# Mitogen- and stress-activated protein kinase-1 activation is involved in melanocortin-induced *BDNF* expression in Neuro2a neuronal cells

Weibo Zhang and Yue Wu

Melanocortins are neuropeptides exerting versatile functions in the nervous system. Melanocortin 4 receptor (MC4R) is primarily expressed in the brain and is thought to be a major mediator for melanocortin. Brain-derived neurotrophic factor (BDNF) may be a crucial downstream molecule of MC4R activation, to yield neurite outgrowth, neuroregenerative, anorexigenic and other actions. In this study, we stimulated Neuro2a murine neuronal cells with an α-melanocyte stimulating hormone (α-MSH) analog, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone (NDP-MSH). In Neuro2a cells, NDP-MSH promoted neurite outgrowth. Upon NDP-MSH administration, *BDNF* expression was greatly enhanced. Furthermore, this effect was effectively reversed by the MC4R antagonist, JKC-363. We found that NDP-MSH treatment activated the ERK cascade and its downstream kinase MSK1 (mitogen- and stress-activated protein kinase-1). Antagonism of the MSK1 cascade by a specific inhibitor or overexpression of a defective MSK1 mutant interrupted the phosphorylation of the transcription factor cAMP-response element binding protein (CREB), blocking *BDNF* upregulation. In addition, MSK1 activation triggered an epigenetic alteration in histone H3 (Ser10), facilitating the expression of the *BDNF* gene. Taken together, our results showed that MSK1 kinase positively activates MC4R-induced *BDNF* expression via modulating the phosphorylation of CREB and histone H3 in Neuro2a neuronal cells. *NeuroReport* 31: 1007–1014 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

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## Introduction

Melanocortins are a group of neuropeptides, including α-, β- and γ-melanocyte-stimulating hormones (MSHs) and adrenocorticotropic hormone (ACTH). Melanocortins are well recognized to exert anti-inflammatory, antiapoptotic, antipyretic, anorexigenic and neuroregenerative actions in the brain [1]. There are five melanocortin receptors (MCRs), each numbered MC1R to MC5R. MC1R, as a classical MSH receptor, is mainly expressed in skin and hair follicles and is involved in the regulation of pigmentation. MC2R is found to be a classical ACTH receptor, and is required for adrenal steroidogenesis in the adrenal cortex. MC5R is expressed widely and implicated in modulating exocrine gland secretions. Of the five known MCRs, MC3R and MC4R are the predominant subtypes expressed in the central nervous system. In addition, MC4R distribution is relatively much broader than MC3R distribution. MC4R is abundantly expressed in almost every region of the brain and is the only subtype expressed in astrocytes [2,3].

As an important MCR, MC4R plays a vital role in energy homeostasis and glucose balance. Targeted deletion of the *MC4R* gene generates obesity-diabetes syndrome. It is well-known that MC4R activation elicits the expression of a subset of downstream genes to impose catabolic effects by reducing food intake and favoring energy expenditure [4,5]. The α-MSH-MC4R cascade can be activated after nerve injury. α-MSH-induced MC4R activation is neuroprotective, promoting memory and learning and facilitating neurite-like outgrowth [6–9]. Meanwhile, MC4R can repress the inflammatory response by the inhibition of cytokine expression and prostaglandin release [10–12]. A study revealed that α-MSH-MC4R cascade could exert antipyretic and neuroprotective effects, and reverse IL-1β-induced amnesia [13]. In contrast, accumulating evidence has suggested that MC4R is primarily involved in the initiation and maintenance of neuropathic pain [14]. However, the mechanisms underlying MC4R actions are still poorly understood.

Brain-derived neurotrophic factor (BDNF) may be an important downstream molecule mediating MC4R actions [15,16]. BDNF is a versatile neurotrophic factor involved in neuronal development, survival and synaptic plasticity. BDNF is capable of repressing inflammation

and apoptosis in a spinal cord injury model [17]. BDNF also participates in injury-induced compensatory and maladaptive processes, including pain. The contribution of BDNF to the initiation and maintenance of chronic and neuropathic pain has been validated in a mice model of chronic constrictive injury (CCI) of the sciatic nerve [18]. BDNF is also identified as a hypothalamic neuron-derived neurotrophic factor to modulate appetite. It was revealed that MC4R activation affects food intake by evoking the secretion of BDNF in the hypothalamus, supporting that BDNF is a signal mediator of MC4R action [15,16]. Moreover, MC4R activation can protect striatal neurons and glial cells from cytotoxic damage of 3-nitropropionic acid through BDNF induction [19].

