

Original Article

Downregulation of Sirtuin1 in the rat nucleus accumbens was beneficial for the alleviation of heroin addiction

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Abstract: The sirtuin1 (SIRT1) was demonstrated to be involved in the cocaine and morphine addiction, however, its role in the heroin addiction has not been explored yet. In this study, rat heroin addiction model was established for testing the role of SIRT1 by the small interfering technique. The results indicated that SIRT1 expression was significantly increased in the nucleus accumbens (NAc) of rats with heroin addiction. In addition, the expression of SIRT1 upstream protein Δ FosB was significantly increased in rats with heroin addiction, whereas the SIRT1 downstream target acetylated-H3 (AC-H3) was significantly decreased. After the treatment of SIRT1 siRNA in rats with heroin addiction, the addictive symptoms caused by heroin administration were significantly alleviated, while SIRT1 agonist resveratrol (RES) increased behavioral responses to heroin. These results indicated that the induction of SIRT1 protein may play a critical role in the development of heroin addiction and its mediation may be through Δ FosB-SIRT1-AC-H3 pathway. The strategy for the inhibition of SIRT1 might help to lessen the heroin addiction.

Keywords: Heroin, addiction, sirtuin1, nucleus accumbens, rat

Introduction

Heroin, derived from morphine, is considered as one of the most hazardous drugs of abuse due to its high lipid solubility, membrane permeability, and rapid physical dependence and tolerance [1, 2]. Heroin can lead to both psychological and physical addiction due to activation of some brain regions that produce euphoric sensations [3]. The heroin addiction is characterized by continuous demand for the heroin, tolerance (i.e. craving for larger doses) and painful withdrawal. Heroin addiction is intensively related to the deviant behaviors and crime [4, 5]. Recent studies have shown that heroin addiction is also associated with profound alterations in brain structure and composition [6]. Until recently, the molecular and cellular mechanisms regarding heroin addiction have not yet been fully elucidated.

Continuous administration of addictive drugs can result in the cellular adaptations in some neurons of specific brain region, and the alterations in gene expression are considered as a critical mechanism of drug abuse [7]. Nucleus

accumbens (NAc) is a critical component of the mesocorticolimbic system, a brain circuitry involved in reward and motivation. Recent work has demonstrated that repeated cocaine administration causes long-lasting changes in gene expression within the NAc [8]. Investigation of epigenetic mechanisms revealed that numerous posttranslational modifications of histones modulate gene transcription in drug addiction. Robison et al reported that repeated exposure to the cocaine promotes histone acetylation, which is tightly regulated through the actions of numerous histone acetyltransferases and histone deacetylases (HDACs) in the NAc [9].

SIRT1 (silent information regulator 2 homolog 1), one of the class III HDACs, has been shown to be upregulated in the NAc after chronic cocaine administration [10, 11]. It is also reported that long-term exposure to cocaine or morphine can increase SIRT1 expression in the NAc [12]. However, few studies have examined the activation of SIRT1 in heroin addiction. In the present study, we aimed to examine the expression of SIRT1 in the NAc of rats with heroin addiction. By using SIRT1 small interfering

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RNA (siRNA), we further explored the potential treatment for heroin addiction through the down-regulation of SIRT1.

Materials and methods

Animals

A total of 48 male Sprague Dawley rats (200-220 g) were purchased from the Department of Laboratory Animal Science of Guizhou Medical University (Guiyang, China). All rats were housed under a 12 h/12 h day/night cycle with free access to food and water. Rats were randomly divided into 6 groups: normal control (N.C., n=8), saline control (S.C., n=8), heroin-addition (H.A, n=8), heroin-addition+SIRT1 siRNA (H.A+si, n=8), heroin-addition+SIRT1 control siRNA (H.A+C.si, n=8), and heroin-addition+RES groups (H.A+R, n=8). Among the group, 4 animals were used for behavior observation, 4 for conditioned place preference (CPP) test. All animal protocols and experimental procedures were approved by the Ethics Committee of Guizhou Medical University.

