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Exposure to Cocaine alters Dynorphin-mediated Regulation of Excitatory Synaptic Transmission in Nucleus Accumbens Neurons

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Abstract

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 kappa type opioid receptors, are up-regulated within the NAc upon exposure to cocaine administration, and the increased dynorphin-signaling in NAc has been critically implicated in negative mood observed in cocaine- or stress-exposed animals. Despite such apparent behavioral significance of the NAc dynorphins, understanding of how dynorphins regulate excitatory synaptic transmission in NAc remains incomplete. Here, 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 kappa receptor-selective antagonist nor-Binaltorphimine. These results implicate kappa receptor-independent mechanisms in dynorphin B-mediated synaptic effect in NAc. Furthermore, repeated exposure to cocaine (15 mg/kg/day via intraperitoneal injection for 5 days, with 1 or 2 days withdrawal) completely abolished dynorphin A-mediated modulation of EPSCs in NAc MSNs, whereas the effect of dynorphin B remained largely unchanged. Given the quantitatively higher abundance of dynorphin B in the NAc, our present results suggest that the dynorphin B-mediated, kappa receptor-independent pathways predominate in the overall effect of dynorphins in cocaine-pretreated animals, and potentially in cocaine-induced alterations in mood.

Keywords

membrane excitability; cocaine; nucleus accumbens; action potential; withdrawal

Introduction

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 that 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 are a number 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 up-regulates the gene expression of prodynorphin in the NAc, and experimental up-regulation of NAc dynorphin-signaling produces aversive behaviors (6). In contrast to the behaviorally manifested importance of NAc dynorphins, it remains incompletely understood how dynorphins regulate excitatory synaptic transmission to NAc neurons.

The two key dynorphins derived from prodynorphin are dynorphin A (DynA) and B (DynB). Early immunohistochemical results show that in NAc and dorsal striatum, DynB is more abundant than DynA (9). Nonetheless, based on the affinity studies, both DynA and DynB are thought to be endogenous agonists of kappa receptors, although their selectivity for kappa receptors over other opioid receptors is poor (10). Using a more selective kappa receptor agonist (U69593), recent studies demonstrate that activation of kappa receptors acutely decreases excitatory postsynaptic currents (EPSCs) in NAc MSNs, an effect likely mediated by inhibition of presynaptic release of glutamate (11). However, it is not clear whether this effect holds for DynA and DynB, and whether this kappa 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 kappa 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 kappa receptor-independent mechanism. Moreover, following repeated exposure to cocaine, the effect of DynA on EPSCs in NAc MSNs was completely abolished, whereas the effect of DynB remained largely intact. Therefore, DynA and DynB may utilize different cellular and molecular mechanisms in regulating excitatory synaptic transmission to NAc MSNs, and thus may differentially regulate the NAc-based emotional and motivational responses.

Materials and Methods

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 12:12 hr light:dark cycle. Rats were then either kept in the home cage for additional 7 days or received cocaine/saline administration for 5 days, followed by a 1 – 2 d of 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-d procedure of repeated cocaine administration, which was similar to earlier studies (12–14). Briefly, rats received one intraperitoneal injection (i.p.) of either (–)cocaine HCl (15 mg/kg) or the same volume of saline per day for 5 d. Injections were performed within the home-cage at ~9 AM each day. Treated rats were then sacrificed for electrophysiological recordings 1–2 days 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 aCSF (in mM: 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4 , 1.2 MgCl_2 , 2.5 CaCl_2 , 18 NaHCO_3 , and 11 glucose, 295–305 mOsm, equilibrated at 31–34°C with 95% O_2 /5% CO_2).

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 using a MultiClamp 700B amplifier (Molecular Device) through an electrode (2–6 $\text{m}\Omega$) in all electrophysiological experiments. Voltage-clamp recordings were used to measure EPSCs in NAc MSNs. The intracellular and extracellular solutions used can be found in our published papers (15, 16). The extracellular solution routinely contained picrotoxin (0.1 mM) to block GABA_A receptor-mediated currents. Presynaptic stimuli (intensity, 200–500 μA ; duration, 300–600 μs ; frequency, 0.1 Hz) were applied through a bipolar microelectrode. The stimulating electrode was placed close to recorded neurons (~ 3 –4 cells away) and the amplitude of EPSCs was adjusted within ~ 80 –150 pA; both of these efforts were made to minimize the potential spatial effect (18). Amplitudes of EPSC were calculated by averaging 25 EPSCs at -70 mV (holding potential) and measuring the peak (1 ms window) compared to the baseline (1 ms window). All chemicals were purchased from Sigma-Aldrich. Dynorphin peptides, nor-BNI, and U69593 were provided by the Drug Supply Program of NIA NIDA.

