Significance of the effects of LCCG1, LY354740, and LAP4 on synaptic transmission in neurons from kindled animals and/or animals chronically treated with cocaine is determined using an F test to analyze the linear regression fitted to the concentration-response data (Prism 3.0, Graph Pad Software, San Diego, CA). Linear analysis is done to show “no effect” and applies only to the cocaine group. Concentration-dependence effects of drugs on the slope conductances are evaluated with repeated measures ANOVA followed by post hoc t-tests where appropriate. Statistical significance is accepted at the level P < 0.05. EC50 and 95% confidence intervals are calculated from sigmoid curves fitted to the cumulative concentration-response data by non-linear regression using the formula y = A + (B - A)/(1 + (X/EC50)^n); where A = bottom plateau, B = top plateau, C = log (EC50), D = slope coefficient. Using the linear curve fit function of pCLAMP6 software (Axon Instruments), slope conductances (in nS) in the absence and presence of agonists are calculated from the linear portion of the current-voltage (I-V) relationships recorded in voltage-clamp mode.

**RESULTS**

Animals in these studies belonged either to the control group (consisting of naïve untreated animals = 18, neurons = 37; saline injected rats = 8, neurons = 12; or implanted, nonstimulated rats = 6, neurons = 10) or to the fully kindled animal (n = 14; neurons = 23) or chronic cocaine (n = 12; neurons = 22) animal treatment groups. Membrane properties and characteristics of synaptic transmission (Table 1) were compared, in naïve control, saline-injected control and control implanted, nonstimulated animals. No differences were found between the control groups and the data were pooled and termed “controls” (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1. Chronic cocaine treatment and kindling in vivo alter membrane properties and synaptic transmission in central amygdala neurons recorded in vitro</th>
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<tbody>
<tr>
<td>Control Neurons</td>
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<tr>
<td>Resting membrane potential, mV</td>
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<tr>
<td>Input resistance, MΩ</td>
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<tr>
<td>Slope conductance, nS</td>
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<tr>
<td>Synapse</td>
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<td>VAP-CeA</td>
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<td>EPSC threshold V</td>
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<td>Spike threshold V</td>
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<tr>
<td>BLA-CeA</td>
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<tr>
<td>EPSC threshold V</td>
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<td>Spike threshold V</td>
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</tbody>
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Values are means ± SE. Parentheses enclose number of neurons. Control neurons are comprised of naïve control, saline-injected, and implanted, nonstimulated; *P < 0.05; †P < 0.01; compared to control neurons, one-way ANOVA followed by Dunnett’s multiple comparison test. VAP, ventral amygdaloid pathway; CeA, central amygdala; BLA, basolateral amygdala; EPSC, excitatory postsynaptic current.
Membrane properties and synaptic transmission in neurons from animals chronically treated with cocaine showed several characteristics that distinguished them from control neurons. The resting membrane potential of CeA neurons in brain slices from rats treated chronically with cocaine was, on average, significantly hyperpolarized (Table 1; $P < 0.01$, Dunnett’s test) and the input resistance was significantly lower ($P < 0.05$, Dunnett’s test) compared with control neurons. Accordingly, the average slope conductance calculated from the linear portion of the current-voltage ($I$-$V$) relationship was greater in neurons from animals chronically treated with cocaine, on average, ($P < 0.01$, Dunnett’s test). Chronic cocaine also altered the input-output relationship at the VAP-CeA synapse (Table 1).

**Kindling alters synaptic transmission but not membrane properties in CeA neurons**

Recordings were compared in neurons from control animals and from kindled rats 3–7 days after the last stage-five kindled seizure (Table 1). In contrast to those from animals chronically treated with cocaine, neurons from kindled animals did not show an alteration in resting membrane potential or input resistance or in the linear portion of the input-output relationship (Table 1; Fig. 1B; 2-way ANOVA: $F = 0.11_{1,672}$; $P > 0.05$). The amplitudes of EPSCs at the VAP-CeA synapse increased from threshold to generation of an action potential current over a stimulus intensity range of 8–18 V in control and from 8 to 15 V in neurons from kindled animals (Fig. 1B). EPSC thresholds (Table 1) did not differ between the groups although thresholds for synthetically evoked action potentials (APs) compared with control shams ($n = 23$; 19 naive, 4 implanted) but the EPSC thresholds do not differ between the 2 experimental groups. A and B: EPSCs were evoked by electrical stimulation of VAP afferents and peak EPSC amplitudes were averaged (means ± SE) for each sample of neurons and plotted as a function of the stimulus intensity. Neurons were held at −60 mV.