Drug administration

In this study, the heroin (provided by Public Security Department of Guizhou Province) was dissolved in 0.9% NaCl solution immediately before injection. The rats were intraperitoneally injected with heroin twice a day with an increasing dose for 9 consecutive days. The dose for each injection was 3 mg/kg on day 1, and then increased by 3 mg/kg each day. On day 9, the dose was 27 mg/kg. This pattern of heroin administration has been shown to induce physiological dependence in rats [13]. In control group rats received an equal volume of normal saline. In SIRT1 siRNA group, SIRT1 siRNA (1.5 μ L; Viraltherepy Technologies, Wuhan, China) was diluted in 5 μ L transfection reagent and then injected intracerebroventricularly into heroin-dependent rat NAc as previously described [14]. In the control siRNA group, heroin-dependent rats were treated with the same volume of control siRNA. In the SIRT1 agonist resveratrol (RES) group, heroin-dependent rats were injected intraperitoneally with 20 mg/kg RES (Sigma-Aldrich, USA, dissolved in 5% dimethyl sulfoxide, DMSO) once a day for 7 days. For SIRT1 siRNA treatment, the anesthetized rats were placed on a stereotaxic instrument and then the siRNA was injected into right lateral ventricle (coordinates 1.2 mm posterior to the breg-

ma, 1.5 mm lateral to the midline, 7.0 mm ventral to the surface of the skull) over 5 min using a Hamilton microsyringe 1 week before the intraperitoneal injection of heroin. Naloxone injection (0.8 mg per rat, Chongqing Yaoyou Pharmaceutical Co., LTD, China) was intraperitoneally given to heroin-dependent rats 24 hours after the final heroin injection [15].

Sample preparation

In the heroin dependent group and control group, brains were extracted after the last injection of heroin or saline. In the naloxone-induced withdrawal group, brains were extracted 30 min after the injection of naloxone. In the SIRT1 siRNA and RES treatment group, brains were extracted 24 hours after the last heroin injection. The rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.), and sacrificed by intracardiac perfusion of 4% paraformaldehyde in PBS. The brains were harvested and embedded in Tissue Tek for the further analysis.

The NAc was punched with a 14-gauge needle, and then removed in liquid nitrogen until they were assayed by western blotting.

Behavioral observations

Behavioral observations were carried out as described previously [13]. In rats with heroin administration, behaviors were recorded during visual checks for 2 hours, starting after the first daily injection. Typical heroin-induced behaviors including explore, rear, walk, hyperactivity, straggling, erecting, licking the hair, eat/drink, biting paws were recorded for each subject. Heroin withdrawal reactions such as jumping, upright position (rearing), face washing, wet dog shaking, teeth chatting, stretching and weight loss were recorded after naloxone intraperitoneal injection and lasted for 30 minutes. The numbers of upright position, face washing, wet dog shaking, teeth chatting, stretching and weight loss in each group were scored.

Conditioned place preference test

Conditioned place preference (CPP) test was performed as reported by others [16, 17]. The rats were placed into a photo beam-monitored box to observe the bias of two chambers in CPP instrument (Zhongshidichuang Science and Technology development Co., Ltd, Beijing).

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A Naloxone Withdrawal Reactions (mean \pm SD)

Withdrawal signs	S.C.	H.A.
jumping	2.0 \pm 0.71	3 \pm 1.58
rearing	6.4 \pm 1.67	7.2 \pm 2.17
face washing	2.8 \pm 0.84	10.0 \pm 1.58*
wet dog shaking	0.8 \pm 0.84	16.8 \pm 1.64*
teeth chatting	0.6 \pm 0.55	7.8 \pm 0.84*

* $P < 0.05$ Vs. S.C. group

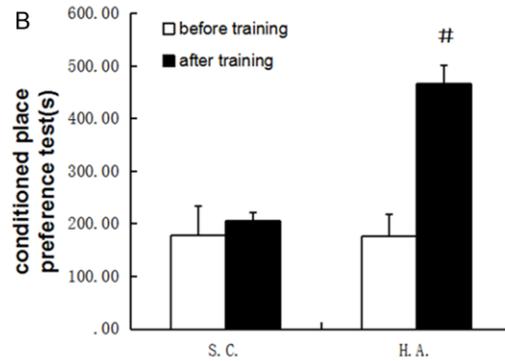


Figure 1. Establishment of rat heroin addiction model. A. On day 10 after naloxone administration, the rats with heroin addiction had higher numbers of face washing, wet dog shaking, and teeth chatting than the saline-treated controls ($P < 0.05$). B. On day 9, the CPP test revealed that the rats treated with heroin spent more time in the white chamber compared with those of vehicle-treated animals (202.33 ± 14.12 vs 475.50 ± 19.77 , $P < 0.05$).