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. Numbers of cells (n) was used in all statistics. All results are shown as mean $\pm$ SEM. Paired t-test was used for all results involving comparisons of the amplitudes of EPSCs before, during perfusion of pharmacological manipulations, and during the wash-out period (i.e., perfusion of dynorphins or U69593).

Results

To examine the effects of dynorphins on excitatory synaptic transmission within the NAc, we used whole-cell voltage-clamp technique 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 μM) through recording bath. Upon application of DynA, the amplitude of EPSCs in NAc MSNs was significantly decreased (amplitude relative to baseline: $70.9 \pm 6.6\%$, $n = 6$; $p < 0.01$, paired t-test; Fig. 1A, B). 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: $76.4 \pm 12.9\%$; $p = 0.01$, vs. baseline, paired t-test; Fig. 1A, B). Accompanying the DynA-induced decreased in the amplitude, the paired pulse ratio (PPR, amplitude of the 2nd EPSC over amplitude of 1st EPSCs, inter-pulse interval, 50 ms) of EPSCs was increased (baseline, 1.12 ± 0.13 ; DynA, 1.34 ± 0.14 , $n = 6$; $p = 0.03$, paired t-test; Fig. 1A, C), suggesting a presynaptic action of DynA. Similar to DynA, bath application of DynB (1 μM) also decreased the peak amplitude of EPSCs (relative to baseline: $58.5 \pm 7.2\%$, $n = 6$; $p < 0.01$, paired t-test; Fig. 1D, E) and increased the PPR of EPSCs (baseline, 1.23 ± 0.08 ; DynB, 1.58 ± 0.22 , $n = 6$; $p = 0.04$, paired t-test; Fig. 1D, F) in NAc MSNs. Similar to DynA (Fig. B), the effect of DynB was also only partially washed out (relative to baseline: $87.2 \pm 9.9\%$; $p = 0.23$, vs. baseline,

paired t-test; Fig. 1E). Thus, DynA and DynB exhibited similar modulatory effects on EPSCs in NAc MSNs in naïve rats.

Opioid kappa receptors are enriched in the presynaptic terminals in dorsal and ventral striatum, and both DynA and DynB exhibit higher affinity to kappa receptors over other opioid receptor subtypes (19, 20). We thus examined whether the above effects of DynA were mediated by kappa receptors. The approach we used was to test the effects of kappa receptor-selective compounds on EPSCs in NAc MSNs. If kappa receptors were the key of the observed effects, activation of kappa receptors with highly selective agonists should mimic DynA-mediated effects whereas preventing the activation of kappa receptors with highly-selective antagonists should inhibit these effects. U69593 has been demonstrated as a highly selective agonist for kappa receptors over other opioid receptors at submicromolar concentrations (19). In the same experimental setup as above, bath application of U69593 (1 μ M) decreased the peak amplitude of EPSCs (relative to baseline: $71.7 \pm 5.6\%$, $n = 5$; $p = 0.02$, paired t-test; Fig. 2A, B) and increased the PPR of EPSCs in NAc MSNs (baseline, 1.20 ± 0.11 ; U69593, 1.75 ± 0.27 , $n = 5$; $p = 0.02$, paired t-test; Fig. 2A, C), an overall effect similar to the that of DynA and DynB.

