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Effect of rhynchophylline on conditioned place preference on expression of NR2B in methamphetamine-dependent mice

Junkui Li [a,1], Wei Liu [a,1], Qiuxian Peng [a,b,1], Mingjin Jiang [a], Chaohua Luo [a], Youli Guo [a], Yi Liu [a], Miao Fang [a], Zhixian Mo [a,*]

[a] School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, China
[b] Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong

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ABSTRACT

Objective: To study the effect of rhynchophylline on N-methyl D-aspartate receptor subtype 2B subunit in hippocampus of Methamphetamine-induced conditioned place preference (CPP) mice.
Methods: Place preference mice models were established by methamphetamine; the expression of NR2B was observed by immunohistochemistry technique and Western blot.
Results: Methamphetamine (4 mg/kg)-induced place preference mice model was successfully established; ketamine (15 mg/kg), rhynchophylline (40 mg/kg) and rhynchophylline (80 mg/kg) can eliminate place preference; Immunohistochemistry showed that the number of NR2B-positive neurons in hippocampus was increased in the methamphetamine model group, whereas less NR2B-positive neurons were found in the ketamine group, low and high dosage rhynchophylline group. Western blot showed that the expression of NR2B protein was significantly increased in the model group, whereas less expression was found in the ketamine group, low and high dosage rhynchophylline group.
Conclusions: NR2B plays an important role in the formation of methamphetamine-induced place preference in mice. Rhynchophylline reversed the expression of NR2B in the hippocampus demonstrates the potential effect of mediates methamphetamine induced rewarding effect.

1. Introduction

Methamphetamine is a highly addictive stimulant drug that activates certain systems in the brain, including Central nervous system. Although belong to amphetamine family whose members are generally much more potent, longer lasting, and more harmful to the central nervous system [1]. Methamphetamine may also affect the expression of functional proteins in multiple regions in the brain, including hippocampus, prefrontal cortex, ventral tegmental area, striatum, nucleus accumbens and amygdale [2]. The accurate mechanism responsible for the addiction is complicated and uncovered. As drug addiction has becomed a worldwide problem and numerous studies are focusing on the risk, prevention, and intervention for addictions, an effective compound which can stop people from drug abusing is highly expected.

Several plant-derived compounds have been shown to have significant anti-drug addiction in preclinical studies, but the exact mechanisms of these compounds are still unclear [3]. Rhynchophylline (Fig. 1) is an alkaloid found in certain Uncaria species, rhynchophylline is a non-competitive NMDA antagonist and calcium channel blocker [4]. In the central nervous system, rhynchophylline could be beneficial for memory impairment induced by the dysfunction of cholinergic systems in brain [5,6]. Rhynchophylline is also effective in sedation, anti-convulsion, anti-epilepsy [7–11], thus rhynchophylline has been widely used in traditional Chinese medicines prescribed to treat ailments in central nervous system and cardiovascular systems, such as lightheadedness, convulsions, numbness, and hypertension.

Our previous studies showed that rhynchophylline down-regulates the expression of N-methyl D-aspartate receptor subtype 2B in the cortex and reverses the amphetamine-induced rewarding effect in rats [12,13]. These results show a promising application future of rhynchophylline in methamphetamine-induced drug addiction. However, its underlying mechanisms are still largely unknown.

In this study, based on the conditioned place preference animal model, we get the activity routes of mice in CPP compartment. By

* Corresponding author.
E-mail address: cherrymogz@gmail.com (Z. Mo).
[1] These authors contributed equally to these work.

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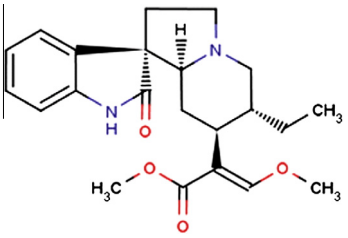

Fig. 1. Molecular structure of rhynchophyllin.

immunohistochemistry and Western blot, we evaluate the expression of NR2B in the hippocampus after treated with rhynchophylline, and our research showed that rhynchophylline reversed the expression of methamphetamine-induced rewarding effect which is mediated by regulating the expression of NR2B in the hippocampus.