The rats which showed significant preference to any chambers were excluded. During the training period, heroin (3 mg/kg, i.p.) were injected into the rats in the morning, which were placed into one chamber (white chamber) for 30 min, and then the animals were injected with normal saline and placed into the other chamber (black chamber) for 30 min in the afternoon. After 9 days, the animals were placed into the CPP apparatus and the chamber preference was recorded. The baseline locomotive activities for saline injection were evaluated. CPP scores were calculated through the following formula: the time in the saline-paired chamber - the time in the heroin-paired chamber.

Immunohistochemistry staining

The coronal sections containing the bilateral NAc (10 μ m thickness) were obtained using a cryostat (Leica Microsystems, Bannockburn, IL, USA). The sections were blocked with the serum for 1 h, and then incubated with rabbit polyclonal antibody SIRT1 (sc-19857), goat polyclonal antibody Δ FosB (sc-48868), goat polyclonal antibody AC-H3 (sc-8655) (1:200, Santa Cruz Biotechnology, TX, USA) overnight at 4°C. The sections were incubated with the corresponding ABC Kit (Santa Cruz Biotechnology, TX, USA). The peroxidase activities were showed by 3-diaminobenzidine (DAB) for 5 min. The serum instead of the corresponding primary antibody was used as negative control.

Western blot

At 24 h after heroin administration, the total protein of NAC was extracted using RIPA lysis

buffer (Beijing Applygene Technology Co. Ltd, Beijing). The protein concentration was measured using detergent compatible Bradford protein assay (Bio-Rad, CA). The samples (80 mg) were loaded onto the Tris-glycine gel, and then electrophoresed and transferred onto the nitrocellulose membrane. The membrane was then incubated with 5% defatted milk for 2 hrs followed by the same SIRT1, Δ FosB, and AC-H3 primary antibodies used for immunostaining overnight at 4°C. The membrane was then treated with corresponding horseradish peroxidase conjugated secondary antibody for 1 h. The bands were observed by the chemiluminescent kit (Santa Cruz Biotechnology, TX, USA) and exposed to Investigator Prolmage. The densitometries of bands were analyzed using Image J 5.0 software (National Institutes of Health, Bethesda, MD, USA). The β -actin of the same membrane was also blotted as a loading control.

Statistical analysis

SPSS19.0 statistical software was applied for the statistical analyses. The values were expressed as mean \pm standard deviation, and one-way analysis of variance (ANOVA) with the Tukey-Kramer post hoc tests was used. A P value of < 0.05 was considered significant.

Results

Establishment of rat heroin addiction model

Seven days after heroin administration, the typical addictive symptoms were found in the heroin treated rats compared to the vehicle-

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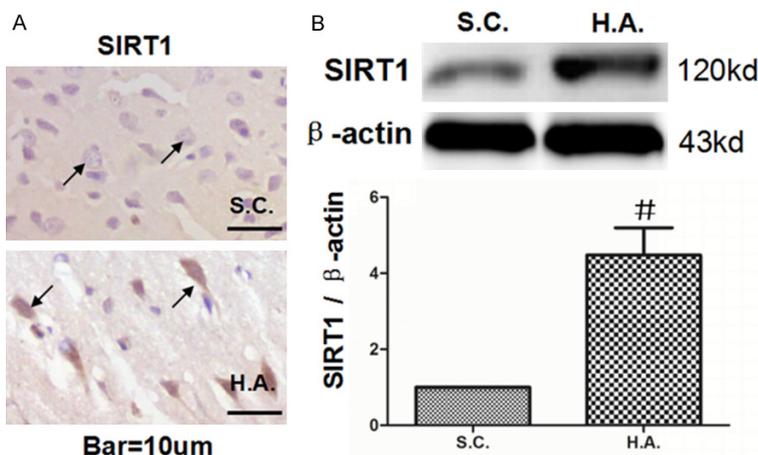