It has been demonstrated that nor-BNI is a highly selective kappa receptor antagonist (19). Our results show that when perfused alone, nor-BNI (0.1 μ M) did not significantly affect either the peak amplitude (relative to baseline: $108.3 \pm 7.8\%$, $n = 5$; $p = 0.45$, paired t-test; Fig. 2D, E) or the PPR (baseline, 1.37 ± 0.11 ; nor-BNI, 1.23 ± 0.06 , $n = 5$, $p = 0.47$; Fig. 2D, F) of EPSCs in NAc MSNs. Consistent with previous results (11, 21), application of nor-BNI reversed the effects of U69593 on both the peak amplitude (relative to baseline: U69593, $77.5 \pm 4.7\%$, $p < 0.01$, vs. baseline; U69593 + nor-BNI, $95.4 \pm 8.4\%$, $p = 0.67$, vs. baseline; $p = 0.33$, vs. U69593; $n = 5$; Fig. 2G, H) and PPR (baseline, 1.02 ± 0.11 ; U69593, 1.66 ± 0.22 , $p = 0.02$; U69593 + nor-BNI, 1.30 ± 0.17 , $p = 0.08$; $n = 5$; Fig. 2G, I) of EPSCs in NAc MSNs. Thus, if the selectivity of U69593 and nor-BNI holds in the NAc slice as demonstrated in cell lines (19), our above results suggest that kappa receptor-mediated modulation of EPSCs can be relatively selectively induced by U69593 and reversed by nor-BNI.

With nor-BNI, we examined whether the effects of DynA and DynB on EPSCs in NAc MSNs could be inhibited by inhibition of kappa receptors. Our results show that the effects of DynA on both peak amplitude (Relative to baseline: DynA, $69.8 \pm 4.5\%$, $p < 0.01$, vs. baseline; DynA + nor-BNI, $90.2 \pm 10.7\%$, $p = 0.38$, vs. baseline; $n = 6$; Fig. 2J, K) and PPR (baseline, 1.35 ± 0.11 ; DynA, 1.68 ± 0.18 , $p = 0.02$, vs. baseline; DynA + nor BNI, 1.44 ± 0.14 , $p = 0.17$, vs. baseline, $n = 5$; Fig. 2J, L) of EPSCs were reversed by application of nor-BNI. In contrast, the effects of DynB on the peak amplitude (relative to baseline: DynB, $60.2 \pm 8.7\%$, $p < 0.01$, vs. baseline; DynB + nor-BNI, $83.8 \pm 5.6\%$, $p = 0.02$, vs. baseline; $n = 6$; Fig. 2M, N) and PPR (baseline, 1.29 ± 0.14 ; DynB, 1.76 ± 0.24 , $p = 0.02$, vs. baseline; DynB + norBNI, 1.67 ± 0.10 , $p = 0.02$, vs. baseline; $n = 5$; Fig. 2M, O) of EPSCs in NAc MSNs were only partially reversed. These results taken together suggest that the effects of DynA on EPSCs in NAc MSNs are primarily mediated by activation of kappa receptors whereas the effects of DynB are mediated not only activation of kappa receptors but also kappa receptor-independent mechanisms.

Exposure to cocaine increases the level of dynorphins in NAc, and this elevated tone of dynorphins has been implicated in several NAc-based behavioral alterations (6, 22). We asked whether DynA/B-mediated modulation of excitatory synaptic transmission in NAc was affected by exposure to cocaine. Using the same experimental setup as described above, we observed that the effects of DynA on the peak amplitude (relative to baseline: DynA, $116.1 \pm 8.2\%$, $n = 7$, $p = 0.11$; Fig. 3A, B) and PPR (baseline, 1.36 ± 0.12 ; DynA, $1.16 \pm$

0.04, $n = 7$, $p = 0.36$; Fig. 3A, C) were abolished in cocaine-pretreated rats (1–2 days withdrawal after 5-d repeated i.p. injection). In contrast, exposure to cocaine did not significantly affect the effects of DynB (relative EPSC amplitude: DynB, $75.9 \pm 10.4\%$, $n = 7$, $p = 0.03$; PPR: baseline, 1.24 ± 0.10 ; DynB, 1.39 ± 0.02 , $n = 7$, $p < 0.01$; Fig. 3 D–F). To selectively examine kappa receptor-mediated regulation, we test the effect of U69593. The effects of the selective kappa receptor agonist U69593 ($1 \mu\text{M}$) were also abolished in cocaine-pretreated rats (relative EPSC amplitude: U69593, $97.7 \pm 2.6\%$, $n = 7$, $p = 0.45$; Fig. 3 G, H), similar to that for DynA. These results suggest that the kappa receptor-mediated modulation of excitatory synaptic transmission in NAc was abolished after exposure to cocaine, whereas the kappa receptor-independent effects of DynB remain intact.

