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Research report

Microinjection of histone deacetylase inhibitor into the ventrolateral orbital cortex potentiates morphine induced behavioral sensitization

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ABSTRACT

Accumulating evidence indicates that epigenetic regulation, such as changes in histone modification in reward-related brain regions, contributes to the memory formation of addiction to opiates and psychostimulants. Our recent results suggested that the ventrolateral orbital cortex (VLO) is involved in the memories of stress and drug addiction. Since addiction and stress memories share some common pathways, the present study was designed to investigate the role of histone deacetylase (HDAC) activity in the VLO during morphine induced-behavioral sensitization. Rats received a single exposure to morphine for establishing the behavioral sensitization model. The effect of HDAC activity in the VLO in morphine induced-behavioral sensitization was examined by microinjection of HDAC inhibitor Trichostatin A (TSA). Furthermore, the protein expression levels of extracellular signal-regulated kinase (ERK) and phosphorylated ERK (p-ERK), histone H3 lysine 9 acetylation (aceH3K9) and brain-derived neurotrophic factor (BDNF) in the VLO in morphine-induced behavioral sensitization were examined. The results showed that the bilateral VLO lesions suppressed the expression phase, but not the developmental phase of morphine-induced behavioral sensitization. Microinjection of TSA into the VLO significantly increased both the development and expression phases. Moreover, the protein levels of p-ERK, aceH3K9 and BDNF except ERK in the VLO were significantly upregulated in morphine-treated rats in the expression phase. These effects were further strengthened by intra-VLO injection of TSA. Our findings suggest that HDAC activity in the VLO could potentiate morphine-induced behavioral sensitization. The upregulated expression of p-ERK, aceH3K9 and BDNF in the VLO might be the underlying mechanism of histone acetylation enhancing the morphine-induced behavioral sensitization.

1. Introduction

Opiate addiction usually results in severe social and physical repercussions (Raehal et al., 2005; Renthal and Nestler, 2009). One of the major clinical challenge of opiate addiction is its persistence even after long periods of drug abstinence, which is highlighted by high rates of drug relapse. Behavioral sensitization is a long-lasting form of neuronal plasticity that plays a key role in certain aspects of drug addiction such as drug-seeking behavior and relapse (Guegan et al., 2015; Khan et al., 2015; Vanderschuren and Kalivas, 2000; Vanderschuren et al., 2001). The behavioral sensitization phenomenon has been confirmed as a very useful model for studying the mechanisms underlying addiction. However, the neurobiological mechanism of opioid drug induced behavioral sensitization is not yet fully elucidated at the anatomic and molecular levels.

Clinical and laboratory observations have converged on the hypothesis that addiction represents the pathological usurpation of neural processes that normally serve reward-related learning and memories (Hyman et al., 2006). The ventrolateral orbital

Abbreviations: aceH3K9, histone H3 lysine 9 acetylation; BDNF, brain-derived neurotrophic factor; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; HDAC, histone deacetylase; *i.p.*, intraperitoneally; m.i., microinjection; min, minute(s); Mor, morphine; NAc, nucleus accumbens; p-ERK, phosphorylated ERK; PFC, prefrontal cortex; Sal, saline; TSA, Trichostatin A; VLO, ventrolateral orbital cortex; vmPFC, ventromedial prefrontal cortex; VTA, ventral tegmental area

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cortex (VLO), as a major subdivision of prefrontal cortex (PFC), has been identified as part of the limbic-thalamic-cortical circuits that are strongly implicated in morphine-mediated pain modulation, morphine- and methamphetamine-induced addiction-related and stress-related memory formation in our recent studies (Tang et al., 2009; Wei et al., 2016; Zhao et al., 2013; Zhao et al., 2015). The role of VLO in drug addiction remains to be further studied. Accumulating evidence suggests that epigenetic mechanisms (e.g. histone acetylation) are attractive candidates in the brain reward pathway, mainly including the ventral tegmental area (VTA), nucleus accumbens (NAc), PFC and hippocampus, for regulation of drug addiction and formation of memory (Feng and Nestler, 2013; Levenson et al., 2004; Malvaez et al., 2009; Renthal and Nestler, 2009). Histone acetylation is one of the most important epigenetic modifications which are vital for opiate addiction and aversive memory in the PFC (Freeman et al., 2008; Stafford et al., 2012; Tesone-Coelho et al., 2015). Acetylation at lysine 9 on histone H3 (aceH3K9) is a common histone modification enriched at transcriptionally active genes (Adachi and Monteggia, 2009). Liang et al. has revealed that promoter regions of brain-derived neurotrophic factor (BDNF) was strongly associated with aceH3K9 after morphine and selective histone deacetylase (HDAC) inhibitor treatment (Liang et al., 2014). Administration of HDAC inhibitor, sodium butyrate, enhances morphine-induced locomotor sensitization and conditioned place preference (Sanchis-Segura et al., 2009). All these findings suggest that aceH3K9 contributes greatly to morphine addiction.