**Group II and group III mGluR agonists depress synaptic transmission in CeA control neurons**

**GROUP II mGluR AGONISTS.** In control neurons, a group II agonist, LCCG1, depressed the peak amplitude of EPSCs evoked at the BLA-CeA (Fig. 2A) and the VAP-CeA (not shown) synapses. LCCG1 (100 nM) reversibly depressed EPSCs to 50% of predrug control ($P = 0.0001$). LCCG1 (100 nM) also reduced the EPSC to 50% of control ($P = 0.0001$) and EPSC threshold ($P < 0.01$) were also altered at the BLA-CeA synapse (Table 1).
shown) synapses. The effect of LCCG1 was slowly reversible, usually within 20 min of washing with ACSF. A comparison of mGluR agonist EC_{50}s in Table 2 showed that LCCG1 was more potent at the BLA-CeA synapse than at the VAP-CeA synapse. Since LCCG1 can have agonist activities at other mGluR subtypes, including mGluR1, 5, 4a, and 8 (Conn and Pin 1997; Saugstad et al. 1997; Schoepp et al. 1997), we also tested the novel orally active compound LY354740, which is a highly selective group II mGluR agonist (Schoepp et al. 1997). LY354740 was more potent than LCCG1 but had qualitatively similar effects. LY354740 reversibly depressed EPSCs evoked at the BLA-CeA synapse (Fig. 2B) more potently than at the VAP-CeA synapse (Table 2). In addition, the maximum inhibition produced by the group II agonists was not significantly different from each other as evidenced by the overlapping 95% confidence intervals at the BLA-CeA synapse (LCCG1: 31.82–46.38%; LY354740: 21.15–51.22%). Similar maximum effects of LCCG1 and LY354740 were measured at the VAP-CeA synapse.

**GROUP II mGluR AGONIST AND GROUP I ANTAGONIST.** Recently it has been shown that activation of protein kinase C by group I mGluRs blocks the inhibitory effect of group II and group III mGluR agonists (Macek et al. 1998). It is possible that tonic activation of group I mGluRs could influence the inhibitory effect of group II mGluRs agonists. To examine this issue, the effect of group-II-selective agonist, LY354740, was tested on EPSC amplitude (Fig. 3A) in the absence (left) and presence (right) of selective antagonists for group I, mGluR1, CPCCOEt, (50 μM), and mGluR5, SIB-1893 (10 μM), in naïve slices. Application of LY354740 (1 μM) for 15 min significantly decreased the EPSC amplitude by 57.8% (paired t-test, \(P < 0.001, n = 4\)), an effect that was reversible in all cells tested after 15 min of wash with ACSF. In the presence of mGluR1 and -5 antagonists, CPCCOEt and SIB-1893, respectively, LY354740 (1 μM) showed a similarly significant inhibitory effect on EPSC amplitude (45.8% decrease; paired t-test, \(P < 0.001, n = 4\)). Analysis of the input-output relationships using a one-way ANOVA with post hoc Bonferroni comparisons showed no significant difference between the effects of LY354740 in the presence and absence of the group I mGluR antagonists. These results suggest that group II mGluR inhibition in the CeA is not affected by endogenous activation of group I mGluRs.

