Expression of PSD-95 in hippocampal CA1 region of morphine withdrawal rats in different dependent times

Junge Han[1,*], Hua Zhu[1], Chengbao Wang[1], Peng Chen[2], Liangming Luo[2], Guanghua Ye[1]

[1]*Department of Forensic Medicine, Wenzhou Medical College, Wenzhou, Zhejiang* 325035*, China;* [2]*Wenzhou Public Security, Wenzhou, Zhejiang* 325035*, China*

Received May 29, 2008

Abstract

Objective. To observe the expression of postsynaptic density-95 (PSD-95) protein in hippocampal CA1 region of the withdrawn rats depended on morphine for different times and investigate the influence of morphine-withdrawal on addiction memory. *Methods.* Animal models of the withdrawn rats in different morphine dependent times (1 week, 2 weeks, 4 weeks) were established. The rats' behaviors and livings were observed. The expressions of PSD-95 protein with immunohistochemistry and PSD-95 mRNA with RT-PCR in hippocampal CA1 region were identified. *Results.* The expression of PSD-95 in hippocampal CA1 region decreased ($P < 0.01$) in the withdrawn group dependent on morphine for 1 week as compared with that in normal saline (NS) group, and it increased ($P < 0.05$) in the withdrawn group depended on morphine for 2 weeks as compared with that in morphine-dependent group for 1 week, but still decreased ($P < 0.01$) as compared with that in NS group, and it decreased the most in the withdrawn group depended on morphine for 4 weeks as compared with the other three groups ($P < 0.01$). *Conclusion.* The expression of PSD-95 in hippocampal CA1 region of the withdrawn rats depended on morphine decreased and might play a role in addiction memory. [Life Science Journal. 2008; 5(3): 27 – 39] (ISSN: 1097 – 8135).

Keywords: morphine-withdrawal; hippocampal; PSD-95

1 Introduction

It was discovered that memory and addiction share neural circuitry and molecular mechanisms in the recent studies[1] and memory plays the important role in drug addiction. The study on morphine dependence is scare but on cocaine dependence a lot at present[2,3]. The expressions of postsynaptic density-95 (PSD-95) protein with immunohistochemistry and PSD-95 mRNA with RT-PCR were identified in hippocampal CA1 region of the withdrawn rats in different morphine dependent times (1 week, 2 weeks, 4 weeks), and investigate the influence of morphine-withdrawal on addiction memory in this study.

2 Materials and Methods

2.1 Animals

16 healthy Sprague Dawley rats (weight, 300 ± 20 g) were randomly allocated into 4 groups with 4 rats in each group: rats of control group treated with normal saline for 1 week (N), rats treated with morphine (Shenyang No.1 Pharmaceutical Factory, China) for 1 week (N1), rats treated with morphine for 2 weeks (N2), and rats treated with morphine for 4 weeks (N3). They were allowed for free eating and drinking, and their behaviors and diet, drinking, excrement etc. were observed. All rats were provided by experimental animal centre of Wenzhou Medical College (Wenzhou, Zhejiang, China).

2.2 Management

The animal model of withdrawn rats in different morphine dependent times (1 week, 2 weeks, 4 weeks, respectively) were established by hypodermic injecting the morphine hydrochloride into the back of rats with increasing dose twice per day for 7 days (8:00, 16:00), and trained the rats for conditioned place preference (CPP) at the

*Corresponding author. Email: hanjunge@wzmc.net

same time. The CPP box combined two identical volume: the black part and the white part with an operative board between them and a camera connected to the computer in the wall. In the preliminary experiment we discovered that the rat stayed longer in the black part than the white part. The rat did not prefer to stay in the white part, so we selected the white part as the site to train the rat for the proneness experiment process. The dose of morphine for the first day: 5 mg/kg; the second day: 10 mg/kg; the third day: 15 mg/kg; the fourth day: 20 mg/kg; the fifth day: 30 mg/kg; the sixth day: 40 mg/kg; the seventh day: 50 mg/kg. After injecting the morphine the rats were put into the white part of the CPP box for 50 minutes. All the rats were weight every day. On the eighth day the rats were injected the naloxone hydrochloride (5 mg/kg) to induce the withdrawal symptom before injecting the morphine. We evaluated the withdrawal symptom with scores according to the way of Maledonade's through observing the behaviors of wet dog shaking, stretching, swallowing, diarrhea, saliva etc. Four rats were selected randomly as N1 and were put into the CPP box with the board drawn away for 15 minutes to test how long they stayed in the white part. N2 and N3 continued to be treated with morphine for 1 week or 3 weeks respectively once a day (8:00 A.M.). On the next day (10:00 A.M.) after the last injection, they were put into the CPP box with the board drawn away for 15 minutes to test how long they stayed in the white part. N was treated as N1 but normal saline instead of morphine.

2.3 Immunohistochemistry

The rat's brain was obtained and filled with 4% paraformaldehyde solution. The hippocampal CA1 region was striped according to the stereo-allocation atlas of rats and embed. The process of immunohistochemistry (Beijing Biosynthesis Biotechnology Co., Ltd, China) was done under the instruction of the kit.