In addition, extracellular signal-regulated kinase (ERK) plays an important role in synaptic plasticity and long-lasting behavioral alterations induced by opiate abuse (Li et al., 2008; Lyons et al., 2013). ERK activation by phosphorylation (p-ERK) serves as an intracellular bridging mechanism that facilitates neuronal communication and plasticity (Zamora-Martinez and Edwards, 2014). Moreover, ERK activation is necessary for BDNF function such as inducing spine growth in hippocampal CA1 pyramidal neurons (Alonso et al., 2004; Koo et al., 2015; Pizarro et al., 2004). BDNF is associated with cellular and structural changes that occur during nervous system development and contributes to learning-related synaptic transmission and plasticity (Bredy et al., 2007). Recently, it has been found that activation of ERK signaling pathway induced an increase of acetylation of histone H3 at the promoters of BDNF gene and subsequently upregulated BDNF mRNA and protein expression in the ventromedial prefrontal cortex (vmPFC) of rats in the extinction of aversive memory of morphine withdrawal (Wang et al., 2012). However, whether histone acetylation in the VLO participates in morphine-induced behavioral sensitization, as well as ERK activation, aceH3K9 and BDNF in the VLO involve in this effect have not been studied.

The aim of the present study was to investigate the effect of microinjection of HDAC inhibitor Trichostatin A (TSA) into the VLO on morphine-induced behavioral sensitization in rats. The expression levels of ERK, p-ERK, aceH3K9 and BDNF were further examined in the VLO in an attempt to gain insights into the potential molecular mechanism of morphine-induced behavioral sensitization and addiction.

2. Results

Rats received saline once daily from Day -2 to Day -1. On Day 1, Rats were given saline or morphine (10 mg/kg, *i.p.*) and then put into test chambers individually to measure the locomotor activity for 240 min. After a 7-day drug-free period (on Day 8), all rats were challenged with morphine (5 mg/kg, *i.p.*) and locomotor activity was recorded for 240 min. The schedule of morphine-induced behavioral sensitization and drug administration as shown in Fig. 1.

2.1. The effects of bilateral VLO lesions on locomotor activity and morphine-induced behavioral sensitization

To further confirm the role of the VLO in morphine-induced behavioral sensitization, the bilateral VLO lesions (VLOL) were used in the present study. The results showed that no significant difference was observed between the bilateral VLO lesions and sham rats on locomotor activity in the total distance travelled in 240 min (Student's *t*-test, $P=0.744$), which indicates that the bilateral VLO lesions did not affect the locomotor activity of the rats (Fig. 2(A)).

However, the analysis of the morphine-induced behavioral sensitization revealed significant effects of treatment ($F_{(3, 58)}=40.33$, $P<0.0001$), test condition ($F_{(1, 58)}=30.43$, $P<0.0001$) and their interaction ($F_{(3, 58)}=4.454$, $P<0.01$). Post-hoc analyses revealed that the Sham-Morphine group exhibited a significant effect on the accumulated locomotor activity in both the development and expression phases ($P<0.001$). As reflected by the total distance in 240 min, the VLOL-Morphine group had significant effect on the accumulated locomotor activity in the development phase ($P<0.001$) but not in the expression phase ($P>0.05$) compared with VLOL-Saline group. Compared with Sham-Morphine group, there was no significant effect of the accumulated locomotor activity in VLOL-Morphine group in the development phase. However, in the expression phase, the accumulated locomotor activity of VLOL-Morphine group in 240 min was significantly lower than that of the Sham-Morphine group ($P<0.001$), which indicated that the VLO lesions could suppress morphine-induced behavioral sensitization (Fig. 2(B)).

2.2. The effect of microinjection of HDAC inhibitor TSA into the VLO on the locomotor activity

Microinjection of HDAC inhibitor TSA (165 μM, 0.5 μl/side) into

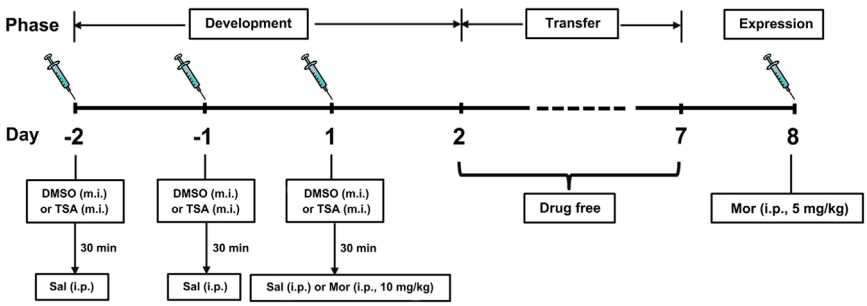

Fig. 1. The experimental schedule of morphine-induced behavioral sensitization and drug administration. Abbreviations: DMSO, dimethyl sulfoxide; *i.p.*, intraperitoneally; m.i., microinjection; min, minute. Mor, morphine; Sal, saline; TSA, Trichostatin A.