Previous studies have revealed that the activation of the MC4R receptor in astrocytes induced *BDNF* expression through the cAMP-PKA cascade, and the transcription factor cAMP-response element binding protein (CREB) has been proposed to participate in this event [20]. Another study showed that *BDNF* expression induced by melanocortin/MC4R signal in the rat hypothalamus was dependent on the ERK-cFos pathway [21]. Herein, we provide evidence that MSK1 (mitogen- and stress-activated protein kinase-1), as a downstream kinase of ERK cascade, contributes to MC4R-induced *BDNF* expression. Blockade of MSK1 cascade by a specific inhibitor or overexpression with a kinase-defective mutant effectively abolished the upregulation of *BDNF*. The activity of MSK1 is required for CREB phosphorylation. Moreover, MSK1 activation elicited dynamic phosphorylation of histone H3 (Ser10) to facilitate *BDNF* expression. Thus, our results suggest that MSK1 activation is involved in *BDNF* upregulation induced by MC4R activation in the neuroblastoma cell line Neuro2a.

## Materials and methods
### Reagents and plasmids
[Nle(4), D-Phe(7)]melanocyte-stimulating hormone (NDP-MSH), JKC-363 and SB-747651A were purchased from Tocris Bioscience. PD98059 was purchased from Selleck Chemicals. Antibodies against phosphorylated ERK, MSK1 (Thr581), CREB (Ser133), histone H3 and phosphorylated histone H3 (Ser10) were obtained from Cell Signaling Technology. Antibodies specific for total ERK, MSK1 and CREB were purchased from Santa Cruz Biotechnology. pDC316 plasmids encoding EGFP or C-terminal kinase-dead (CKD) MSK1 were kind gifts of Dr. Tao Li (Department of Biology, College of Chemistry and Life Sciences, Zhejiang Normal University).

### Cell culture
The murine neuroblastoma Neuro2a cells were purchased from American Type Culture Collection and were grown in Dulbecco's modified Eagle's growth medium (DMEM, Gibco) with 10% fetal bovine serum (FBS, Gibco) and 100 U/ml penicillin plus 100 µg/ml streptomycin under a humidified atmosphere containing 5% $CO_2$ maintained at 37°C. For cell growth, DMEM supplemented with 10% FBS was used. For differentiation experiments, medium was replaced with DMEM supplemented with 1% FBS. Neurite outgrowth was observed under a phase-contrast light microscope (Olympus Corporation, Japan) at a magnification of 200×. HEK-293 cells were maintained in DMEM supplemented with 10% FBS.

### Real-time PCR
Total RNA was extracted using Trizol reagent (Invitrogen). cDNAs were synthesized using the M-MLV reverse transcription kit (Promega) following the manufacturer's instructions. Real-time PCR was performed on an ABI 7500 system (Applied Biosystems) using SYBR Green real-time Master Mix (Toyobo, Japan). The expression of each target gene was normalized to the *Gapdh* gene and calculated using the $2^{-\Delta\Delta Ct}$ method. RNA was from three wells of one group and each sample has been examined in triplicate. Primer sequences were as follows: *BDNF* 5′-TGCAGGGGCATAGACAAAAGG-3′ (forward), 5′-CTTATGAATCGCCAGCCAATTCTC-3′ (reverse); *Gapdh* 5′-CATGTTCCAGTATGACTCCACTC-3′ (forward), 5′-GGCCTCACCCCATTTGATGT-3′ (reverse).