**GAMMA-AMINOBUTYRIC ACID (GABA) INHIBITION AND GROUP II mGluRs.** Our studies were typically performed in the absence of GABA antagonists since inhibition of GABA in the amygdala resulted in epileptiform bursting. To examine whether GABA inhibition affected group II mGluR inhibition, we tested LY354740 (1 μM) on EPSC amplitude before and after treatment with the GABA_{A} antagonist, picrotoxin (PTX, 50 μM) and the GABA_{B} antagonist, CGP 55845 (2 μM, Fig. 3B). Under these conditions, slices become prone to spontaneous bursting. To prevent bursting a low concentration of the non-N-methyl-D-aspartate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX, 3 μM), was added to the superfusing solution. In Fig. 3B is shown the inhibitory effect of LY354740 on the input-output relationship for EPSC amplitude in ACSF (left) and after perfusion with GABA_{A} and _B antagonists for 15 min (right). The data showed that the inhibitory effect of LY354740 on EPSCs elicited with a 12-V stimulus intensity is not different in the absence (72% de-

### Table 2. Summary of mGluR agonists ED_{50}s in control, kindled, and chronic cocaine-treated animals

<table>
<thead>
<tr>
<th></th>
<th>LA-BLA, nM</th>
<th>BLA-CeA, nM</th>
<th>VAP-CeA, nM</th>
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<tbody>
<tr>
<td>LCCG1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control/implanted</td>
<td>36</td>
<td>65.8</td>
<td>157.6</td>
</tr>
<tr>
<td>Kindled</td>
<td>1.2</td>
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<tr>
<td>LY354740</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control/saline</td>
<td>57.8</td>
<td>719</td>
<td></td>
</tr>
<tr>
<td>Chronic cocaine (NE)</td>
<td>(NE)</td>
<td>(NE)</td>
<td></td>
</tr>
<tr>
<td>Control/implanted</td>
<td>2.1</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>Kindled</td>
<td>10.8</td>
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<td>3.7</td>
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<tr>
<td>LAP4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control/saline</td>
<td>297</td>
<td>54.8</td>
<td>17.6</td>
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<tr>
<td>Chronic cocaine (NE)</td>
<td>(NE)</td>
<td>(NE)</td>
<td></td>
</tr>
<tr>
<td>Control/implanted</td>
<td>80.7</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>Kindled</td>
<td>(NE)</td>
<td>(NE)</td>
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* Data from Neugebauer et al. (1997). NE, no effect; LA, lateral amygdala; mGluR, metabotropic glutamate receptor; LCCG1, (S,1’S,2’S)-2-(carboxycyclopropyl)glycine; LY354740, (+)-2-amino-3-(1H,4H)-hexane-2,6-dicarboxylic acid; LAP4, (1S,2R)-2-amino-4-phosphobutyrate.

**Fig. 3.** mGluR group I antagonists or GABA_{A} and _B antagonists do not alter the inhibitory effect of LY354740 on EPSC amplitude in the CeA. A: bath application of LY354740 (1 μM) decreased the amplitude of the EPSC as shown by the input-output curve (left). Right: the inhibitory effect of LY354740 in the presence of antagonists of mGluR1 [7-(hydroxyimino)cyclcopropyl]chromen-1a-carboxylate ethyl ester (CPCCOEt), 50 μM and mGluR5 [2-methyl-6-(2-phenoxyethyl)pyridine (SIB-1893), 10 μM] in the same cell as left panel. B: input-output curve before (●) and after LY354740 application (○) in the absence (left) and presence (right) of GABA_{A} and _B antagonists, picrotoxin (PTX, 50 μM) and [l(15)-1-(3,4-dichlorophenyl)ethyl amino-2-hydroxypropyl]phenylmethyl phosphonic acid (CGP 55845, 2 μM), respectively, and 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX, 3 μM). Each set of graphs represents data points from a single cell. In both experiments (A and B), cells were held at −64 mV.
Neither the group II agonists LCCG1 (up to 100 nM) nor LY354740 in the presence and absence of GABA antagonists, suggesting that GABA inhibition did not influence group II mGluR inhibitory effects.