Figure 2. Increased SIRT1 expression in the rats with heroin addiction. A. Immunostaining showed the SIRT1 expression was found to be mainly located in the nucleus, and the expression levels of SIRT1 in the nucleus were markedly increased in the NAc of rats with heroin administration. B. Western blot showed that there were significant differences in amount of SIRT1 between heroin-treated group and saline control group (SIRT1/β-actin: 4.234 ± 0.256 vs 1.0 ± 0.0 , $P < 0.05$).

treated controls, such as decreased activity, freezing and depression, etc. These behaviors lasted for 30-80 minutes, while the duration of exciting responses including hyperactivity, straggling, erecting, licking the hair, drinking water, biting paws and so on was about 1.5 hours. To confirm the validity of heroin model, naloxone withdrawal reaction was performed. At day 10 after naloxone administration, the rats previously treated with heroin had higher numbers of jumping, rearing, face washing, wet dog shaking, and teeth chatting than the vehicle-treated controls ($P < 0.05$) (Figure 1A). In addition, at day 9 the rats treated with heroin spent more time in the white chamber than those of vehicle-treated animals using CPP test (202.33 ± 14.12 vs 475.50 ± 19.77 second, $P < 0.05$) (Figure 1B). These results indicated that the reliable rat model was established for studying heroin addiction.

Increased SIRT1 expression in the rats with heroin addiction

It has been reported that increased SIRT1 expression in the NAc can be induced after chronic cocaine or morphine administration [9, 12]. Thus, we examined the SIRT1 expression after heroin addiction model establishment. Immunostaining showed the SIRT1 expression was found to be mainly located in the nucleus, and the expression levels of SIRT1 in the nucle-

us were markedly increased in the NAc of rats with heroin addiction. There were significant differences in amount of SIRT1 between heroin-treated group and saline control group (SIRT1/β-actin: 4.234 ± 0.256 vs 1.0 ± 0.0 , $P < 0.05$) (Figure 2A, 2B). These data demonstrated that heroin addiction could induce the activation of SIRT1.

Increased ΔFosB and decreased AC-H3 expressions in the rats with heroin addiction

ΔFosB is a stable transcription factor among Fos family. It has been revealed that SIRT1 induction is associated with increased ΔFosB binding to the SIRT1 promoters in NAc

[12]. It has also reported that SIRT1 exerts its function usually by deacetylating histone [18]. Thus, the expression of ΔFosB and acetylated-H3 (AC-H3) was measured in heroin-treated rats. As shown in Figure 3A, the ΔFosB expression was mainly concentrated in the nucleus of rats with heroin addiction, and using Western blot significant differences in amount of ΔFosB were found between heroin-treated rats and saline control group (ΔFosB/β-actin: 3.352 ± 0.585 vs 1.0 ± 0.0 , $P < 0.05$) (Figure 3B). While the AC-H3 protein was abundantly aggregated in the nucleus in the saline treated rats, after heroin addiction model establishment, the expression levels of AC-H3 protein in the NAc was significantly reduced (AC-H3/β-actin: 0.2592 ± 0.034 vs 1.0 ± 0.0 , $P < 0.05$) (Figure 3C, 3D).

Alleviation of rat heroin addiction by SIRT1 siRNA

To further explore the effect of SIRT1 on the rat heroin addiction, SIRT1 siRNA was intracerebroventricularly injected into heroin-treated rat NAc. The western blot results indicated that the SIRT1 siRNA could significantly decrease the expression level of SIRT1 in the NAc (SIRT1 siRNA/β-actin: 0.2949 ± 0.063 vs 1.0 ± 0.0 , $P < 0.05$) (Figure 4A). After siRNA treatment, CPP test showed that the time was significantly decreased in the SIRT1 siRNA treat-