### Western blot assay
The cells ($1 \times 10^6$) were washed with cold PBS and lysed on ice in 100 µl RIPA lysis buffer (Beyotime Biotech) containing protease inhibitors (100 µM phenylmethylsulfonyl fluoride, 10 µM leupeptin, 10 µM pepstatin and 2 mM EDTA). Protein concentrations were measured using a BCA Protein Assay Kit (Beyotime, China). Then, the proteins were separated by 10% SDS-PAGE electrophoresis and transferred onto polyvinylidene difluoride (PVDF) (Millipore). After blocking with 5% nonfat milk solution for 1 h, the PVDF membranes were incubated with primary antibodies against phosphorylated or total ERK, MSK1 and CREB proteins at 4°C overnight. Next, the membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Detection of specific proteins was carried out with an ECL chemiluminescence detection kit (Millipore) according to the manufacturer's instructions. Each experiment was repeated in triplicate from independent sample with similar results.

### Infection of recombinant adenovirus
Adenoviral shuttle plasmids expressing EGFP or CKD MSK1 were constructed as described previously [22]. HEK-293 cells were cotransfected with the shuttle plasmid pDC316-MSK1 and the helper plasmid pBHGlox-ΔE1,3Cre. After showing the appearance typical of viral cytopathic effect, cells were collected, followed by repeated freezing and thawing to liberate virus. Neuro2a cells were infected with the recombinant adenoviruses Ad-CKD or

Ad-EGFP at a multiplicity of infection (MOI) of 20. After 24 h, cells were subjected to NDP-MSH treatment.

## Chromatin immunoprecipitation assay

A chromatin immunoprecipitation (ChIP) assay was performed with a SimpleChIP Enzymatic Chromatin IP kit (Cell Signaling Technology) following the manufacturer's manual. Briefly, Neuro2a cells ($3.9 \times 10^7$ cells, 10 cm dish) were cross-linked with 1% formaldehyde at 37°C for 10 min, rinsed twice with ice-cold PBS and lysed in buffer containing protease inhibitors. Cell lysates were sonicated to shear DNA, sedimented and diluted supernatants were immunoprecipitated overnight at 4°C with 4 µg of total histone H3 or phosphorylated histone H3 (Ser10) antibodies. Immunoprecipitated DNA samples were used for quantitative PCR of *BDNF* promoter (exon IX) as described [23]: 5′-TAGATAATGACAGGCTTGG-3′ (forward) and 5′-GCCTCGAAATAGACACTCT-3′ (reverse). DNA immunoprecipitated by anti-histone H3 antibody served as an internal input. These data are normalized on the basis of the corresponding input.

## Statistical analysis

All data in this study were displayed as means ± SD. Comparisons were analyzed by Student's *t*-test or one-way ANOVA. The significance was analyzed with SPSS10.0 software and a *P*-value <0.05 was considered to be statistically significant.

## Results

### Involvement of melanocortin 4 receptor in [Nle(4), D-Phe(7)]melanocyte-stimulating hormone-induced *BDNF* expression in Neuro2a cells

The previous study showed that α-MSH was capable of stimulating neurite outgrowth in Neuro2a cells [24]. As BDNF is an important mediator for neurite outgrowth, in this study, we treated Neuro2a cells with the α-MSH analog NDP-MSH and estimated the *BDNF* expression in differentiation medium. After exposure to increasing concentrations of NDP-MSH for 24 h, RNA was isolated from Neuro2a cells and processed for real-time PCR quantification. As shown in Fig. 1a, *BDNF* mRNA levels were elevated by NDP-MSH treatment. About 100 nM NDP-MSH was adequate to upregulate *BDNF* expression more than eight-fold. The previous study has demonstrated the presence of *MC4R* mRNA in Neuro2a cells using the RNase protection assay [25]. To ascertain whether MC4R is the receptor involved in the effects of NDP-MSH on *BDNF* expression in Neuro2a cells, we employed the specific MC4R antagonist, JKC-363, to treat cells before NDP-MSH administration. As shown