**GROUP III mGluR AGONIST.** LAP4 depressed the peak amplitude of EPSCs in control neurons (n = 7), and this effect was readily reversible within 10–12 min of washing with control ACSF (Fig. 2C). Unlike the group II agonists, however, the concentration response curve revealed that LAP4 had a lower potency at the BLA-CeA synapse than at the VAP-CeA synapse (Table 2), but the maximum effect was similar at both synapses. Furthermore, the effects of LAP4 in CeA neurons are more potent than on BLA neurons (cf. Neugebauer et al. 1997a) (Table 2).

**Chronic cocaine treatment renders group II and group III mGluRs functionally ineffective.**

The inhibitory effects of the group II and the group III agonists on synaptic transmission of CeA neurons were studied in brain slices from rats treated chronically with cocaine. Neither the group II agonists LCCG1 (up to 100 μM; n = 5; see Figs. 4A and 5A) and LY354740 (up to 10 μM; n = 5; see Fig. 5B) nor the group III agonist LAP4 (≤100 μM; n = 5; see Figs. 4B and 5C) were able to alter, significantly, evoked synaptic transmission at the BLA-CeA synapses in neurons from animals chronically treated with cocaine. Linear regression analysis of the concentration response curves using a F test and the runs test (Graphpad Prism 3.0) showed that the slope was not significantly different from zero and the data were not significantly nonlinear (P > 0.05 in each case). These tests indicated that linear regression analysis was appropriate and there was no significant change of EPSC amplitude associated with the different agonist concentrations.

**Effects of group II and group III agonists on membrane conductance in neurons from control and from chronic cocaine and kindled animals.**

**GROUP II AGONIST.** The effects of LCCG1 and LAP4 on synaptic transmission were studied in brain slices from kindled animals 3–7 days after the last of three consecutive stage-five seizures.

**GROUP II AGONIST.** In kindled neurons, LCCG1 (100 nM) decreased EPSC amplitude to a greater extent than in control neurons (Fig. 6, A and C). Analysis of the concentration–response relationship (Fig. 6C) showed that the EC50 was shifted twofold to the left at the BLA-CeA synapse in kindled (32.8 nM) compared with control (65.8 nM) neurons. In contrast, at the VAP-CeA synapse, the EC50 was shifted 11-fold to the left (Table 2). The maximal effect of LCCG1 on EPSC amplitude was also enhanced in kindled neurons. At the BLA-CeA pathway, LCCG1 depressed EPSC amplitude in control neurons to a maximum value of 38 ± 6% of control; the depression of EPSC was greater after kindling with only 7 ± 4% of the initial EPSC amplitude remaining (Fig. 6C). Likewise, significant differences were found between the different concentrations (F = 45.26, P < 0.0001) and between control and kindled neurons (2-way ANOVA, F = 25.45, P < 0.0001). Similar alterations were recorded at the VAP-CeA synapse.

**GROUP III AGONIST.** We compared the depression of synaptic transmission induced by L-AP4 in control and kindled neurons at the two synapses in the central amygdala. LAP4 (1 nM) significantly altered EPSCs in kindled neurons to approximately the same extent as a higher concentration (100 nM) did in control neurons (Fig. 6B; n = 5). The concentration–response curve for L-AP4 at the BLA-CeA pathway was shifted almost 50-fold to the left in CeA neurons from kindled animals (Fig. 6D; Table 2). Maximal inhibition of EPSC amplitude was not altered with kindling (Fig. 6D). At the VAP-CeA synapse, a fivefold increase in potency was recorded (Table 2). At the BLA-CeA synapse, the differences in the effects of L-AP4 at the two different concentrations were significant (2-way ANOVA, F = 20.85, P < 0.0001) and between control and kindled neurons (F = 14.86, P < 0.0005).