2. Materials and methods

2.1. Animals

Kunming mice (weight: 18–20 g; NO: SCXK GD 2011-0015) were provided by Experimental Animal Center of Southern Medical University (Guangzhou, China). All animals were adapted to the experimental conditions (temperature: 20 ± 2 °C, humidity: 60 ± 5% and 12 h dark/light cycle) for one week. All mice had free access to tap water and chew diet. The ethical aspects of the research plan and experimental procedures have been approved by Science and Technological Committee and the Animal Use and Care Committee of Southern Medical University.

2.2. Drugs and reagents

Rhynchophylline (No. H1I2, purity 99.7%) was bought from Matsuura Yakugyo Co., Ltd., Japan. Ketamine hydrochloride (50 mg/mL) was purchased from Jiangsu Hengrui Medicine Co., Ltd., China. Methamphetamine was obtained from the National Narcotics Laboratory, China (No. 1212-9802). Both rhynchophylline and methamphetamine sulfate were dissolved in physiological saline to final concentrations and injected in a volume of 80 mg/kg and 4 mg/kg. Western blot Kit: NR2B antibody (Rabbit anti human, Millipore company, American), PVDF membrane (Millipore), Tris-buffer, skimmed milk powder, Secondary antibody (Santa Cruz), Mark (Amersham), Immunohistochemical reagents: NR2B antibody (Rabbit anti rat, Abcam, USA), anti NMDA-2B (NR2B) detection kit and DAB Kit (Zhongshan Biotechnology Co., Ltd., Beijing, China).

2.3. Apparatus

The CPP apparatus consists of two equal-sized compartments (15 × 15 × 15 cm), one with a white interior and the other a black interior separated by a wall with a sliding door. For testing purpose, the sliding door was raised 15 cm above the floor to allow the rat free access to both sides of the box (Ethovision XT 8.5).

2.4. Conditioned place preference (CPP) procedure

CPP test consists of three phases and was proceeded within 5 consecutive days. For the pre-conditioning phase, the mice was placed under the door which was left opened to allow free access to the entire box for 5 min each day. On day 2 and day 3, the amount of time spent on each compartment was calculated and averaged to use as the pre-conditioning time of each animal. The mice that showed place preference for the white compartment in pre-conditioning phase were excluded from further analysis. During the conditioning phase (days 1–5), the door was closed so that the two compartments were separated. The mice were divided into methamphetamine-paired group and control groups, and underwent two conditioning sessions each day. The first session was performed in the morning, when the rats received methamphetamine (4 mg/kg, s.c.) in methamphetamine-paired group or sterile physiological saline (10 ml/kg, s.c.) in saline-paired group, and were immediately confined to the white compartment for 1 h. After an interval of 8 h, the second session of the day began. All the rats received saline (0.15 ml, s.c.) and were confined to the black compartment immediately for 1 h during the second session. In the morning of the second day, 15 mg/kg ketamine, 40 mg/kg rhynchophylline and 80 mg/kg rhynchophylline were injected to the treatment group respectively. Twenty-four hours after the last drug-paired conditioning trial (day 5), the post-conditioning phase was carried out and was exactly the same as the pre-conditioning phase. A 5-min trial was recorded to observe the time that the mice spent on the drug-paired side (the white compartment).

2.5. Experimental design

In order to assess the effect of rhynchophylline on the expression of methamphetamine-induced CPP and the expression of protein NR2B, mice were divided into five groups randomly with 10 mice in each group: control group, methamphetamine model group, methamphetamine with rhynchophylline (40 mg/kg), methamphetamine with rhynchophylline (80 mg/kg), methamphetamine with ketamine (15 mg/kg). An intraperitoneal injection of vehicle 10 ml/kg(sterile physiological saline) and rhynchophylline were given for 3 days, 12 h after the methamphetamine injection on day 3, day 4, and day 5. The mice of the rhynchophylline-paired group received rhynchophylline (40 mg/kg or 80 mg/kg, i.p.) instead of methamphetamine (4 mg/kg, s.c.) during the conditioning phase. The dose and time points of drug administration were selected according to our previous work [14]. Control mice was injected with the vehicle (10 ml/kg, i.p.) under the same schedule and served as a control.