**Fig. 1**
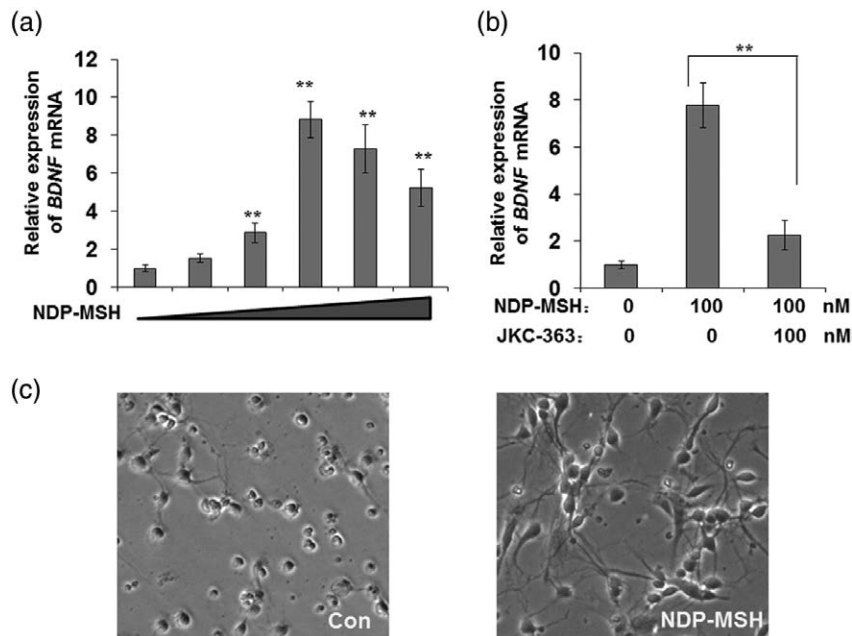


NDP-MSH induces *BDNF* expression in Neuro2a cells through MC4R activation. (a) Neuro2a cells were cultured in differentiation medium and then treated with NDP-MSH (1, 10, 100, 1000, 10 000 nM) for 24 h. (b) Neuro2a cells were preincubated for 15 min with MC4R antagonist (JKC-363, 100 nM), and then treated with 100 nM NDP-MSH for 24 h. Real-time PCR was performed to estimate expression alterations of *BDNF*. *Gapdh* expression was used as an internal control. Values represent the normalized *BDNF* expression compared to the control group of triple independent experiments (**$P < 0.01$, *$P < 0.05$). (c) Neurite outgrowth was analyzed after 48 h incubation with 100 nM NDP-MSH. Cell morphology was observed under a light microscope (200×). BDNF, brain-derived neurotrophic factor; MC4R, melanocortin 4 receptor; NDP-MSH, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone.

**Fig. 2**
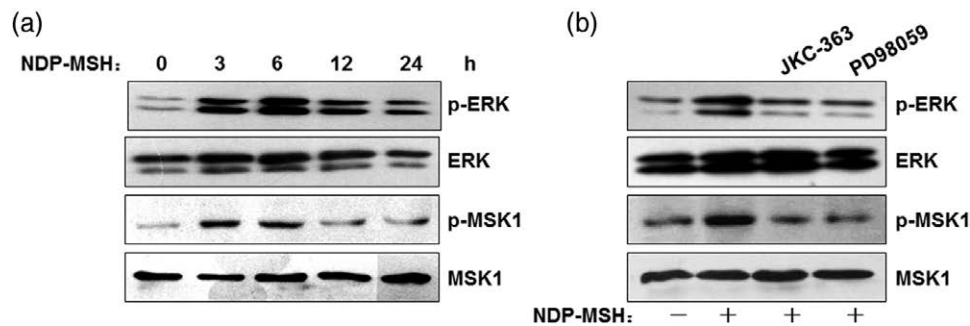


NDP-MSH activates MSK1 via MC4R and ERK. (a) Neuro2a cells were cultured in differentiation medium and then treated with 100 nM NDP-MSH for indicated periods. Then cells were harvested, and western blot assay was performed for the evaluation of phosphorylation levels of ERK and MSK1. (b) Neuro2a cells were preincubated with MC4R antagonist (JKC-363, 100 nM) or ERK inhibitor (PD98059, 10 μM) for 15 min and then treated with 100 nM NDP-MSH for 3 h. Cell lysates were subjected for western blot to display ERK and MSK1 phosphorylation. Representative images from at least three independent experiments are shown. MSK1, mitogen- and stress-activated protein kinase-1; MC4R, melanocortin 4 receptor; NDP-MSH, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone.

in Fig. 1b, JKC-363 almost completely antagonized the promoting effect of NDP-MSH on *BDNF* expression. MC4R activation is involved in *BDNF* induction elicited by NDP-MSH in Neuro2a cells. Subsequently, we analyzed morphological changes of Neuro2a cells after NDP-MSH treatment for 48 h in differentiation medium. As shown in Fig. 1c, the formation of extensive neurites was observed in cells treated by NDP-MSH. Thus, we assume that MSH can induce *BDNF* expression to favor neurite outgrowth.