2.4 RT-PCR

The rat's brain was obtained after its decapitation and the hippocampal CA1 region was striped quickly according to the stereo-allocation atlas of rats. Total RNA was isolated from the hippocampal CA1 region. The process of One-Step RT-PCR was done under the instruction of the RT-PCR kit (Shanghai Promega Biological Products Limited, China). Primer sequence (Shanghai Songon Biological Engineering Technology & Services Co., Ltd, China): PSD (upstream: 5'-CAAGACCGACATCAC AGGA-3'; downstream: 5'-ACGGCAAGGGCGAAT-3'). β-actin was an internal control (upstream: 5'-TCACCC ACACTGTGCCCATCTACGA-3'; downstream: 5'-CATCGGAACCGCTCGTTGCCAATAG-3'). The amplified fragments were 470 bp for PSD and 346 bp for β-actin. The RT-PCR amplification products were gel eletrophoresed, stained with ethidium.

2.5 Statistical analysis

Statistical analysis was performed with SPSS10.0 software. Group-sample *t* test was for comparison. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Scores of withdrawal symptom and the staying times in the white part of the CPP box

The morphine dependent groups (N1, N2, N3) manifested withdrawal symptom which indicated the animal model was established successfully as compared with the control group (N) through observing the withdrawal symptoms ($P < 0.01$). Rats of N1, N2 and N3 stayed much longer in the white part of the CPP box as compared with rats of N. The scores of withdrawal symptom and the staying times in the white part of CPP box showed in Table 1.

3.2 Immunohistochemistry

As shown in Figure 1, there were buffy particles in the neuron kytoplasm of all the rats' hippocampal CA1 region. Buffy particles were obvious and thick in N (Figure 1A), and a little thick in N1 and N2 (Figure 1B), and thin and low in N3 (Figure 1C).

3.3 RT-PCR

PSD-95 was expressed at high level in all rats' hippocampal CA1 region (Figure 2 and Table 2). It decreased in N1 as compared with that in N ($P < 0.01$), and it increased in N2 as compared with that in N1 ($P < 0.05$), but still decreased as compared with that in N ($P < 0.01$), and it decreased the most in N3 as compared with the other three groups ($P < 0.01$).

Table 1. Scores of withdrawal symptom and the staying times in the white part of the CPP box ($\bar{X}$ ± SD)

Group	Scores	Staying time (s)
N	2.1 ± 2.5	328.2 ± 67.4
N1	28.2 ± 5.1*	486 ± 107.6*
N2	24.5 ± 3.2*	494.8 ± 31.3*
N3	22.5 ± 3.9*	470.6 ± 56.7*

N: The control group; N1: The withdrawn group treated with morphine for 1 week; N2: The withdrawn group treated with morphine for 2 weeks; N3: The withdrawn group treated with morphine for 4 weeks. *: *vs.* N, $P < 0.01$.

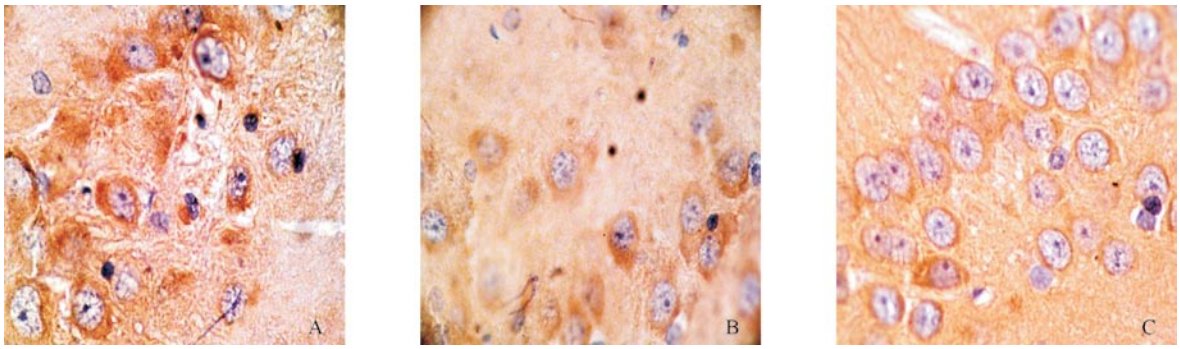

Figure 1. The expression of PSD-95 in hippocampal CA1 region of different groups. A: The control group; B: Rats treated with morphine for 1 week and 2 weeks; C: Rats treated with morphine for 3 weeks.

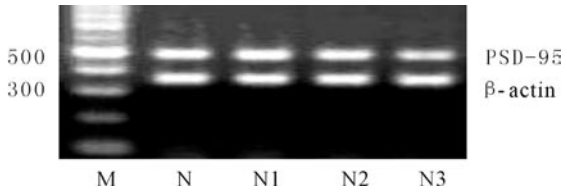

Figure 2. Expression of PSD-95 in hippocampal CA1 region of different groups. M: Maker; N: Control group; N1: Rats treated with morphine for 1 week; N2: Rats treated with morphine for 2 weeks; N3: Rats treated with morphine for 3 weeks.