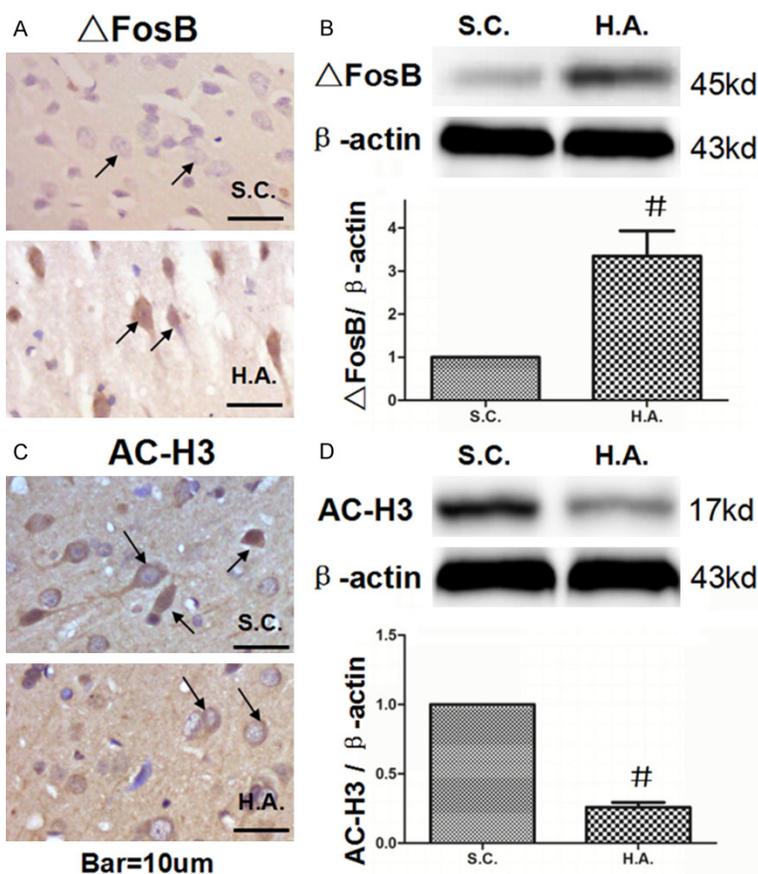


Figure 3. Increased Δ FosB and decreased AC-H3 expressions in the rats with heroin addiction. A. Immunostaining showed that the Δ FosB expression in the nucleus was markedly increased and mainly concentrated in the NAc nucleus of rats with heroin addiction. B. Western blot demonstrated that there were significant differences in amount of SIRT1 between heroin-treated rats and saline control group (Δ FosB/ β -actin: 3.352 ± 0.585 vs 1.0 ± 0.0 , $P < 0.05$). C. Immunostaining showed that AC-H3 protein in the saline-treated animals was abundantly aggregated in the nucleus, while the expression levels of AC-H3 protein was decreased after heroin addiction model establishment. D. The expression levels of AC-H3 protein in the nucleus was significantly reduced in the rats with heroin addiction (AC-H3/ β -actin: 0.2592 ± 0.034 vs 1.0 ± 0.0 , $P < 0.05$).

ed rats than those with SIRT1 control siRNA (279.03 ± 27.79 vs 486.60 ± 31.7 second, $P < 0.05$) (**Figure 4B**). Furthermore, after the SIRT1 agonist resveratrol (RES) treatment, the animals with heroin addiction stayed in the heroin-paired white chamber for a longer time (581.78 ± 30.26 vs 465.80 ± 35.39 second, $P < 0.05$) (**Figure 4B**). These results indicate that downregulation of SIRT1 by siRNA may help to alleviate heroin addiction.

Discussion

Our investigation reveals that the increasing doses of heroin administration can be used to

develop the heroin addiction in rats and SIRT1 expression was significantly increased in the NAc of rats with heroin addiction. Furthermore, the increased expression of upstream protein Δ FosB and decreased expression of AC-H3 of SIRT1 confirm that the induction of SIRT1 protein may be involved in the development of heroin through Δ FosB-SIRT1-AC-H3 pathway. Specifically, the addictive symptoms caused by heroin administration were significantly alleviated by SIRT1 siRNA.