### Mitogen- and stress-activated protein kinase-1 activation upon [Nle(4), D-Phe(7)]melanocyte-stimulating hormone exposure

MC4R belongs to the superfamily of G protein-coupled receptors (GPCRs). It is well known that MC4R activation leads to the activation of cAMP-PKA and ERK-RSK pathways. Indeed, MSK1 is also a downstream kinase of ERK cascade. In this study, we investigated the function of MSK1 during NDP-MSH treatment. First, we found NDP-MSH administration triggered the phosphorylation of ERK and MSK1 kinases (Fig. 2a). ERK and MSK phosphorylation were very significant at 3 and 6 h of NDP-MSH treatment. Second, both of MC4R antagonist (JKC-363) and the specific inhibitor against ERK (PD98059) greatly abolished the activation of MSK1 (Fig. 2b). This suggested that MC4R activation could stimulate the phosphorylation of MSK1 via the ERK pathway.

### The effects of mitogen- and stress-activated protein kinase-1 inhibition on cAMP-response element binding protein activation and *BDNF* expression

Furthermore, we estimated the effect of MSK1 inhibition on *BDNF* expression. As shown in Fig. 3a, the treatment of MSK1 inhibitor SB-747651A effectively blocked *BDNF* upregulation induced by NDP-MSH administration.

This result indicates that the MSK1 kinase is involved in MC4R-mediated effect on *BDNF* expression in Neuro2a cells. *BDNF* expression is regulated by the transcription factor CREB, which is a known MSK1 downstream substrate. Subsequently, we evaluated the effect of MSK1 inhibition on CREB phosphorylation. Upon NDP-MSH stimulation for 3 h, the CREB protein was rapidly activated by phosphorylation. As expected, SB-747651A repressed the phosphorylation of CREB protein in the presence of NDP-MSH (Fig. 3b). Altogether, these data indicate that MC4R activation can induce CREB phosphorylation to stimulate *BDNF* expression via the MSK1 cascade.

### The interruption of cAMP-response element binding protein phosphorylation and *BDNF* expression after overexpressing an MSK1 mutant

To further confirm the effects of MSK1 on NDP-MSH-induced *BDNF* expression, we specifically overexpressed a CKD MSK1 mutant in Neuro2a cells. As shown in Fig. 4a, adenovirus-mediated overexpression of CKD significantly decreased NDP-MSH-induced *BDNF* expression, whereas it almost unaffected the basic expression of *BDNF* in the absence of NDP-MSH. Accordingly, we also observed that CKD overexpression interrupted the phosphorylation of CREB protein triggered by NDP-MSH stimulation (Fig. 4b). Therefore, our results further validated that MSK1 activity is required for NDP-MSH-mediated *BDNF* induction. Responsive to NDP-MSH stimulation, MC4R activation might evoke the phosphorylation of transcription factor CREB via ERK-MSK1 pathway, thereby driving *BDNF* expression.

### Epigenetic change of histone H3 on the *BDNF* promoter

ERK and MSK1 activation can regulate epigenetic modification of histone H3 by phosphorylation of