Table 2. Expression of PSD-95 in hippocampal CA1 region of different groups ($\bar{X}$ ± SD)

Group	Case	PSD-95
N	4	0.782 ± 0.052
N1	4	0.575 ± 0.024[a]
N2	4	0.666 ± 0.031[a,b]
N3	4	0.328±0.023[a,b,c]

N: Control group; N1: Rats treated with morphine for 1 week; N2: Rats treated with morphine for 2 weeks; N3: Rats treated with morphine for 3 weeks. [a]: compared with N, $P < 0.01$; [b]: compared with N1, $P < 0.05$; [c]: compared with N1 and N2, $P < 0.01$.

4 Discussion

Hippocampal is a key part in cerebral region on memory and plays an important role in drug dependence. PSD-95 is a substructure in the postsynaptic membrane, which is sensitive about stress and changes in some cerebral regions at some kinds of stimulus. It has been proved that knockdown of spinal cord PSD-95 protein prevents the development of morphine tolerance in rats[6], which indicates that PSD-95 takes a part in the process of morphine addiction.

Rats of N1, N2 and N3, stayed much longer in the white part of the CPP box than the rats of N, which indicated the rats of withdrawn group had formed addiction memory. The results of immunohistochemistry and RT-PCR showed that the expressions of PSD-95 in hippocampus CA1 region of N1 decreased firstly, then N2 increased a little as compared with N1, and N3 decreased again as compared with N2, but all the groups decreased as compared with N. The expressions of PSD-95 in hippocampus CA1 region of N1, N2 and N3 decreased as compared with N, which indicated morphine damaged the neurons in hippocampus severely; but it increased in N2 as compared with that in N1, which might due to morphine tolerance. After injecting morphine for 1 week the rats had already been in morphine dependence. We didn't increase the dose of morphine but keep the last dose once a day for the rest of the experiment from the eighth day, which induced the rats in morphine tolerance. With the extension of time hippocampus was destroyed more by morphine, the expression of PSD-95 in hippocampal CA1 region of N3 also decreased more.

The influence of morphine on memory has double sides. On one side, it harms memory and on the other side, it emphasizes some bad adaptive memory that makes drugger crave for drugs and relapse after treating more morphine which induces memory to come back and strengthen[6,7]. PSD-95 acts on memory mainly through changing the plasticity of dendritic spine and the synapse. In the process of morphine dependence, the expression of PSD-95 increased in order to maintain the normal function of the body because of morphine damaging the neurons. Treating the rat with morphine longer, damage of the neurons and the memory was more serious. But this process of memory on the euphoria induced by morphine and craving for drugs was strengthened gradually. Then at the same time the plasticity of dendritic spine and the synapse in hippocampal CA1 region also changed gradu-

ally, and the changes of the plasticity were strengthen and modified by degrees with the extension of time. After morphine withdrawal, morphine stops damaging the neurons, and the expression of PSD-95 decreased, but the plasticity of dendritic spine and the synapse connected to the euphoria induced by morphine and craving for drugs had been steady, so the addiction memory on the euphoria induced by morphine and craving for drugs expressed significantly. The numbers of synapse near neuropil increased significantly in rats depended on morphine for 4 weeks in our previous study[8], which also indicated that addiction memory was more and more firm with the extension of the morphine dependent time.

References

1. Kelley AE. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 2004; 44(Suppl 1): 161 – 79.
2. Bolanos CA, Nestler EJ. Neurotrophic mechanisms in drug addiction. Neuromolecular Med 2004; 5(1): 69 – 83.
3. Itzhak Y, Anderson KL. Memory reconsolidation of cocaine-associated context requires nitric oxide signaling. Synapse 2007; 61(12): 1002 – 5.
4. Maldonade R, Negus S, Koob GF. Precipitation of morphine withdrawal syndrome in rats by administration of mu-delta-and-kappa-selective opioid antagonists. Neuropharmacology 1992; 31(12): 1231 – 5.
5. Liaw WJ, Zhang B, Tao F, *et al*. Knockdown of spinal cord postsynaptic density protein-95 prevents the development of morphine tolerance in rats. Neurosci 2004; 123(1): 11 – 5.
6. Zarrindast MR, Farahmandfar M, Rostami P, *et al*. The influence of central administration of dopaminergic and cholinergic agents on morphinr-induced amnesia in morphine-sensitized mice. J Psychopharmacol 2006; 20(1): 59 – 66.
7. Zarrindast MR, Khalilzadeh A, Rezayat SM. Influence of intracerebroventricular administration of histaminergic drugs on morphine state-dependent memory in the step-down passive avoidance test. Psychopharmacology 2005; 74(2): 106 – 12.
8. Zhu H, Li LH,Yang JL, *et al*. Microscopic and electron microscopic observation on dependence-related cerebral regions in morphine-dependent rats. Chinese Journal of Forensic Medicine 2006; 21(1): 9 – 13.