2.6. Immunohistochemistry NR2B expression

After test of the CPP, the mice were killed by cervical dislocation, followed by quickly stripping the meninges, cut along the brain sagittal midline, removal of the brain tissue, and placing the tissue in 4% paraformaldehyde. Dehydrated and embedded the brain tissue, the hippocampus slices were selected under the microscope. Immunohistochemistry was performed as is described previously [15]. Brain tissues were excised and sent for paraffin wax-embedding and processing. Sections (3 μm thick) were deparaffinized with xylene, then dehydrated in decreasing concentrations of alcohol. An endogenous peroxidase activity was blocked by hydrogen peroxidase (3%) in Tris-buffered saline (TBS) for 10 min. The sections were then boiled for 2 min under pressure in citrate buffer for antigen retrieval. Nonspecific binding was blocked with 5% goat serum in TBS for 20 min and the tissues were incubated with the first NR2B antibodies (diluted 1:100; Abcam, USA) in TBS containing 1% bovine serum albumin for one night. The sections were then washed with TBS and incubated with secondary antibody (horseradish peroxidase labeled Goat anti rabbit IgG) for 60 min at room temperature; the slides were removed from the incubation chamber, followed by washing with TBST for three times. 10 clear sections were selected randomly from each group, and then three horizons were selected in the light microscope (40×) randomly to observe the distribution of positive parti-

cles in the cell at 400× magnification. Brown particles appeared to represent positive cells. Image-Pro plus 6.0 image analysis software was used to measure positive cells integrated optical density (IOD), and the mean value of the group is regarded as the relative content of NR2B.

2.7. Western blot for NR2B expression

The sample of membrane were prepared according to the instruction of membrane protein extraction kit. After determinate the concentration of the protein by BCA method. The sample were preserved at 20 degrees below zero degree. Protein samples were added to 2× sample buffer, and 12% sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE), electrophoresed at 100V for 2 h and transferred to PVDF membranes. The membranes were incubated in PBS with 3% nonfat dry milk. Primary antibodies (1:1000 anti-NR2B, (Santa Cruz)) were diluted in PBS with 3% milk, and then added to the member overnight with agitation at 4 °C. The membrane was washed three times with PBS, each time for 5 min, incubated with a horseradish peroxidase-conjugated secondary antibody(goat anti-rabbit IgG) at a 1:3000 dilution for 1 h, and then washed with PBST (0.05% Tween 20 in PBS) and developed by using a Super Enhanced chemiluminescence detection kit and recorded on X-ray film (Eastman Kodark, Rochester, NY, USA). Relative intensities of all bands were quantified using image analysis software (Gel Media System, China).

2.8. Statistical analysis

Values are expressed as mean ± SD. All data were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test (two-tailed). All statistical analyses were performed using SPSS software (version19.0). $P < 0.05$ was considered to be statistically significant.

Table 1
Change of activity time of mice in non-preferred compartment by injection of methamphetamine ($n = 10$).

Group	Dose (mg/kg)	Mice activity time in non-preferred compartment(s)
Control	–	96.29 ± 5.85
Methamphetamine model	4	154.81 ± 4.08**
Methamphetamine + rhynchophylline	40	97.69 ± 5.27▲▲
Methamphetamine + rhynchophylline	80	94.29 ± 4.62▲▲
Methamphetamine + ketamine	15	96.96 ± 3.02▲▲