In recent years, long term abuse of heroin, regarded as opioid important derivatives, induces pathological changes in nervous system [19]. Following the repeated heroin administration, the reward of heroin addiction can be associated with external behavioral abnormalities [20]. The conditioned place preference (CPP) has been reported to evaluate the motivation impacts of heroin and measure the incentive value attributed to heroin by a dependent rat [13]. In this study, using CPP test, we show that the rats treated with heroin spent markedly more time in the heroin-paired chamber

compared to the saline treated rats, suggesting that the rats expressed stronger heroin preference in response to the increasing doses of heroin administration. In addition, naloxone administration was performed to induce withdrawal reaction which is considered as a core feature of the drug dependence [21]. After naloxone administration, we demonstrate that the rats previously treated with heroin had significantly higher numbers of face washing, wet dog shaking, and teeth chatting compared to saline-treated rats. These typical withdrawal reactions were consistent with other works [22, 23]. Therefore, our results suggest that the reliable rat model can be established by the injec-

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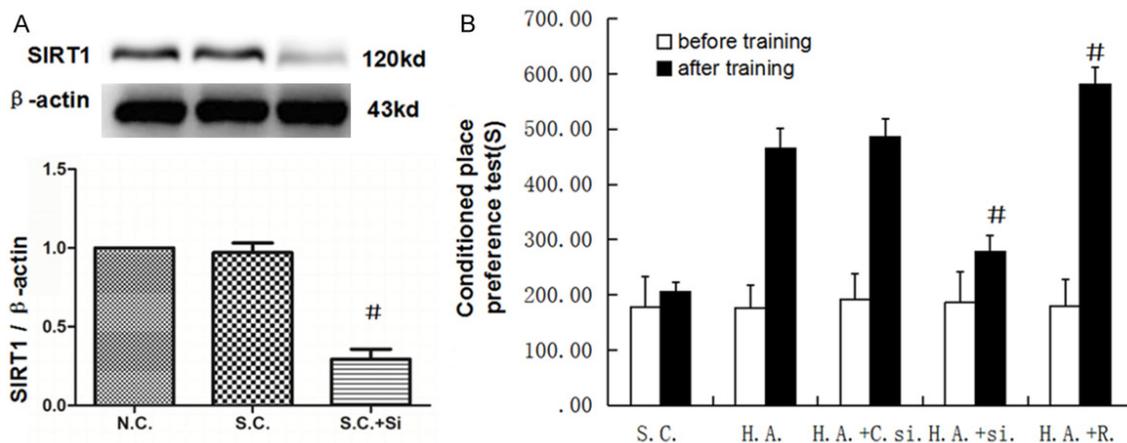


Figure 4. Alleviation of rat heroin addiction by SIRT1 siRNA. A. Western blot results showed that the SIRT1 siRNA could significantly decrease the expression level of SIRT1 in the NAC of rats (SIRT1 siRNA/ β -actin: 0.2949 ± 0.063 vs 1.0 ± 0.0 , $P < 0.05$). B. CPP test showed that the time was significantly decreased in the SIRT1 siRNA treated rats than those with SIRT1 control siRNA (279.03 ± 27.79 vs 486.60 ± 31.7 second, $P < 0.05$), while after the SIRT1 agonist resveratrol (RES) treatment, the animals with heroin addiction stayed in the white chamber for a longer time (581.78 ± 30.26 vs 465.80 ± 35.39 second, $P < 0.05$).

tion of increasing doses of heroin and confirmed by CPP test and naloxone withdraw reaction.

The NAc is a critical region in brain mesocorticolimbic system that creates reward, pleasure and motivation. In this system, the NAc is an important component in charge of natural and drug-induced reinforcement behaviors [8]. It is reported that the chronic exposure to morphine and cocaine resulted in the changes in gene expression and neuronal morphology in the NAc [9]. Recent studies examining the molecular mechanisms controlling drug-induced gene expression have indicated a direct role for chromatin modifications in the regulation of drug-mediated gene program [8]. The accumulating evidence has suggested that after cocaine administration, the increased levels of histone H3 and/or H4 acetylation have been reported in some brain regions [24-26]. SIRT1, one of the class III histone deacetylases, can modulate the DNA repair, chromosomal stability and gene transcription through catalyzing the deacetylase reaction [27, 28]. Chronic exposure to cocaine and morphine will result in increased SIRT1 expression in the NAc [26]. In this study, using immunostaining and Western blot, we revealed that the SIRT1 expression was predominantly distributed in the nucleus of NAc and was significantly increased following heroin addiction. Moreover, using SIRT siRNA, we iden-