**Fig. 3**
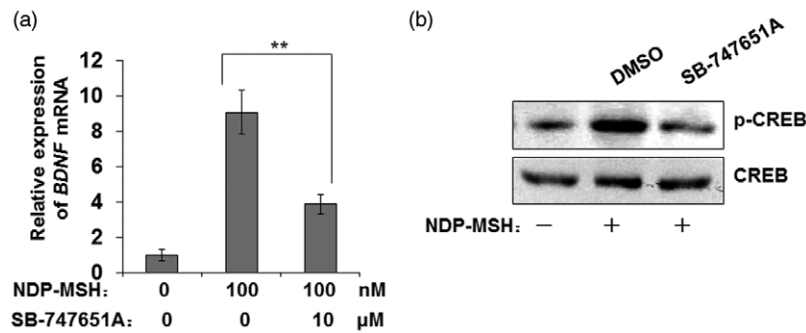


MSK1 inhibition blocks CREB phosphorylation and *BDNF* expression induced by NDP-MSH. (a) Neuro2a cells were cultured in differentiation medium and pretreated with 10 μM of MSK1 inhibitor SB-747651A for 15 min, and then incubated with 100 nM NDP-MSH for 24 h. *BDNF* expression was analyzed by real-time PCR. Data are shown as relative gene expression with means ± SD (n = 3). (b) Neuro2a cells were pretreated with 10 μM of MSK1 inhibitor SB-747651A for 15 min and then incubated with 100 nM NDP-MSH for 3 h. CREB phosphorylation was analyzed by western blot assay. One representative blot of three independent experiments was shown. BDNF, brain-derived neurotrophic factor; CREB, cAMP-response element binding protein; MSK1, mitogen- and stress-activated protein kinase-1; NDP-MSH, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone.

**Fig. 4**
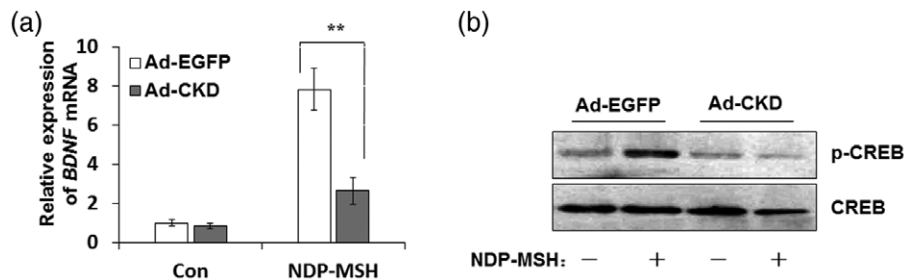


CKD overexpression represses CREB phosphorylation and *BDNF* expression induced by NDP-MSH. (a) Neuro2a cells infected by Ad-EGFP or Ad-CKD were exposed to 100 nM NDP-MSH in differentiation medium for 24 h. *BDNF* expression was analyzed by real-time PCR. Data are shown as relative gene expression with means ± SD (n = 3). (b) Neuro2a cells expressing EGFP or CKD were incubated with 100 nM NDP-MSH for 3 h. CREB phosphorylation was analyzed by western blot assay. One representative blot of three independent experiments was shown. BDNF, brain-derived neurotrophic factor; CKD, C-terminal kinase-dead; CREB, cAMP-response element binding protein; NDP-MSH, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone.

serine 10 to facilitate gene expression. As shown in Fig. 5a, NDP-MSH directly enhanced the total phosphorylation levels of histone H3 at Ser10. CKD overexpression interrupted the phosphorylation of histone H3 triggered by NDP-MSH stimulation. To further determine whether MSK1-mediated epigenetic modification contributes to *BDNF* induction, we performed a ChIP assay. In the EGFP-overexpressing cells, NDP-MSH addition directly increased the phosphorylation level of histone H3 on *BDNF* promoter. By comparison, overexpression of CKD significantly attenuated histone H3 phosphorylation induced by NDP-MSH (Fig. 5b). Therefore, our findings revealed that MSK1-mediated histone modification also participates in *BDNF* expression induced by MSH-MC4R activation. It further strengthens the role of MSK1 signaling in MSH-MC4R actions.