**$P < 0.01$, v.s. control group; ▲$P < 0.05$, ▲▲$P < 0.01$, v.s. methamphetamine group.

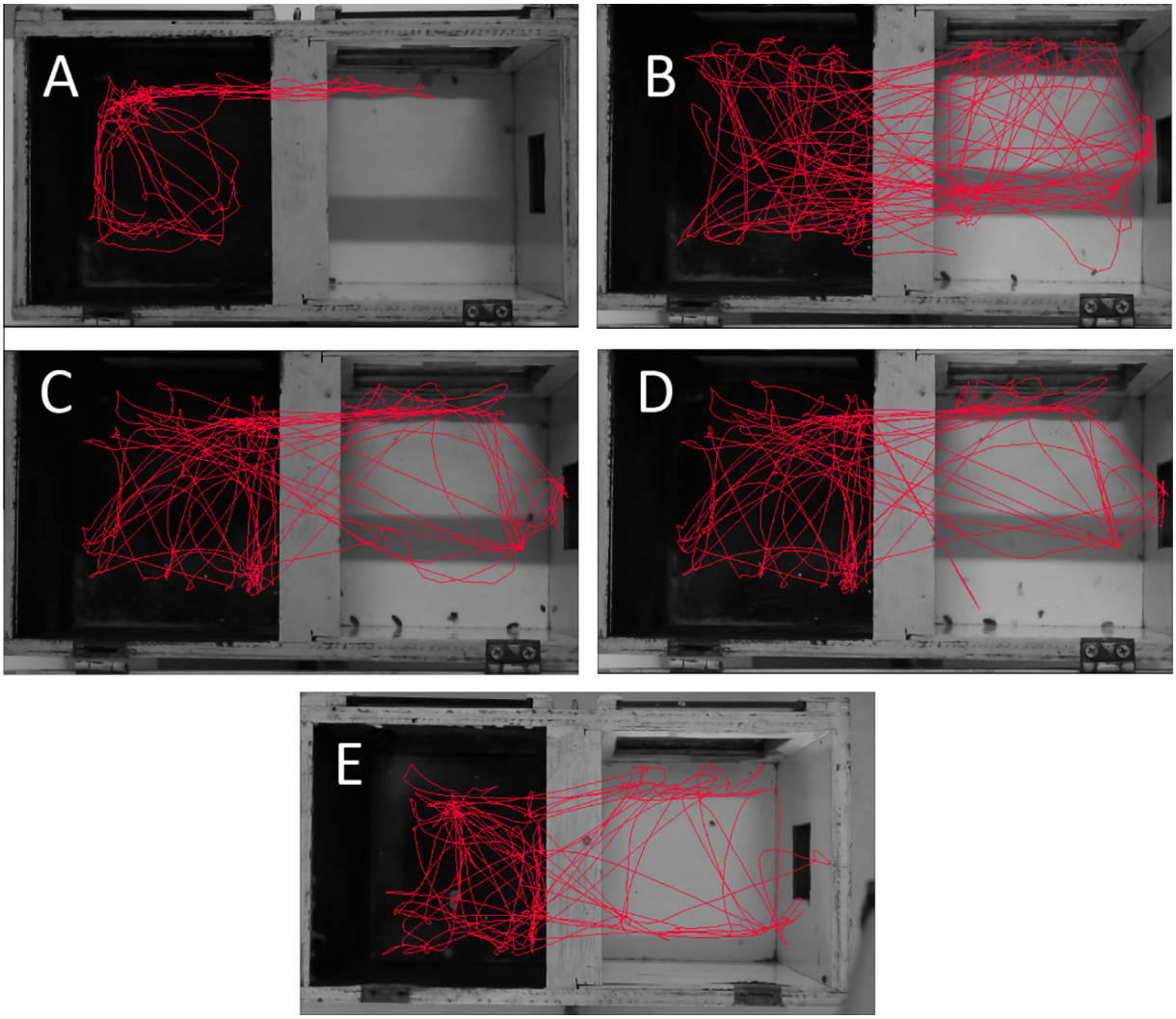

Fig. 2. Activity routes of mice in CPP compartment. (A) Control group; (B) methamphetamine model group; (C) low dose of rhynchophylline; (D) high dose of rhynchophylline; (E) ketamine group.

3. Results

3.1. Rhynchophylline blocked the behavioral alteration induced by methamphetamine

The effect of rhynchophylline on methamphetamine-induced CPP mice was shown in Table 1. Methamphetamine significantly increased the time spent on the drug-paired side (the white compartment) compared with that of the control mice ($P < 0.01$). Rhynchophylline and ketamine reduced the time spent by CPP mice in the white compartment, and the value is similar to that in control group ($P < 0.01$).

