Exposure to Cocaine Alters Dynorphin-Mediated Regulation of Excitatory Synaptic Transmission in Nucleus Accumbens Neurons

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Background: Dysregulation of excitatory synaptic input to nucleus accumbens (NAc) medium spiny neurons (MSNs) underlies a key pathophysiology of drug addiction and addiction-associated emotional and motivational alterations. Dynorphin peptides, which exhibit higher affinity to κ type opioid receptors, are upregulated within the NAc upon exposure to cocaine administration, and the increased dynorphin-signaling in the NAc has been critically implicated in negative mood observed in cocaine- or stress-exposed animals. Despite such apparent behavioral significance of the NAc dynorphins, the understanding of how dynorphins regulate excitatory synaptic transmission in the NAc remains incomplete.

Methods: We used electrophysiological recording in brain slices to examine the effects of dynorphins on excitatory synaptic transmission in the NAc.

Results: We focused on two key dynorphins, dynorphin A and B. Our current results show that dynorphin A and B differentially regulated excitatory postsynaptic currents (EPSCs) in NAc MSNs. Whereas perfusions of both dynorphin A and B to NAc slices decreased EPSCs in MSNs, the effect of dynorphin A but not dynorphin B was completely reversed by the κ receptor-selective antagonist nor-binaltorphimine. These results implicate κ receptor-independent mechanisms in dynorphin B-mediated synaptic effects in the NAc. Furthermore, repeated exposure to cocaine (15 mg/kg/day via intraperitoneal injection for 5 days, with 1, 2, or 14 days withdrawal) completely abolished dynorphin A-mediated modulation of EPSCs in NAc MSNs, whereas the effect of dynorphin B remained largely unchanged.

Conclusions: Given the quantitatively higher abundance of dynorphin B in the NAc, our present results suggest that the dynorphin B-mediated, κ receptor-independent pathways predominate in the overall effect of dynorphins in cocaine-pretreated animals and potentially in cocaine-induced alterations in mood.

Key Words: Action potential, cocaine, membrane excitability, nucleus accumbens, withdrawal

The nucleus accumbens (NAc) has long been hypothesized as a key brain site that mediates emotional and motivational responses (1,2). Medium spiny projection neurons (MSNs) contribute to >90% of the neuronal population within the NAc (3). Malfunction of these NAc MSNs underlies a key pathophysiology of emotional and motivational distortions associated with cocaine addiction (1,2). The functional output of NAc MSNs heavily relies on excitatory synaptic input; it is the synchronous excitatory synaptic inputs that drive MSNs into functionally active states in which MSNs execute their actions by firing action potentials (4). Excitatory synaptic transmission to NAc MSNs is regulated by a myriad of neuromodulator systems. Distortion of these regulations is presumably one way in which pathogenic stimulations, such as exposure to cocaine or stress, reshape the functional output of NAc, resulting in emotional and motivational alterations (1,5).

Among the neuromodulator-based regulation of NAc MSNs, dynorphin-signaling is particularly important for stress- or addiction-associated negative mood (6,7). Dynorphins comprise a family of biologically active peptides derived from the common precursor prodynorphin (8) and are enriched in the NAc (9). Exposure to stress or drugs of abuse upregulates the gene expression of prodynorphin in the NAc, and experimental upregulation of NAc dynorphin-signaling produces aversive behaviors (6). Despite our understanding of the behavioral effects of dynorphins, much less in known about how dynorphins regulate excitatory synaptic transmission in the NAc.

The two key dynorphins derived from prodynorphin are dynorphin A (DynA) and B (DynB). Early immunohistochemical results from the NAc and dorsal striatum show that DynB is more abundant than DynA (9). Additionally, both DynA and DynB are thought to be endogenous agonists of κ receptors on the basis of affinity studies, although their selectivity for κ receptors over other opioid receptors is poor (10). Using a more selective κ receptor agonist (U69593), recent studies demonstrated that activation of κ receptors acutely decreases excitatory postsynaptic currents (EPSCs) in NAc MSNs, an effect likely mediated by inhibition of presynaptic glutamate release (11). However, it is not clear whether this effect holds for DynA and DynB and whether this κ receptor-mediated synaptic modulation is affected by dynorphin-associated pathophysiological conditions. Here we demonstrated that, similar to U69593, DynA inhibited EPSCs in NAc MSNs, an effect that could be reversed by κ receptor-selective antagonist nor-binaltorphimine (nor-BNI). In contrast, although DynB also inhibited EPSCs in NAc MSNs, this effect was not completely reversed by nor-BNI, suggesting a κ receptor-independent mechanism. Moreover, after repeated exposure to cocaine, the effect of DynA on EPSCs in NAc MSNs was abolished, whereas the effect of DynB was still present. Therefore, DynA and DynB might use different cellular and molecular mechanisms in regulating excitatory synaptic transmission to NAc MSNs and thus might differentially regulate NAc-based emotional and motivational responses.