Discussion

Current understanding about dynorphin-signaling-mediated regulation of synaptic transmission within NAc is primarily derived from kappa receptor-based studies. In behaving animals, it is dynorphin peptides that are released during behavioral responses and initiate dynorphin-signaling. Thus, to depict a thorough picture of dynorphin-signaling, it is important to also examine the precise role of dynorphin peptides in addition to kappa receptors.

Our present study focused on DynA and DynB, two key dynorphin products from prodynorphin. Our results show that inhibiting kappa receptors did not completely prevent DynB-mediated synaptic inhibition (Fig. 2), suggesting in addition to activation of kappa receptors, other mechanisms are also involved in dynorphin-signaling-mediated regulation of synaptic transmission in NAc. These results provide a cellular explanation why different dynorphins exhibit differential psychopharmacological effects (23, 24). Furthermore, DynB is much more abundant than DynA in the NAc (9). Thus, kappa receptor-independent dynorphin-signaling should play a significant role in the NAc upon upregulation of prodynorphin.

Then, what are the potential kappa receptor-independent mechanisms that mediate the effect of DynB? One candidate is mu receptors. Mu receptors are also enriched in the NAc, and activation of mu receptors inhibits excitatory synaptic transmission in NAc MSNs with a seemingly similar presynaptic mechanism (19). Although dynorphins including DynB exhibit preferential affinity to kappa receptors over mu and other opioid receptors, the selectivity for kappa receptors is limited over mu receptors (10). Thus, it is possible that the nor-BNI-resistant effect of DynB on EPSCs is achieved by activation of mu receptors. Indeed, the mu receptor activity of dynorphins has also been implied in other brain sites (25).

Another important finding of the present study is that repeated exposure to cocaine abolished the effect of DynA on excitatory synaptic transmission in NAc. In contrast, the effect of DynB remained largely intact in cocaine-pretreated animals (Fig. 3). As suggested by our results, the effect of DynA is primarily mediated by kappa receptors. As such, the lack of effect of DynA can be mediated by cocaine-induced alteration in kappa receptors or kappa receptor-coupled intracellular signaling pathways. For example, if kappa receptors are downregulated in NAc following exposure to cocaine, a diminished effect of DynA would be expected. However, results related to the effects of cocaine on the NAc kappa receptors are highly inconsistent; no effect (26, 27), upregulation (28–30), or downregulation (31, 32) have all been reported in cocaine-treated animals. Such discrepant results may reflect the dynamic nature of the NAc kappa receptors upon different cocaine procedures. Nonetheless, in addition to kappa receptors, pre-stimulation of kappa receptor-coupled signaling pathways may also be a contributing factor. It has been consistently shown that the level of dynorphins

is increased by exposure to cocaine and other drugs of abuse (33–37). Thus, in the NAc of cocaine-exposed animals, increased dynorphins may strongly and persistently stimulate kappa receptors, resulting in either receptor desensitization or a saturation of activation of their coupled signaling pathways. Consequently, an additional application of DynA could not produce further effects.

On the other hand, our results also suggest that the kappa receptor-independent component of the DynB-mediated effects remain substantially intact in cocaine-exposed rats, albeit the maximal effect is diminished (Fig. 3). If the kappa receptor-independent effect of DynB is indeed mediated by mu receptors (see above), this result would suggest that mu receptors and their coupled signaling in the NAc are still functional upon exposure to cocaine. It has long been hypothesized that the NAc mu receptors act to promote the development/maintenance of the addictive state whereas the NAc kappa receptors act the opposite way (6, 38). Thus, the fine balance between kappa and mu receptor might be disrupted in the NAc following exposure to cocaine. Whether this loss of balance is pro-addiction remains to be determined.

Functionally, the NAc dynorphins significantly contribute to stress and drug withdrawal-induced depressive emotional state, likely through their coupled kappa receptor-signaling (39, 40). If the NAc kappa receptor-coupled signaling is functionally saturated in cocaine-exposed animals as discussed above, kappa receptor antagonists, or, ideally inverse agonists, may be effective for treating stress and drug withdrawal-induced depression. Furthermore, it has been hypothesized that activation of dynorphin-kappa receptors may act to counteract the development and maintenance of the addictive state (6). Thus, if this system is functionally saturated upon exposure to drugs of abuse, further application of kappa receptor antagonists as a clinical approach may not achieve a desired therapeutic efficacy.