3.2. Mice activities routes in CPP

As is shown in Fig. 2, compared with the mice in control group, the activity routes in the non-preferred compartment in methamphetamine model was increased significantly. Compared with methamphetamine model group, both the low dosage and the high dosage of rhynchophylline reduced the activity routes significantly, and the similar results could be observed in mice treated with ketamine.

3.3. Immunohistochemistry for NR2B expression

DAB chromogenic color, microscopic control chromogenic time; Wood grain redyeing, dehydration, transparent, sealing, PBS solution instead of a resistance as a negative control. Slice observation and judgment results: positive expression of membrane and showing stained brown granules in the cytoplasm, the deeper the color, the stronger expression, brown particles is negative does not appear.

As is shown in Fig. 3, compared with the rats in control group, the number of NR2B positive cells in the hippocampus region in methamphetamine-treated mice was increased significantly. Both the low dosage and the high dosage of rhynchophylline reduced the number of positive NR2B cells, but the high-dosage of rhynchophylline group and the positive drug group reduced significantly ($P < 0.01$). The numbers of NR2B immunoreactivity cells in the hippocampus were shown in Table 2.

3.4. Western blot for the expression of NR2B

Compared with the control group, the NR2B expression in the brain of mice in model group were significantly increased ($P < 0.01$). Compared with the model group methamphetamine, rhynchophylline low-dose and high-dose group and ketamine