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Methods and Materials

Animal Use

Male Sprague–Dawley rats at an age of 22–24 days were allowed to acclimate to their home-cage (housed individually) for 5–7 days with free access to food and water under a 12:12-hour light/dark cycle. Rats were then either kept in the home cage for an additional 7 days or received cocaine/saline administration for 5 days, followed by a 1–2-day withdrawal period. Rats at 35–40 days (with or without cocaine treatment) were used for electrophysiological recording.

Intraperitoneal Injection of Cocaine

We used a 5-day procedure of repeated cocaine administration, which was similar to earlier studies (12–14). Briefly, rats received one intraperitoneal injection (IP) of either (-)cocaine hydrogen chloride (15 mg/kg) or the same volume of saline/day for 5 days. Injections were performed within the home-cage at approximately 9:00 AM each day. Treated rats were then killed for electrophysiological recordings 1–2 days or 2 weeks after the last injection.

NAc Slice Preparation, Cell Selection, and Electrophysiology

Detailed procedures for obtaining NAc slices can be found in our previous publications (13,15–17). Briefly, coronal NAc slices of 250–300-μm thickness were cut such that the preparation contained the signature anatomical landmarks that delineated the NAc subregions. Slices were submerged in a recording chamber and were continuously perfused with regular oxygenated artificial cerebrospinal fluid (in mmol/L: 126 sodium chloride, 1.6 potassium chloride, 1.2 sodium dihydrogen phosphate, 1.2 magnesium chloride, 2.5 calcium dichloride, 18 sodium bicarbonate, and 11 glucose, 295–305 mOsm, equilibrated at 31–34°C with 95% oxygen/5% carbon dioxide).

Electrophysiological recordings were preferentially made from the MSNs located in the ventral-medial subregion of the NAc Shell (referred to as NAc MSNs in this study). Standard whole-cell recordings were made with a MultiClamp 700B amplifier (Molecular Device, Foster City, California) through an electrode (2–6 mΩ) in all electrophysiological experiments. Voltage-clamp recordings were used to measure EPSCs and inhibitory postsynaptic currents (IPSCs) in NAc MSNs. The intracellular and extracellular solutions used can be found in our published papers for EPSC (15,16) and IPSC (18,19) recording. To record EPSCs and miniature excitatory postsynaptic currents (mEPSCs), the extracellular solution routinely contained picrotoxin (.1 mmol/L) to block γ-aminobutyric acid–A receptor-mediated currents. To record IPSCS, the extracellular solution contained 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; 5 μmol/L) and D(-)2-amino-5-phosphonovaleric acid (D-APV; 50 μmol/L) to block α-amino-3-hydroxy-5-methylisoxazole propionate receptor- and *N*-methyl-D-aspartate receptor-mediated currents. For evoked EPSCs and IPSCs, presynaptic stimuli (intensity, 200–500 μA; duration, 300–600 μs; frequency, .1 Hz) were applied through a monopolar microelectrode. The stimulating electrode was placed close to recorded neurons (approximately 3–4 cells away), and the amplitude of EPSCs was adjusted within approximately 80–150 pA; both of these efforts were made to minimize the potential spatial effect (20). Evoked EPSCs were recorded at a holding potential of −70 mV, and evoked IPSCs were held at −10 mV. Amplitudes of evoked EPSCs and IPSCs were calculated by averaging 30 traces and measuring the peak (1-msec window) compared with the baseline (1-msec window). All chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri). Dynorphin peptides, nor-BNI, and U69593 were provided by the Drug Supply Program of the National Institutes of Health National Institute on Drug Abuse.