tify that the expression level of SIRT1 in the NAc was markedly decreased and heroin addictive symptoms was significantly alleviated. While SIRT1 activator RES administration was found to significantly aggravate the addictive symptoms in heroin treated rats. These results suggest the SIRT1 activation might be involved in the heroin addiction and downregulation of SIRT1 expression may aid in the development of novel therapeutics for future treatment of heroin addiction.

It is thought that SIRT1 induction is associated with increased Δ FosB binding at their gene promoter [9]. The expression of Δ FosB is enhanced in mouse corpus striatum in response to cocaine and morphine [29]. Furthermore, the Δ FosB in the NAc can increase cocaine-seeking behaviors [30]. In this study, we showed that the expression level of Δ FosB in the NAc of rat with heroin administration was significantly increased using immunostaining and Western blot, which suggested that induction of Δ FosB protein may mediate heroin-induced increased SIRT1 expression in the NAc. In addition, we also demonstrate that the expression level of AC-H3 in the NAc was markedly reduced after heroin addiction. This result is consistent with previous studies demonstrating that the chronic cocaine can increase histone H3 acetylation in the NAc [9, 31]. However, in the morphine administration study, the histone H3 acetyla-

tion was significantly decreased [12]. Therefore, there might be various mechanisms between heroin, morphine and cocaine addiction in the NAc.

In summary, significantly increased SIRT1 expression was found in the NAc of rats with heroin addiction and SIRT1 siRNA can be used to alleviate the addictive symptoms caused by heroin administration. The activated SIRT1 played an important role in the heroin addiction through Δ FosB-SIRT1-AC-H3 pathway. The strategy for the inhibition of SIRT1 may potentially have therapeutic implication for heroin addiction.

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Disclosure of conflict of interest

None.

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References

[1] Maurer HH, Sauer C, Theobald DS. Toxicokinetics of drugs of abuse: current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine, heroin, morphine, and codeine. *Ther Drug Monit* 2006; 28: 447-453.

[2] Boening JA. Neurobiology of an addiction memory. *J Neural Transm (Vienna)* 2001; 108: 755-765.

[3] Cunha-Oliveira T, Rego AC, Garrido J, Borges F, Macedo T, Oliveira CR. Neurotoxicity of heroin-cocaine combinations in rat cortical neurons. *Toxicology* 2010; 276: 11-17.

[4] Brown R. Heroin dependence. *WMJ* 2004; 103: 20-26.

[5] Kenny PJ, Chen SA, Kitamura O, Markou A, Koob GF. Conditioned withdrawal drives heroin

consumption and decreases reward sensitivity. *J Neurosci* 2006; 26: 5894-5900.

[6] Cadet JL, Bisagno V. Glial-neuronal ensembles: partners in drug addiction-associated synaptic plasticity. *Front Pharmacol* 2014; 5: 204.

[7] Vassoler FM, Sadri-Vakili G. Mechanism of transgenerational inheritance of addictive-like behaviors. *Neuroscience* 2014; 264: 198-206.

[8] Maze I, Feng J, Wilkinson MB, Sun H, Shen L, Nestler EJ. Cocaine dynamically regulates heterochromatin and repetitive element unsilencing in nucleus accumbens. *Proc Natl Acad Sci U S A* 2011; 108: 3035-3040.

[9] Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci* 2011; 12: 623-637.

[10] Canto C, Auwerx J. Targeting sirtuin 1 to improve metabolism: all you need is NAD (+)? *Pharmacol Rev* 2012; 64: 166-187.

[11] Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT. Multiple roles of HDAC inhibition in neurodegenerative conditions. *Trends Neurosci* 2009; 32: 591-601.