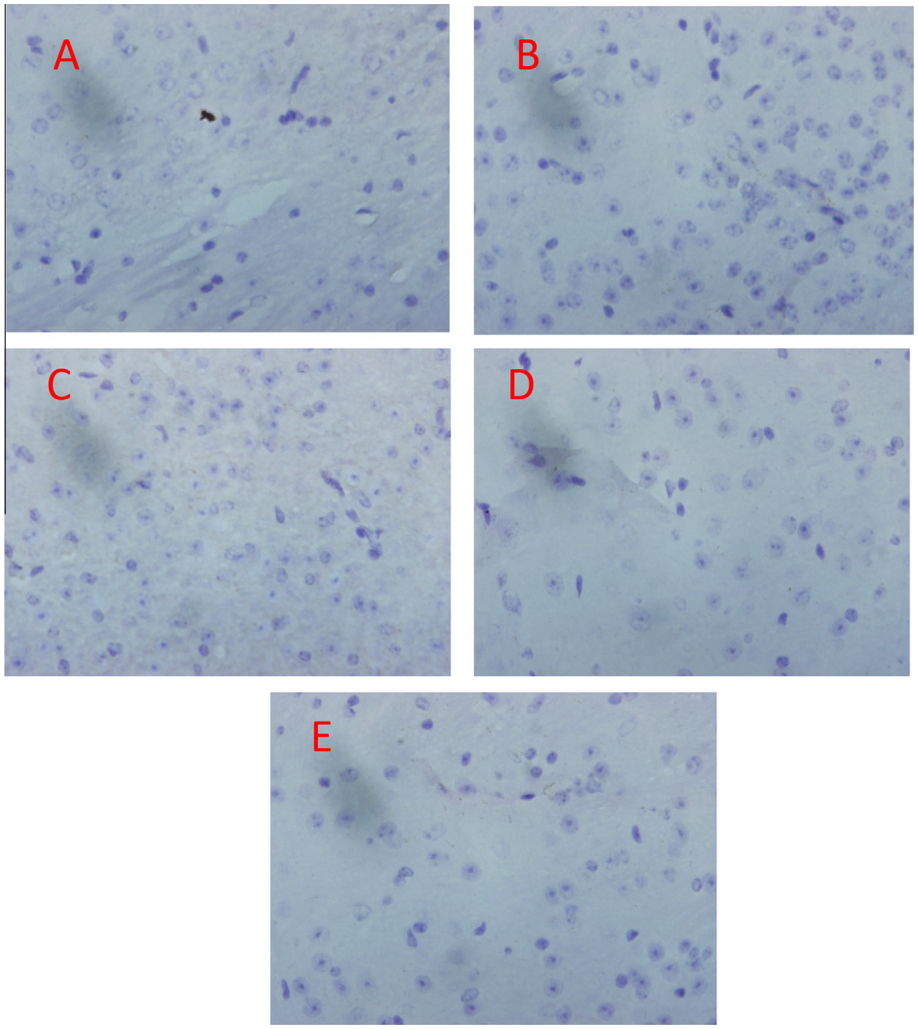

Fig. 3. Micrographs of NR2B positive cells in the hippocampus. (A) Control group; (B) methamphetamine model group; (C) low dose of rhynchophylline; (D) high dose of rhynchophylline; (E) ketamine group.

Table 2
The IOD of NR2B positive cells in area of hippocampus of mice in each group ($n = 10$).

Group	Dose (mg/kg)	(NR2B) IOD
Control	–	4137.91 ± 1555.73
Methamphetamine model	4	126018.01 ± 13366.97**
Methamphetamine + rhynchophylline	40	5658.92 ± 1845.40▲▲
Methamphetamine + rhynchophylline	80	4588.08 ± 1415.03▲▲
Methamphetamine + ketamine	15	5063.96 ± 1613.09▲▲

**$P < 0.01$ v.s. control group, ▲▲$P < 0.01$ v.s. methamphetamine model group.

Table 3
The OD of NR2B expression in mice brain ($n = 10$).

Group	Dose (mg/kg)	(NR2B) IOD
Control	–	5532.77 ± 1103.62
Methamphetamine model	4	11091.57 ± 1012.26**
Methamphetamine + rhynchophylline	40	8118.28 ± 543.12▲▲
Methamphetamine + rhynchophylline	80	8571.60 ± 447.59▲▲
Methamphetamine + ketamine	15	8260.11 ± 828.02▲▲

**$P < 0.01$ v.s. control group, ▲▲$P < 0.01$ v.s. methamphetamine model group.

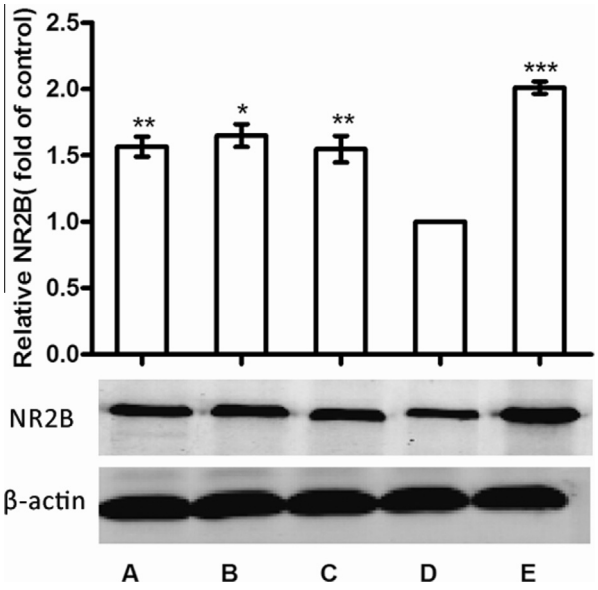

Fig. 4. The expression of NR2B by Western blotting. The bar graph shows quantification of data normalized in each experiment to the zero NR2B condition as means ± SD. ($n = 3$). (A) Ketamine group; (B) high dose of rhynchophylline; (C) low dose of rhynchophylline; (D) control group; (E) methamphetamine model group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ v.s. control group.

group NR2B expression in the brain of mice were significantly decreased ($P < 0.01$), as shown in Table 3 and Fig. 4.

4. Discussion

Amphetamines can excite central nervous system and is therefore easily getting addiction. Methamphetamine is an amphetamine with much longer and higher addictive, and the toxic effects on the central nervous system is more pronounced [16]. Methamphetamine can induce neural impairment in other brain areas, such as the piriform and parietal cortexes, thalamus and hippocampus, areas receiving only a sparse dopaminergic input, as well as in non- dopamine striatal elements [17].