## Discussion

Melanocortins consist of α-MSH, β-MSH, γ-MSH and ACTH. α-MSH is mainly produced by the pars intermedia of the pituitary gland, and its synthesis also can be found in several other peripheral tissues. α-MSH functions by binding to MC4R [1]. MC4R exerts versatile roles in the regulation of development and physiological function of the nervous system, including energy homeostasis. Gene expression studies in both mice and humans have demonstrated that MC4R is predominantly expressed in the central nervous system, including the thalamus, hypothalamus, cortex, hippocampus and brainstem. Evidence so far suggests that MC4R mediates α-MSH effects on synaptic plasticity, inflammation, neuroprotection and reproduction, as well as astrocyte functions. Activation of MC4R has been shown to elicit anti-inflammatory effects in the pathogenesis of

**Fig. 5**
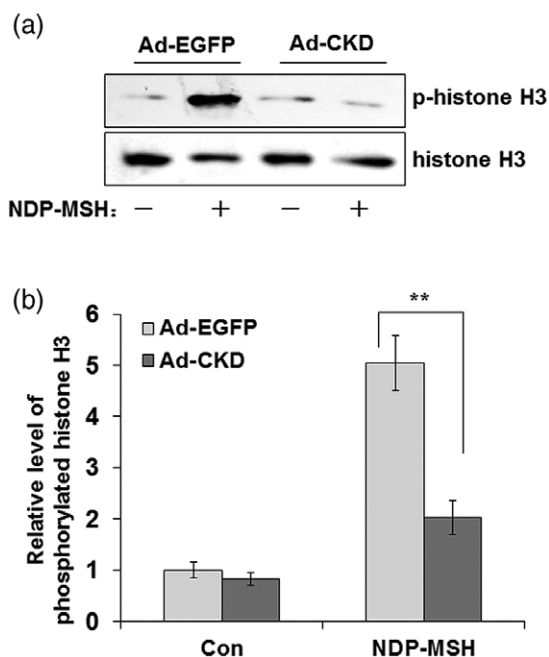


CKD overexpression affects histone H3 phosphorylation on *BDNF* promoter. (a) Neuro2a cells expressing EGFP or CKD were incubated with 100 nM NDP-MSH for 3 h. Western blot was analyzed to detect phosphorylated histone H3 levels induced by NDP-MSH. Total histone H3 was used to confirm equal protein loading. (b) Then cells were collected and ChIP assays were conducted with specific antibody against total or phosphorylated (Ser10) histone H3. Immunoprecipitated DNA fragments were amplified by real-time PCR for *BDNF* promoter. DNA immunoprecipitated by anti-histone H3 antibody served as an internal input. The relative abundance of phosphorylated histone H3 in the control group was set as 1. Normalized values relative to the control were expressed as mean ± SD from three independent experiments. BDNF, brain-derived neurotrophic factor; ChIP, chromatin immunoprecipitation; CKD, C-terminal kinase-dead; NDP-MSH, [Nle(4), D-Phe(7)]melanocyte-stimulating hormone.

intracerebral hemorrhage-induced secondary brain injury [25]. MC4R activation also protects striatal neurons and glial cells from 3-nitropropionic acid toxicity [19]. Moreover, α-MSH ameliorates cognitive performance in animals with attenuated memory consolidation caused by IL-1β, while this effect is reversed by MC4R antagonist HS014 [13]. Neurite outgrowth induced by α-MSH in mouse dorsal root ganglia neurons is completely blocked by the MC4R-selective inhibitor [26]. Moreover, functional blockade of spinal MC4R delays the development of neuropathic pain induced by peripheral nerve injury [14,27]. These data revealed that the MC4R is a crucial mediator of painful stimuli.

It remains unclear how MC4R mediates diverse effects in regulating energy intake, neuropathic pain, anti-inflammation and neuroprotection. Several studies investigated the mechanisms that MC4R activation modulates BDNF and PPAR expression, activates CREB and induces TGF-β release [12,19]. BDNF release from the

hypothalamus is indispensable for MC4R's effect on energy balance, suggesting that BDNF is a downstream mediator of MC4R activation in regulating food intake [15,16]. Moreover, MC4R activation induces BDNF to protect striatal neurons and glial cells from the cytotoxicity of 3-nitropropionic acid [19]. A study showed that MC4R agonist RO27-3225 could alleviate brain injury induced by intra-abdominal hypertension, reduce the expression of inflammatory cytokines IL-1β and TNF-α, and decrease the permeability of blood-brain barrier [10]. Regarding the initiation and maintenance of neuropathic pain, MC4R in vivo evokes pain behavior in animals after peripheral nerve injury in a p38-dependent manner [28]. Paradoxically, MC4R activation in the rat model of CCI might contribute to the expression of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, while decreasing the level of the anti-inflammatory cytokine, IL-10 [29].