Taken together, we demonstrate differential effects of dynorphins in the NAc as a function of exposure to drugs of abuse, suggesting the existence of complex cellular behavior of dynorphins in the NAc. These results provide a new way in understanding intra-NAc dynorphins and their potentially differential implications in the development of drug addiction, addiction-associated anhedonic emotional states, and treatment of these emotional and motivational disorders.

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Figure 1.

Inhibition of EPSCs in NAc MSNs by DynA and DynB. **A** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of DynA. EPSCs were elicited in a paired-pulse manner. **B** Summarized results showing that perfusion of DynA inhibited the amplitude of EPSCs in NAc MSNs, and the effect could not be washed. **C** Summarized results showing that accompanying the DynA-mediated inhibition of EPSC amplitude, the PPR was increased. **D** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of DynB. **E** Summarized results showing that perfusion of DynB inhibited the amplitude of EPSCs in NAc MSNs, and the effect could not be washed. **F** Summarized results showing that accompanying the DynA-mediated inhibition of EPSC amplitude, the PPR was increased.

Figure 2.

Differential involvements of kappa receptors in DynA- and DynB-mediated regulation of EPSCs in NAc MSNs. **A** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of U69593. EPSCs were elicited in a paired-pulse manner. **B** Summarized results showing that perfusion of U69593 inhibited the amplitude of EPSCs in NAc MSNs, and the effect could not be washed. **C** Summarized results showing that accompanying the U69593-mediated inhibition of EPSC amplitude, the PPR was increased. **D** Examples showing that the amplitude of EPSCs in NAc MSNs was not affected by perfusion of nor-BNI. **E** Summarized results showing that perfusion of nor-BNI did not significantly affect the amplitude of EPSCs in NAc MSNs. **F** Summarized results showing that the PPR of EPSCs was not significantly affected by perfusion of nor-BNI. **G** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of U69593, and this effect was reversed by application of nor-BNI. **H** Summarized results showing that U69593-induced inhibition of EPSC amplitude in NAc MSNs could be reversed by application of nor-BNI. **I** Summarized results showing that U69593-induced increase in PPR could be reversed by application of nor-BNI. **J** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of DynA, and this effect was reversed by application of nor-BNI. **K** Summarized results showing that DynA-induced inhibition of EPSC amplitude in NAc MSNs could be reversed by application of nor-BNI. **L** Summarized results showing that DynA-induced increase in PPR could be reversed by application of nor-BNI. **M** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of DynB, and this effect was not completely reversed by application of nor-BNI. **N** Summarized results showing that DynB-induced inhibition of EPSC amplitude in NAc MSNs could be partially reversed by application of nor-BNI. **O** Summarized results showing that U69593-induced increase in PPR could be partially reversed by application of nor-BNI.

Figure 3.

DynA-, but not DynB-, mediated regulations of EPSCs in NAc MSNs was disrupted by exposure to cocaine. **A** Examples showing that the amplitude of EPSCs in NAc MSNs was not affected by perfusion of DynA in cocaine-pretreated rats. EPSCs were elicited in a paired-pulse manner. **B** Summarized results showing that DynA-induced inhibition of EPSC amplitude in NAc MSNs was not present in cocaine-pretreated rats. **C** Summarized results showing that DynA-induced increase in PPR was not present in cocaine-pretreated rats. **D** Examples showing that the amplitude of EPSCs in NAc MSNs was decreased by perfusion of DynB in cocaine-pretreated rats. **E** Summarized results showing that DynB-induced inhibition of EPSC amplitude in NAc MSNs was still present in cocaine-pretreated rats. **F** Summarized results showing that DynB-induced increase in PPR was still present in cocaine-pretreated rats. **G** Examples showing that the amplitude of EPSCs in NAc MSNs was not affected by perfusion of U69593. **H** Summarized results showing that U69593-induced inhibition of EPSC amplitude in NAc MSNs was not present in cocaine-pretreated rats.