Data Acquisition, Analysis, and Statistics

One to three cells were obtained from each rat. For experiments involving cocaine/saline administration, at least four rats were used in each treatment group. The numbers of cells (*n*) was used in all statistics. All results are shown as mean ± SEM. Paired *t* test was used for all results involving comparisons of the peak amplitudes of EPSCs or IPSCs before, during, and after (wash-out period) perfusion of pharmacological manipulations.

Results

To examine the effects of dynorphins on excitatory synaptic transmission within the NAc, we used whole-cell voltage-clamp techniques in acute brain slices to record evoked EPSCs in NAc MSNs. We first examined the effect of DynA. After establishing a stable baseline of EPSCs, we perfused the brain slice with DynA (1 μmol/L) through recording bath. Upon application of DynA, the amplitude of EPSCs in NAc MSNs was significantly decreased (amplitude relative to baseline: 70.9 ± 6.6%, $n = 6$; $p < .01$, paired *t* test) (Figures 1A and 1B). This DynA-induced effect could not be washed out; it persisted after being perfused by DynA-free bath for the rest of the experiment (> 20 min) (relative to baseline: 76.4 ± 12.9%; $p = .01$, vs. baseline, paired *t* test) (Figures 1A and 1B). Accompanying the DynA-induced decreased in the amplitude, the paired pulse ratio (PPR) (amplitude of the second EPSC peak over the amplitude of first EPSC peak, 50-msec interpulse interval) of EPSCs was increased (baseline, 1.12 ± .13; DynA, 1.34 ± .14, $n = 6$; $p = .03$, paired *t* test) (Figures 1A and 1C), suggesting a presynaptic action of DynA. Similar to DynA, bath application of DynB (1 μmol/L) also decreased the peak amplitude of EPSCs (relative to baseline: 58.5 ± 7.2%, $n = 6$; $p < .01$, paired *t* test) (Figures 1D and 1E) and increased the PPR of EPSCs (baseline, 1.23 ± .08; DynB, 1.58 ± .22, $n = 6$; $p = .04$, paired *t* test) (Figures 1D and 1F) in NAc MSNs. Similar to DynA (Figure 1B), the effect of DynB was also only partially washed out (relative to baseline: 87.2 ± 9.9%; $p = .23$, vs. baseline, paired *t* test) (Figure 1E). Thus, DynA and DynB exhibited similar presynaptic effects on EPSCs in NAc MSNs in naive rats.

Opioid κ receptors are enriched in the presynaptic terminals in the dorsal and ventral striatum, and both DynA and DynB exhibit higher affinity for κ receptors over other opioid receptor subtypes (21,22). We thus examined whether the aforementioned effects of DynA were mediated by κ receptors. The approach we used was to test the effects of κ receptor-selective compounds on EPSCs in NAc MSNs. If κ receptors were the key to the observed effects, activation of κ receptors with highly selective agonists should mimic DynA-mediated effects, whereas preventing the activation of κ receptors with highly-selective antagonists should inhibit these effects. U69593 has been demonstrated as a highly selective agonist for κ receptors over other opioid receptors at submicromolar concentrations (21). In the same experimental setup as in the preceding text, bath application of U69593 (1 μmol/L) decreased the peak amplitude of EPSCs (relative to baseline: 71.7 ± 5.6%, $n = 5$; $p = .02$, paired *t* test) (Figures 2A and 2B) and increased the PPR of EPSCs in NAc MSNs (baseline, 1.20 ± .11; U69593, 1.75 ± .27, $n = 5$; $p = .02$, paired *t* test) (Figures 2A and 2C), an overall effect similar to that of DynA and DynB.

It has been demonstrated that nor-BNI is a highly selective κ receptor antagonist (21). Our results show that, when perfused alone, nor-BNI (.1 μmol/L) did not significantly affect either the peak amplitude (relative to baseline: 108.3 ± 7.8%, $n = 5$; $p = .45$, paired *t* test) (Figures 2D and 2E) or the PPR (baseline, 1.37 ± .11; nor-BNI, 1.23 ± .06, $n = 5$, $p = .47$) (Figures 2D and 2F) of EPSCs in NAc MSNs.