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Research Report

Molecular mechanisms associated with the antidepressant effects of the class I histone deacetylase inhibitor MS-275 in the rat ventrolateral orbital cortex

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ABSTRACT

Histone modifications mediated by histone acetylation are thought to play an important role in the pathogenesis and treatment of depression. Recent studies have revealed that histone deacetylase inhibitors (HDACis), such as sodium valproate (VPA) and MS-275, may be involved in the pathogenesis of depression and in the underpinnings of antidepressant therapeutic action in several brain regions, including the ventrolateral orbital cortex (VLO). In the present study, we investigated whether the class I histone deacetylase inhibitor MS-275 exerts antidepressant-like effects when infused bilaterally into the VLO of a rat, using the forced swimming test (FST) and tail suspension test (TST) as behavioral measures. We found that chronic intra-VLO infusion of MS-275 significantly reduced immobility time in the FST and TST compared with vehicle-treated controls, similar to the effects of systemically administered fluoxetine. These antidepressant-like effects of MS-275 are associated with an increase in H3 acetylation and elevated CREB and BDNF levels in the VLO. Our findings suggest the possibility that alterations in gene expression due to chromatin remodeling, including upregulation of CREB and BDNF, may be involved in the antidepressant-like effect of HDACis in the VLO.

1. Introduction

Depression is one of the most common mental illnesses and is a major cause of disability worldwide but has largely unknown etiology (Krishnan and Nestler, 2008). Although current treatments, mostly targeting the brain's monoamine systems, are highly effective for some sufferers with depression, about half of all patients remain symptomatic despite receiving standard antidepressant medications. Considering the enormous burden on society, it is a high priority to identify the biological underpinnings of depression and develop more effective treatments.

Aside from monoamine disturbances, recent studies in humans and animal models indicate that epigenetic regulation of gene function, a mechanism known to regulate long-lasting behavioral responses to environmental stimuli, may play an important role in the pathogenesis of depression and in the mechanisms underlying the therapeutic action of

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antidepressants (Hobara et al., 2010). Histone acetylation contributes to the transcriptional activation process by relaxing a repressive chromatin state, which facilitates the sequestration of the basal transcriptional machinery. Therefore, histone acetylation may represent a key target for antidepressant action. For example, it has been shown that histone deacetylase inhibitors (HDACis) such as sodium butyrate (NaBt), which can increase levels of brain-derived neurotrophic factor (BDNF) expression in the frontal cortex (Schroeder et al., 2007), and exert an antidepressant-like effect when administered systemically. Chronic social defeat stress induces a selective lasting decrease in BDNF transcription, which can be reversed by chronic antidepressant treatment via increased histone acetylation at the promoter of the *BDNF* gene (Tsankova et al., 2006). Recently, Xing et al. (2011) have shown an antidepressant-like effect of sodium valproate (VPA), a HDAC inhibitor traditionally used as a mood stabilizer, when microinjected into the ventrolateral orbital cortex (VLO). It has been suggested that the VLO, a sub-region of the orbitofrontal cortex (OFC), is part of the limbic–thalamic–cortical circuit that is highly involved in the pathogenesis of depression (Drevets, 2000). It is possible that the action of VPA and NaBt on CREB, BDNF, or other gene transcriptions and beneficial behavioral effects on the animal models of depression are mediated by mechanisms that are independent of the inhibition of histone acetylation. However, VPA is an inhibitor of HDACs with possible non-specific effects such as elevation of GABA concentration, which is also associated with antidepressant-like action. Experiments with more potent and specific HDACi should help to clarify the underlying molecular mechanisms the action of VPA and NaBt on the regulation of CREB and BDNF expression in the VLO.

Studies have shown that the 2′-aminophenyl-benzamide derivative MS-275 is a potent brain region-selective HDACi. It is 100-fold more potent than VPA for increasing the content of acetylated histone H3 (acH3) in the frontal cortex, and its action is longer lasting than that of VPA (Simonini et al., 2006). When MS-275 is infused into the nucleus accumbens (NAc), it produces an antidepressant-like effect by counteracting learned helplessness and social defeat stress (Covington et al., 2009). Moreover, as revealed by microarray analysis, the changes in gene expression patterns in the NAc induced by chronic defeat stress can be counteracted by intra-NAc infusion, similar to the effects of the antidepressant fluoxetine (Covington et al., 2009).

