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Epigenetic upregulation of PSD-95 contributes to the rewarding behavior by morphine conditioning

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ABSTRACT

Abuse of opiates, including morphine, induced remarkable synaptic adaptation in several brain regions including ventral tegmental area (VTA), which underlay the induction and maintenance of opioid dependence and addiction. Scaffolding protein postsynaptic density protein 95 (PSD-95) is critically involved in the glutamatergic synaptic maturation and plasticity in the central neurons. The present study revealed a significantly increased mRNA and protein expression of PSD-95 in the VTA of the rats conditioned with morphine. The further chromatin immunoprecipitation study found an increased histone H3 acetylation in the promoter region of *Dlg4*. An upregulation of expression of phosphorylated cAMP response element-binding protein (pCREB) and the occupancy of pCREB in the *Dlg4* promoter region were shown in the VTA of the morphine-conditioned rats. Inhibition of pCREB activity significantly decreased the histone H3 acetylation in *Dlg4* promoter region, PSD-95 upregulation, enhancement of glutamatergic strength and the preference to morphine-paired chamber in the rats with morphine conditioning. These results suggested that CREB-mediated epigenetic upregulation of PSD-95 critically contributed to the enhanced glutamatergic transmission and rewarding behavior induced by morphine conditioning.

1. Introduction

Opioid analgesics, such as morphine, are highly addictive and are among commonly abused drugs due to their strong rewarding effect. Repeated exposure to opiates eventually leads to drug dependence and addiction, a psychological disease and disturbing social problem (Koob and Le Moal, 2008; Nestler, 2004). Several brain regions, including the ventral tegmental area (VTA), the nucleus accumbens and the amygdala complex, have been classically implicated in the mechanisms of reward and addiction to various abused drugs including morphine. A lot of evidence demonstrates that remarkable adaptive changes of neurocircuits occur in these brain areas, which are thought to critically contribute to the compulsive behavior of drug abuse induced by repeated exposure to opiates (Koob and Le Moal, 2008; Williams et al., 2001). Significantly enhanced glutamatergic transmission in several brain regions, including the VTA (Ungless et al., 2001) and amygdala (Cai et al., 2013), serves as one of the major components of the central sensitization induced by abused drugs, such as cocaine and morphine, which remarkably reinforces the drug craving and increases the vulnerability of drug relapses. Although many efforts have been made, the molecular mechanism underlying the enhanced glutamatergic transmission in the VTA need to be further elucidated.

Postsynaptic density protein 95 (PSD-95), encoded by the *Dlg4* (disks large homolog 4) gene, is a postsynaptic scaffolding protein with three PDZ domains, an SH3 domain, and a membrane-associated guanylate kinase activity (Feng and Zhang, 2009). PSD-95, partnering with other members of scaffolding proteins, is recruited into the postsynaptic sites to form a multimeric scaffold for the clustering of glutamatergic receptors and associated signaling proteins, and is thus significantly involved in the glutamatergic synapse maturation and plasticity (Opazo et al., 2012). It was previously reported that significantly increased PSD-95-mediated signaling in the postsynaptic sites was essentially required for the dendritic spine growth and synaptic plasticity in the glutamatergic synapses following tetanic electric stimuli (Steiner et al., 2008). Overexpression of PSD-95 robustly enhanced the amplitude of the evoked excitatory postsynaptic current (EPSC) (Elias et al., 2008) as well as the electric stimuli-induced synaptic plasticity in the central neurons (Ehrlich and Malinow, 2004). Conversely, inhibition of the function of PSD-95 remarkably attenuated the glutamatergic strength in the hippocampal neurons (Ehrlich et al., 2007). While previous evidence suggested that

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dynamic adaptation of PSD-95 was potentially associated with the neurocircuit adaptation in several brain regions and the induction of rewarding behavior induced by abused drugs (Roche, 2004), currently the involvement of PSD-95 in drug abuse-induced glutamatergic enhancement in the VTA has not been defined.

Emerging evidence suggest that an epigenetic mechanism, altering the transcription and expression of specific genes without change of the DNA sequence, is extensively involved in the induction and maintenance of drug craving and relapse in several rodent models of drug abuse (Feng and Nestler, 2013). Repeated exposure to morphine induced significant activity of several transcriptional factors with histone acetyltransferase activity, such as ΔFosB and cAMP response element-binding protein (CREB), in several brain regions, and blockade of the activity of these transcriptional factors significantly attenuated the drug craving in the rodents (Vialou et al., 2012). Altered activity of these transcriptional factors may lead to significant modification of the expression and function of several key genes, which is critically involved in the induction and maintenance of synaptic plasticity in the central neurons. However, currently the mechanism underlying how these transcriptional factors participated in the morphine abuse-induced rewarding behavior remains unclear. Hence, the present study aims to elucidate the epigenetic mechanism underlying the glutamatergic enhancement in VTA neurons and the rewarding behavior induced by repeated exposure to morphine.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (weighing 180–220 g) were obtained from the Institutional Center of Experiment Animals, and were housed in standard lab conditions (22 ± 2 °C and 12:12 h light cycle) with food and water provided *ad libitum*. All animal protocols were approved by the Institutional Animal Care and Use Institutional Animal Care and Use Committee in the Third Military Medical University, and were confronted with the guidelines of National Institution of Health.