**Effects of group II and group III agonists on membrane conductance in neurons from control and from chronic cocaine and kindled animals.**

**GROUP II AGONIST.** The group II mGluR agonists LCCG1 (≤10 μM) and LY354740 (≤1 μM) had no significant effect on postsynaptic membrane currents and membrane conductance in control neurons. Current-voltage (I-V) relationships for typical control neurons were not changed during the superfusion of LCCG1 (≤10 μM, n = 9) and LY354740 (≤1 μM, n = 5), and no significant changes in slope conductance (repeated-measures ANOVA with post hoc t-test, P > 0.1) were measured (Fig. 7). Superfusion with higher concentrations of LCCG1 (100 μM, Fig. 7A) and LY354740 (10 μM, Fig. 7B) increased the slope conductance significantly in control neurons (post hoc t-test following repeated-measures ANOVA, P < 0.05). At high concentrations, a small (<20 pA) outward current was observed in four of seven neurons with LCCG1 (100 μM) and in two of five neurons with LY354740 (10 μM); in another two neurons, a small inward current (10–15 pA) was recorded in the presence of LCCG1 (100 μM).
Figure 5. The concentration-dependent inhibition of synaptic transmission by group II and III mGluR agonists is lost following chronic cocaine treatment. A: EC\textsubscript{50} for LCCG1 in control neurons is 57.8 nM, but, in neurons from animals chronically treated with cocaine LCCG1, has no significant effect on synaptic transmission. B: the concentration-dependent inhibition of synaptic transmission by LY354740 is lost in neurons from animals chronically treated with cocaine. In control neurons, LY354740 depressed EPSCs evoked at the BLA-CeA synapse with an EC\textsubscript{50} of 2.1 nM, but in neurons from animals chronically treated with cocaine, LY354740 has no significant effect on synaptic transmission. C: the group III mGluR agonist LAP4 inhibits synaptic transmission in a control neuron at the BLA-CeA synapse (EC\textsubscript{50} = 80.7 nM) but has no effect in neurons from animals chronically treated with cocaine. A–C: the peak amplitudes of the EPSCs obtained with each concentration of agonist in control neurons and neurons from animals chronically treated with cocaine and expressed as percent of predrug control values (100%). Symbols and error bars represent means ± SE. Linear regression analysis of the concentrations response curves using an F test, and the runs test showed that the slope was not significantly different from 0 and the data were not significantly nonlinear (P > 0.1 in A–C). EC\textsubscript{50} values were calculated from the sigmoid curves fitted to the cumulative concentration-response data by nonlinear regression.
and LY354740 (10 μM). These data showed that LY354740 (10 μM), and LCCG1 (<1 μM), while clearly modulating evoked EPSCs, did not affect postsynaptic membrane conductance.

GROUP III AGONIST. In control neurons, LAP4 neither evoked detectable membrane currents nor caused changes in the I-V relationship at concentrations up to 100 μM. However, at higher concentrations (100 μM), LAP4 significantly decreased the slope conductance, whereas an increase in slope conductance was measured with LCCG1 (100 μM) and LY354740 (10 μM). For each neuron the slope conductance was calculated from the current-voltage relationships in the presence and absence of the different agonist concentrations. The averaged differences are shown as means ± SE. *, P < 0.05; ***, P < 0.005, post hoc t-test following repeated-measures ANOVA.
ANOVA, \( P < 0.05 \) and evoked a small (<20 pA) inward current in five of nine control neurons.

The postsynaptic membrane properties of neurons from animals treated chronically with cocaine were not altered by the group II and the group III agonists (Fig. 7). Slope conductance calculated in the absence and presence of the different agonist concentrations was not different using a repeated-measures ANOVA \((P > 0.05, n = 5, \text{each agonist})\). A similar lack of effect was found in neurons from fully kindled animals. No alterations in slope conductance were detected for these agonists at any concentration tested \((10 \text{ nM to } 100 \text{ µM; repeated-measures ANOVA, } P > 0.05, n = 5, \text{for each agonist})\).

**Discussion**

Whole cell voltage-clamp recordings of CeA neurons were utilized to study in vitro the role of mGluR as modulators of synaptic transmission in brain slices from control animals, fully kindled animals, and animals treated chronically with cocaine. The main findings of this study are: synaptic transmission at two synapses, the BLA-CeA and VAP-CeA, are depressed by group II and III mGluRs agonists; chronic cocaine treatment but not kindling alters membrane properties of CeA neurons; synaptic transmission is enhanced in neurons from both kindled and cocaine-treated animals; sensitivity of group II and group III mGluR agonists is increased in kindling; and the mGluR agonists lose their ability to modulate synaptic transmission in neurons from animals treated chronically with cocaine.