Based on those findings, the aim of this study was to further investigate whether HDACi MS-275 could produce an antidepressant-like effect when infused into the VLO, and to explore changes in gene expression after intra-VLO infusion in rats.

2. Results

2.1. Determination of microinjection sites

The injection sites were visually confirmed and marked on diagrams from the atlas (Paxinos et al., 1980). Photomicrographs of coronal brain sections depicting bilateral microinjection sites in the VLO of representative animals are presented in Fig. 1. Only injection sites in the VLO were included in the data analysis (DMSO, n=5; MS-275 (10 μM), n=6; MS-275 (50 μM), n=6; MS-275 (100 μM), n=6).

2.2. Behavioral effects of bilateral intra-VLO administration of MS-275

The effects of MS-275 at different concentrations were assessed behaviorally with the open-field test, the TST and FST and compared with the effect of a known antidepressant, Fluoxetine (FLX). There was no significant effect of FLX or repeated MS-275 treatment, regardless of concentration, on the locomotor activity in the open-field test (Fig. 2A). In the TST, repeated FLX treatment significantly reduced immobility time in the 6-min test period compared with the saline controls [$F_{(5,29)}=16.41$, $p<0.0001$; posthoc, $p<0.05$] (Fig. 2B). When compared with the DMSO vehicle group, repeated MS-275 treatment with 50 and 100 μM, but not 10 μM, significantly decreased the duration of immobility in the TST [$F_{(5,29)}=16.41$, $p<0.0001$; posthoc, $p<0.05$ and $p<0.0001$, respectively]. In the FST, the immobility time was significantly reduced in repeated FLX-treated rats [$F_{(5,29)}=20.02$, $p<0.0001$; posthoc, $p<0.05$]. Conversely, there was no significant effect of FLX in climbing or swimming time compared with the saline group. A significant immobility decrease and climbing time increase were observed in rats receiving MS-275 (100 μM) versus DMSO controls [$F_{(5,29)}=20.02$, $p<0.0001$; posthoc, $p<0.0001$ for both]. In addition, MS-275 (50 μM) showed a limited behavioral effect in climbing time only [$F_{(5,29)}=20.02$, $p<0.0001$; posthoc, $p<0.05$] (Figs. 2C–E).

2.3. Effect of repeated intra-VLO MS-275 treatment on protein levels of acH3, CREB and BDNF

Because there was a significant effect of repeated intra-VLO MS-275 treatment in the TST and FST, we performed Western blot analyses to investigate possible protein acetylation and expression changes contributing to depression-like behaviors. Western blot analysis revealed a significant enhancement of histone H3 acetylation in MS-275 (50 and 100 μM) groups [$F_{(3,19)}=7.044$, $p=0.0022$; posthoc, $p<0.05$ and 0.01, respectively] (Fig. 3). Since it is well known that the sustained acetylation of histones by HDAC inhibitors leads to upregulation of gene transcription, we used Western blotting to examine whether repeated intra-VLO administration of MS-275 affects the total protein expression of CREB and BDNF. MS-275 (100 μM) robustly increased BDNF expression approximately 1.75-fold [$F_{(3,19)}=8.06$, $p=0.0011$; posthoc, $p<0.01$] (Fig. 3) and increased CREB expression approximately 1.96-fold [$F_{(3,19)}=5.593$, $p=0.0064$; posthoc, $p<0.0001$] (Fig. 3).

2.4. Effect of repeated intra-VLO MS-275 treatment on CREB and BDNF mRNA levels

We subsequently investigated the levels of CREB and BDNF mRNA during MS-275 treatment. Consistent with the Western blot analysis, a significant elevation of CREB mRNA was observed in MS-275 (50 and 100 μM) animals compared with DMSO controls [$F_{(3,22)}=51.14$, $p<0.0001$; posthoc, $p<0.01$ and 0.0001, respectively] (Fig. 4). In addition, BDNF mRNA levels