2.2. Cannula implantation and microinjection

The methods for site-specific cannula implantation and microinjection were similar as previously reported (Margolis et al., 2008). Before conditioning treatment, a rat was anesthetized with sodium pentobarbital solution (50 mg/kg). Animals were placed in a stereotaxic frame and were implanted with bilateral 26-gauge stainless steel chronic guide cannulas (Plastics One) into the VTA (AP, −5.8; ML, ±0.5; and DV, −7.0) (Paxinos and Watson, 1998). The guide cannula was then secured to the skull with dental cement and capped. After the implantation surgery, the rat was allowed a recovery period of 1 week before undergoing the conditioning procedure. VTA microinjection of CBP–CREB interaction inhibitor (0.5 μg, Millipore, MA) or vehicle was performed daily 15 min before conditioning with morphine or saline. The agents were delivered into the VTA through a 33-gauge single injector cannula with an infusion pump at a rate of 0.2 μl/min. A total of 0.5 μl was injected on each side. All cannula placements for the VTA were histologically verified afterward.

2.3. Animal model and conditional place preference (CPP)

A standard 3-chamber CPP apparatus (MED Associates, St. Albans, VT) was used for the analysis of morphine-induced rewarding behavior in rats, as reported previously (Bie et al., 2012). The conditioning procedure consisted of four phases for a total of 12 consecutive days (Fig. 1). For phase 1 (habituation; days 1–2), after an intraperitoneal injection of saline, a rat was placed in the center compartment and allowed to move freely in all chambers for 30 min each day. For phase 2 (day 3), a pretest was performed to determine the baseline preference. After an intraperitoneal injection of saline, the rat was placed in the center compartment, and the time the rat spent in each of the two test chambers during a 30-min test period was recorded. For phase 3 (morphine conditioning; days 4–11), the rats were randomly assigned to saline and morphine groups. On day 4, the rat in the morphine group was injected with morphine (10 mg/kg i.p.) and was immediately confined in a test chamber for 20 min. On day 5, the rat was injected with saline and confined in another test chamber for 20 min. The same procedure of morphine and saline conditioning on alternate days was repeated through day 10 and 11. In the saline control group, the rats received saline injection and were confined in alternate chambers on all 8 conditioning days. For phase 4, (post-test; day 12), after an intraperitoneal injection of saline, the rats in both groups were placed in the center compartment and allowed to move freely between the two test chambers for 30 min, and the time the rat spent in each test chamber was automatically recorded to determine CPP behavior. CPP data were presented as the percentage of time that a rat spent in the morphine-paired chamber over the sum of the times spent in both test chambers during a 30 min test period.

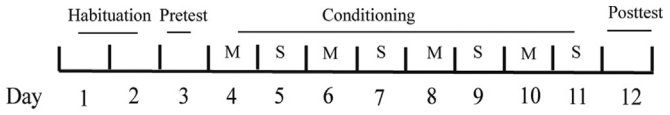

Fig. 1. The experimental paradigm to show the procedure of morphine conditioning. In the morphine conditioning group, the rats were alternatively conditioned with morphine (M, 10 mg/kg) or saline (S) at day 4 through day 11. In the saline control group, the rats received saline injection and were confined in alternate chambers on all 8 conditioning days.

2.4. Brain slice preparations and whole-cell recording

After behavioral test, the rat was anesthetized with inhalation of halothane and then euthanized by decapitation. The preparation of brain slices and whole-cell recording were performed as previously reported (Margolis et al., 2008). The brain of a rat was removed and cut in a Vibratome slicer in cold (4 °C) physiological saline to obtain coronal slices (200–300 μm thick) containing the VTA. A single slice was submerged in a shallow recording chamber and perfused with preheated (35 °C) physiological saline (126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 11 mM glucose, and 25 mM $NaHCO_3$, saturated with 95% O_2–5% CO_2, pH 7.3). Slices were maintained at around 33 °C throughout a recording experiment.

The neurons were visualized through a microscope with infrared illumination (Olympus, Tokyo, Japan) in a slice containing the VTA. Whole-cell recordings were made with a glass pipette (resistance, 3–5 MΩ) filled with a solution containing 126 mM potassium gluconate, 10 mM NaCl, 1 mM $MgCl_2$, 11 mM EGTA, 10 mM HEPES, 2 mM ATP, and 0.25 mM GTP, pH adjusted to 7.3 with KOH; osmolarity 280–290 mOsmol/l. An AxoPatch 200B amplifier and pCLAMP 9.1 software (Molecular Devices, Sunnyvale, CA) were used for data acquisition and analyses. Holding potential was −70 mV for all recordings. A seal resistance of 2 GΩ or above and an access resistance of 20 MΩ or less were considered acceptable. Series resistance was optimally compensated. Electrical stimuli of constant current (0.2 ms, 0.04–0.2 mA) were used to evoke glutamate-mediated excitatory postsynaptic currents (EPSCs) with bipolar stimulating electrodes (FHC Inc., Bowdoinham, ME). All EPSCs were recorded in the presence of the $GABA_A$ receptor