MC4R is a prototypical GPCR with seven transmembrane domains. MC4R has been shown to modulate the activities of various kinases, such as PKA, p38, ERK, PI3K, AMPK, JNK and PKC [2,20,21,25,28]. It was reported that α-MSH and its analog, NDP-MSH, increase *BDNF* expression in rat astrocytes via the production of cAMP. Both the adenylate cyclase inhibitor, SQ22536, and the PKA inhibitor, Rp-cAMP, can abolish MC4R-induced *BDNF* expression. The MC4R signal triggers the classic pathway of cAMP-mediated PKA activation, further leading to CREB phosphorylation and CRE-dependent transcription [20]. In addition, ERK and downstream RSK cascades partly account for MC4R-induced *BDNF* expression in astrocytes via c-Fos induction [21].

Herein, our results suggest that MC4R-ERK-MSK1 signaling is involved in α-MSH-induced *BDNF* expression. MSK1 and MSK2 are downstream kinases of ERK and p38 MAPK pathways. ERK and p38 directly phosphorylate several sites on MSKs, which leads to the activation of the C-terminal kinase domain. It further elicits autophosphorylation and activation of the N-terminal kinase domain, which subsequently phosphorylates the MSK substrate. The best-characterized MSK substrates include CREB and histone H3. MSK1 and MSK2 phosphorylate CREB on Ser133, a site that is also targeted by other kinases, such as PKA. Phosphorylation on Ser10 of histone H3 mediated by MSKs is sufficient to induce chromatin remodeling from condensed heterochromatin to more open euchromatin [30]. Both MSK1 and MSK2 are expressed in nervous tissues and neuronal cells, whereas MSK1 level is relatively higher in most brain structures. Increasing evidence shows that MSK1-mediated phosphorylation of CREB and histone H3 participates in synaptic plasticity, memory formation and long-term neuroadaptation in response to drug addiction [31,32].

Previous and our studies also revealed that MSH stimulation can induce neurite outgrowth in Neuro2a cells [24]. In addition, its effect was completely inhibited by an MC4R antagonist. As BDNF is an important mediator for neurite outgrowth [33]. In this study, our results showed that NDP-MSH stimulated *BDNF* expression via MC4R activation in Neuro2a cells. Further investigation demonstrated that NDP-MSH triggered the ERK-MSK1 cascade to induce CREB phosphorylation. MSK1 activation also altered the post-translational modification of histone H3 on Ser10 located at the *BDNF* promoter. Thus, CREB phosphorylation orchestrated *BDNF* transcription with epigenetic modification of histone H3. Blockade of MSK1 cascade by a specific inhibitor or overexpression of a defective MSK1 mutant interrupted the phosphorylation of CREB and histone H3, thereby impairing *BDNF* induction.

Taken together, our results provide new insights into mechanisms of MC4R action, emphasizing the role of MSK1 in the transduction of the MC4R signal. MSK1 may be an important effector of melanocortin-MC4R in the brain. Intriguingly, it is well known that MSKs can also exert anti-inflammatory or proinflammatory roles dependent on different cellular contexts [30]. Indeed, MC4R exhibits similar features. MC4R can alleviate brain injury via reducing proinflammatory cytokines; in contrast, MC4R also can contribute to the generation of neuropathic pain via affecting the expression of proinflammatory cytokines. Our findings suggest a potential functional association between MSK1 and MC4R in inflammation regulation. Further investigation is essential to explore the functional relationship of MSK1 and MC4R, thereby offering a better understanding of the physiological effects and mechanisms of melanocortins within the brain.

## Conclusion

The present study indicated that ERK-MSK1 activation was involved in MC4R-induced *BDNF* expression in Neuro2a neuronal cells. MSK1 kinase promoted *BDNF* induction via modulating the phosphorylation of CREB and histone H3. Antagonism of MSK1 cascade by specific inhibitor or overexpressing a defective MSK1 mutant interrupted *BDNF* upregulation induced by α-MSH analog, NDP-MSH.

## Acknowledgements

## Conflicts of interest

There are no conflicts of interest.

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