**Effects of mGluR activation on synaptic transmission in the CeA nucleus**

Differences in agonist potencies between the synapses (Table 2) may be due to the distribution of mGluR subtypes. Both LY354740 and LCCG1 are more potent at group II mGluRs but the potency of LCCG1 for mGluR8 in some systems is equivalent to that of group II mGluRs; its effects on group I subtypes is 10 times less than at group II (Schoepp et al. 1999). LY354740 is \sim 10 times more potent than LCCG1 on group II mGluRs (Schoepp et al. 1999). Interestingly, the \( EC_{50} \) ratios LCCG1 to LY354740 for saline-injected control animals at the BLA- and VAP-CeA synapses is 28:1 and 31:1, respectively, suggesting that both agonists may act through a similar group II receptor. LAP4 is more potent on the group III subtypes mGluR4 and mGluR8 than on mGluR7 (Conn and Pin 1997; Schoepp et al. 1999), and on cloned mGluR8, LAP4 is 10 times more potent than LCCG1 (Wu et al. 1998). The agonist potencies for LCCG1 and LAP4 in the amygdala are greater than measured elsewhere (Anwyl 1999: Conn and Pin 1997; Schoepp et al. 1999). It has been shown that potency of agonists in cloned systems is dependent on the amount of receptor expressed (Hermans et al. 1999), and it is possible that there are significant numbers of spare mGluRs in the amygdala. Alternatively, mGluRs in the amygdala may have a highly efficient coupling mechanism to their G-protein-mediated second-messenger effectors where high occupancy of receptors is not needed and/or where amplification of the response occurs (see Kenakin 1996). Definitive determination of mGluR subtypes mediating effects in the CeA awaits the ready availability of specific agonists and antagonists.

**Changes of mGluR subgroups following chronic cocaine exposure**

The intriguing finding of this study is that with chronic cocaine-treatment group II and group III mGluR agonists lose their effectiveness to inhibit synaptic transmission. The functional loss of sensitivity to group II and group III mGluR agonists may be due to multiple mechanisms. One possibility is that this loss is due to changes in the subtype composition. This mechanism is unlikely since both subgroups are affected equally. An alternative possibility is that chronic cocaine exposure may interfere with one or more step(s) in the signal transduction cascade i.e., the G-protein-\((G_{i}/G_{o})\)-mediated inhibition of voltage-dependent calcium currents, negative coupling to adenylyl cyclase, or, a mechanism downstream from calcium influx through voltage-gated calcium channels (Conn and Pinn 1997; Schoppa and Westbrook 1997; Toms et al. 1996). Chronic cocaine treatment has been shown to decrease the levels of the G-protein subunits \(G_{ia}\) and \(G_{oa}\) in brain areas involved in reward mechanisms (Nestler et al. 1990, 1993) and pertussis toxin-sensitive G proteins have been implicated in behavioral sensitization to cocaine (Steckee et al. 1991). Chronic morphine increases levels of \(G_{ia}\) and \(G_{oa}\) in the amygdala (Nestler et al. 1989; Terwilliger et al. 1991) and cocaine sensitization is associated with an upregulation of a beta subunit of a G protein (Wang et al. 1997). Furthermore, it is hypothesized that an altered G-protein-dependent mechanism may underlie the reduced effectiveness of presynaptic metabotropic GABA\(_B\) receptors in the septum after chronic cocaine treatment (Shoji et al. 1997). Alterations in the adenylyl cyclase/cAMP system, on the other hand, may not account for the chronic cocaine-induced changes of group II and III effectiveness because extracellular levels of adenosine, due to elevated cAMP, are increased in the VTA after chronic cocaine treatment (Bonci and Williams 1996). Protein kinase C (PKC) activation has been proposed to inhibit the function of group II and III mGluRs (Macek et al. 1998; Schatz et al. 1993; Tyler and Lovinger 1995) by uncoupling the mGluRs from their G proteins (Macek et al. 1998). Group I mGluR or adenosine A3 receptor activation can increase a G-protein PKC and subsequently may block group II or III inhibition of synaptic transmission (Macek et al. 1998). Block of tonic activation of mGluR1 and -5 with specific antagonists did not affect group II mGluR inhibition of synaptic transmission in control CeA neurons, and the presynaptic sensitivity to adenosine is reduced on cocaine withdrawal (Manzoni et al. 1998). It is possible, however, that the higher levels of adenosine (Bonci and Williams 1996) may activate A3 receptors, increase endogenous PKC, and block the inhibitory action of group II and III mGluR agonists with chronic cocaine treatment. Future experiments are designed to address this hypothesis.