In this research, hippocampus was chosen to be the primary focus, since the nature of hippocampus has been proved to be highly associated with neuropsychiatric conditions, such as drug addiction [18]. Currently, rhynchophylline has been applied to treat central nervous system diseases, and it is also a non-competitive NMDA receptor antagonist [4,8,19]. NMDA receptor subunit of glutamate receptors were focus in the brain cortex and hippocampus, and play an important role in learning and memory [20,21]. NMDA receptor mainly is composed of two subunits: a subunit of NMDA receptor 1 (NR1), another is NMDA receptor 2 (NR2). NR1 is essential to form functional NMDA receptor channel subunits, and NR2 subunits of molecular determinants the diversity of these receptors function. NR2B is mainly expressed in cortex, hippocampus, and striatum in rats and human being [22]. The hippocampus is an important area for learning memory, regulate emotion, and the impairment of hippocampus will lead to many neurodegenerative diseases.

Study has found that ketamine can inhibit opioid withdrawal symptoms. Ketamine has obvious inhibitory effects on amphetamine addicts, which is mainly related with the activity of NMDA receptor in the reward center [23]. Therefore, ketamine is suitable chosed as a positive drug in this experiment. In the present study, through administering for 4 consecutive days, the methamphetamine dependent mice CPP models were established successfully. After three days of ketamine treatment in mice, the CPP effects induced by methamphetamine disappeared. Rhynchophylline low dose (40 mg/kg) and high dose (80 mg/kg) can eliminate the position of methamphetamine-induced preference in mice to a certain extent. Immunohistochemistry results showed that the model group of methamphetamine-positive cells increased significantly in the hippocampus, which indicates that methamphetamine can increase the density of positive cells neurons in the hippocampus of mice, but compared with model group, the low dose and high-dose of rhynchophylline group decreased significantly which indicates that the number of positive hippocampal neurons can be inhibited by rhynchophylline. The increasing of NR2B positive neurons cells in hippocampus of mice may have certain connections with CPP. Western blot detected that NR2B in methamphetamine model group increased significantly compared with control group, while compared with methamphetamine, the low dose, high dose of rhynchophylline group, ketamine group and model group was significantly decreased, which consistent with the immunohistochemical results, showing rhynchophylline and ketamine can restrain the NR2B expression to a certain extent, to make it back to normal levels, also means that rhynchophylline anti-methamphetamine dependence is associated with the expression of NR2B in hippocampus.

In summary, the increased expression of NR2B protein is closely related to the position preference of the mouse which induced by methamphetamine. Further studies are required to elucidate the mechanism of anti-drug dependence effect of rhynchophylline.

Author contributions

Designed the study: J.K.L. W.L. Q.X.P. Z.X.M. Coordinated the study and finalized the manuscript: J.K.L. Q.X.P. Read and approved the manuscript: WL QXP. Performed the experiments: J.K.L. M.J.J. C.H.L. Y.L.G. Y.L. M.F. Analyzed the data: J.K.L. W.L. M.F. Wrote the paper: J.K.L. Q.X.P.

Competing financial interests

The authors declare no competing financial interests.