[12] Ferguson D, Koo JW, Feng J, Heller E, Rabkin J, Heshmati M, Renthal W, Neve R, Liu X, Shao N, Sartorelli V, Shen L, Nestler EJ. Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. *J Neurosci* 2013; 33: 16088-16098.

[13] Seip KM, Reed B, Ho A, Kreek MJ. Measuring the incentive value of escalating doses of heroin in heroin-dependent Fischer rats during acute spontaneous withdrawal. *Psychopharmacology (Berl)* 2012; 219: 59-72.

[14] Yan J, Li L, Khatibi NH, Yang L, Wang K, Zhang W, Martin RD, Han J, Zhang J, Zhou C. Blood-brain barrier disruption following subarchnoid hemorrhage may be facilitated through PUMA induction of endothelial cell apoptosis from the endoplasmic reticulum. *Exp Neurol* 2011; 230: 240-247.

[15] Ghowsi M, Yousofvand N. Impact of morphine dependency and detoxification by methadone on male's rat reproductive system. *Iran J Reprod Med* 2015; 13: 275-282.

[16] Liu X, Liu Y, Zhong P, Wilkinson B, Olsen CM, Bayer KU, Liu QS. CaMKII activity in the ventral tegmental area gates cocaine-induced synaptic plasticity in the nucleus accumbens. *Neuropsychopharmacology* 2014; 39: 989-999.

[17] Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol* 2007; 12: 227-462.

[18] Cheng BB, Yan ZQ, Yao QP, Shen BR, Wang JY, Gao LZ, Li YQ, Yuan HT, Qi YX, Jiang ZL. Association of SIRT1 expression with shear

SIRT1 in rats with heroin addiction

- stress induced endothelial progenitor cell differentiation. *J Cell Biochem* 2012; 113: 3663-3671.
- [19] Pu H, Wang X, Zhang J, Ma C, Su Y, Li X, Liu X, Su L. Cerebellar neuronal apoptosis in heroin-addicted rats and its molecular mechanism. *Int J Clin Exp Pathol* 2015; 8: 8260-8267.
- [20] Evans CJ, Cahill CM. Neurobiology of opioid dependence in creating addiction vulnerability. *F1000Res* 2016; 5.
- [21] Merlin MA, Ariyaprakai N, Arshad FH. Assessment of the safety and ease of use of the naloxone auto-injector for the reversal of opioid overdose. *Open Access Emerg Med* 2015; 7: 21-24.
- [22] Mayo-Michelson L, Young GA. Effects of chronic morphine administration and naloxone on EEG, EEG power spectra and associated behavior in two inbred rat strains. *Pharmacol Biochem Behav* 1992; 42: 815-821.
- [23] Zhou W, Zhang F, Tang S, Liu H, Gu J, Yang G. The dissociation of heroin-seeking patterns induced by contextual, discriminative, or discrete conditioned cues in a model of relapse to heroin in rats. *Psychopharmacology (Berl)* 2005; 181: 197-206.
- [24] Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 2005; 48: 303-314.
- [25] Rogge GA, Wood MA. The role of histone acetylation in cocaine-induced neural plasticity and behavior. *Neuropsychopharmacology* 2013; 38: 94-110.
- [26] Feng J, Nestler EJ. Epigenetic mechanisms of drug addiction. *Curr Opin Neurobiol* 2013; 23: 521-528.
- [27] Imai S, Armstrong CM, Kaerberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000; 403: 795-800.
- [28] Duan W. Sirtuins: from metabolic regulation to brain aging. *Front Aging Neurosci* 2013; 5: 36.
- [29] Kelz MB, Chen J, Carlezon WA Jr, Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranaukas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ. Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. *Nature* 1999; 401: 272-276.
- [30] Zachariou V, Bolanos CA, Selley DE, Theobald D, Cassidy MP, Kelz MB, Shaw-Lutchman T, Berton O, Sim-Selley LJ, Dileone J, Kumar A, Nestler EJ. An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nat Neurosci* 2006; 9: 205-211.
- [31] Wang L, Lv Z, Hu Z, Sheng J, Hui B, Sun J, Ma L. Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKII alpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology* 2010; 35: 913-928.