The loss of mGluR-mediated inhibitory effects may have significant implications in neuroadaptive changes occurring with chronic cocaine use. Evidence suggests that drug dependence is associated with motivational and affective aspects of withdrawal reflected in an altered brain reward system (Koob et al. 1998). The amygdala is involved in stimulus-reward associations (Aggleton 1992; Gallagher and Holland 1994). Group II mGluRs may play a role in anxiety since the orally active group II mGluR agonist, LY354740, has significant anxiolytic properties (Helton et al. 1998; Monn et al. 1997).
Group II mGluR agonists and antagonists also attenuate morphine withdrawal symptoms (Fundytus and Coderre 1997; Fundytus et al. 1997). If group II mGluR activation is necessary to modulate anxiety, their loss may contribute to counter-adaptive changes following chronic cocaine, including anxiety, dysphoria, depression, and irritability (Koob 1996; Koob et al. 1998; Markou and Koob 1991).

**Chronic cocaine-induced changes in electrophysiological properties in CeA neurons**

Chronic cocaine treatment enhanced CeA excitatory synaptic transmission as evidenced by the leftward shift of the input-output relationships recorded at VAP-CeA and BLA-CeA synapses. A lower EPSC threshold was measured at both synapses, while a lower spike threshold was recorded only at the VAP-CeA synapse, suggesting that chronic cocaine differentially altered the gain of CeA synapses. Increase in glutamate release was measured in the nucleus accumbens after chronic cocaine treatment (Pierce et al. 1996). In brain slices from cocaine-treated rats, current-clamped CeA neurons had a more hyperpolarized membrane potential and their input resistance was significantly lower compared with control neurons. These findings would be expected to reduce excitability by shunting the membrane conductance; however, synaptic transmission in chronic cocaine animals is enhanced, suggesting that the membrane shunting is overcome. Chronic exposure to cocaine induced similar changes in membrane potential and input resistance in dorsolateral septum neurons (Shoji et al. 1997, 1998; Simms and Gallagher 1996), and nucleus accumbens neurons (Zhang et al. 1998). If changes in membrane properties and synaptic transmission, as measured in CeA neurons of animals treated chronically with cocaine, also occurs in cocaine-dependent individuals, these changes may contribute to their altered emotional status since the CeA is the common output nucleus for major amygdala functions and modulates various effector systems involved in the expression of emotional responses through connections with the forebrain, limbic system, and brain stem (Aggleton 1992; Alheid et al. 1995; Gallagher and Holland 1996; Maren 1996).

Synapses and times of withdrawal after chronic cocaine produce differing electrophysiological changes in membrane properties and/or synaptic transmission in different preparations. Following a withdrawal of 7–10 days, changes in synaptic transmission were observed in neurons from locus ceruleus and ventral tegmental (Bonci and Williams 1996; Harris and Williams 1992) but were not observed in hippocampal (Manzoni et al. 1998) or the latter two nuclei (Bonci and Williams 1996; Harris and Williams 1992) 1 day after the last chronic cocaine injection. After a withdrawal interval of 3 days changes in voltage-gated sodium current, in membrane potential, and input resistance were detected in isolated nucleus accumbens neurons from rats treated chronically with cocaine (Zhang et al. 1998). Changes in membrane properties and synaptic transmission also occurred after 1 day of withdrawal in the dorsolateral septal nucleus (Shoji et al. 1998; Simms and Gallagher 1996) and in the CeA. The septum and amygdala appear to be sensitive to early withdrawal from chronic cocaine but the persistence of these effects is not known although limited preliminary studies in the amygdala suggest that a decrease in the effects of mGluR agonist are still present after 7 days (Zinebi, Tokarski, and Shinnick-Gallagher, unpublished observations). Future studies are directed at determining the development and withdrawal time courses of these effects.

**Kindling-induced changes in electrophysiological properties in CeA neurons**

In contrast to chronic cocaine treatment, membrane conductance in neurons from kindled animals is not altered although the threshold for action potential generation is lowered in CeA neurons. These data are similar to findings in BLA neurons (Asprodini et al. 1992; Neugebauer et al. 1997a; Rainnie et al. 1992). One difference in synaptic afferent pathways to the BLA and the CeA in kindled animals is that input-output relationships, a measure of information transfer, in the lateral (LA)-BLA synapse occurs over a <2 V range (Asprodini et al. 1992; Neugebauer et al. 1997a; Rainnie et al. 1992) while at the VAP-CeA (or BLA-CeA) synapses the relationships happen over a greater voltage range, suggesting that within the amygdala there may be a greater amplification of signal transfer in kindled neurons at the LA-BLA synapse than at the BLA-CeA synapse.

**Kindling-induced changes in mGluRs in CeA neurons**

In CeA neurons from amygdala kindled animals, the sensitivity to inhibitory effects of mGluR group II and III agonists were enhanced and the magnitudes of change were different for the different agonists. The shift in the EC<sub>50</sub> for LCCG1 and LAP4 were 2- and 50-fold, respectively, in CeA neurons at the BLA-CeA synapse, whereas mGluR group II and III agonists depress synaptic transmission with a respective 30- and 28-fold increase in potency at the LA-BLA synapse in BLA neurons from amygdala-kindled animals (Neugebauer et al. 1997a). Interestingly, the inhibitory effects of 1-serine-O-phosphate (LSOP), a group III agonist, are reduced in hippocampal dentate granule cells with kindling, suggesting that the effects of kindling on mGluRs may be different in different parts of the brain (Klapstein et al. 1999). Comparison between EC<sub>50</sub> s for LAP4 and LCCG1 showed that LAP4 was 30 times more potent than LCCG1 in kindled neurons at the BLA-CeA synapse and ~4 and 8 times more potent at the VAP-CeA and LA-BLA synapses, respectively. These data suggest that the subtypes of mGluRs underlying the enhanced inhibitory effects of agonists at the various amygdala synapses may be different.

Recent data show that the immunoreactivity to mGluR4 is upregulated in patients with temporal lobe epilepsy (Lie et al. 2000). The inhibitory responses to group II and group III agonists are upregulated in kindling, and, in the BLA, these agents can block epileptiform bursting in kindled animals (Neugebauer et al. 1997a). In light of these findings, it is reasonable to suggest that the group II or III agonists may be useful in the treatment of epilepsy.

**Chronic cocaine treatment and kindling**

The data suggest that CeA synaptic transmission is fine-tuned through modifications of mGluR subgroups in different models of synaptic plasticity. Amygdala sensitivity to electrical (Neugebauer et al. 1997a; Shinnick-Gallagher et al. 1998) and cocaine-induced kindling (Post et al. 1987) and similarities between kindling and behavioral sensitization to cocaine sug-
gested that alterations in mGluR agonist action would be analogous in the two models. Chronic cocaine exposure and electrical kindling alter the sensitivity of mGluR subgroups in different directions. These findings suggest that the mechanisms underlying these modifications may be complex. Irrespective of those mechanisms, drugs that activate group II and group III mGluRs may be novel therapeutic tools in the treatment of limbic temporal lobe epilepsy, and those that restore the inhibitory effects may be of value in chronic cocaine dependence.

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