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References

[1] D.S. Carson, A.J. Guastella, E.R. Taylor, I.S. McGregor, A brief history of oxytocin and its role in modulating psychostimulant effects, J. Psychopharmacol. 27 (2013) 231–247.
[2] U.M. Ricoy, J.L. Martinez Jr., Local hippocampal methamphetamine-induced reinforcement, Front. Behav. Neurosci. 3 (2009) 47.
[3] L. Abenavoli, G. Bardazzi, F. Cracolici, C. Quaranta, G. Santini, S. Graziosi, L. Polero, L. Leggio, G. Addolorato, Complementary therapies for treating alcoholism First Annual Meeting by Complementary Medicine Research Group of the Italian Society for Alcohol Studies-May 5, 2006, Florence, Italy, Fitoterapia 79 (2008) 142–147.
[4] T.H. Kang, Y. Murakami, K. Matsumoto, H. Takayama, M. Kitajima, N. Aimi, H. Watanabe, Rhynchophylline and isorhynchophylline inhibit NMDA receptors expressed in Xenopus oocytes, Eur. J. Pharmacol. 455 (2002) 27–34.
[5] A.F. Mohamed, K. Matsumoto, K. Tabata, H. Takayama, M. Kitajima, H. Watanabe, Effects of *Uncaria tomentosa* total alkaloid and its components on experimental amnesia in mice: elucidation using the passive avoidance test, J. Pharm. Pharmacol. 52 (2000) 1553–1561.
[6] D. Yuan, B. Ma, J.Y. Yang, Y.Y. Xie, L. Wang, L.J. Zhang, Y. Kano, C.F. Wu, Anti-inflammatory effects of rhynchophylline and isorhynchophylline in mouse N9 microglial cells and the molecular mechanism, Int. Immunopharmacol. 9 (2009) 1549–1554.
[7] M. Amat, C. Ramos, M. Perez, E. Molins, P. Florindo, M.M. Santos, J. Bosch, Enantioselective formal synthesis of ent-rhynchophylline and ent-isorhynchophylline, Chem. Commun. (Camb.) 49 (2013) 1954–1956.
[8] J.Y. Zhou, J. Chen, S.W. Zhou, Z.X. Mo, Individual and combined effects of rhynchophylline and ketamine on proliferation, NMDAR1 and GluA2/3 protein expression in PC12 cells, Fitoterapia 85 (2013) 125–129.
[9] W. Cao, Y. Wang, X. Lv, X. Yu, X. Li, H. Li, Y. Wang, D. Lu, R. Qi, H. Wang, Rhynchophylline prevents cardiac dysfunction and improves survival in lipopolysaccharide-challenged mice via suppressing macrophage I-kappaBalpha phosphorylation, Int. Immunopharmacol. 14 (2012) 243–251.
[10] J.Y. Zhou, S.W. Zhou, Isorhynchophylline: a plant alkaloid with therapeutic potential for cardiovascular and central nervous system diseases, Fitoterapia 83 (2012) 617–626.
[11] Y. Song, R. Qu, S. Zhu, R. Zhang, S. Ma, Rhynchophylline attenuates LPS-induced pro-inflammatory responses through down-regulation of MAPK/NF-kappaB signaling pathways in primary microglia, Phytother. Res. 26 (2012) 1528–1533.
[12] J.Y. Zhou, Z.X. Mo, S.W. Zhou, Rhynchophylline down-regulates NR2B expression in cortex and hippocampal CA1 area of amphetamine-induced conditioned place preference rat, Arch. Pharm. Res. 33 (2010) 557–565.
[13] W. Liu, Q.X. Peng, X.L. Lin, C.H. Luo, M.J. Jiang, Z.X. Mo, K.K. Yung, Effect of rhynchophylline on the expression of p-CREB and sc-Fos in triatum and hippocampal CA1 area of methamphetamine-induced conditioned place preference rats, Fitoterapia 92 (2014) 16–22.
[14] G.H. Dai, J.C. Sun, D.M. Qi, Effects of rhynchophylla alkaloids on vascular adventitial fibroblast apoptosis and proliferation in the thoracic aorta of spontaneously hypertensive rats, Zhongguo Zhong Xi Yi Jie He Za Zhi 32 (2012) 1233–1237.
[15] J.Y. Zhou, Z.X. Mo, S.W. Zhou, Effect of rhynchophylline on central neurotransmitter levels in amphetamine-induced conditioned place preference rat brain, Fitoterapia 81 (2010) 844–848.
[16] E.J. Jaehne, A. Salem, R.J. Irvine, Pharmacological and behavioral determinants of cocaine, methamphetamine, 3,4-methylenedioxymethamphetamine, and para-methoxyamphetamine-induced hyperthermia, Psychopharmacology 194 (2007) 41–52.
[17] C. Davidson, A.J. Gow, T.H. Lee, E.H. Ellinwood, Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment, Brain Res. Brain Res. Rev. 36 (2001) 1–22.
[18] C.S. Lansink, P.M. Goltstein, J.V. Lankelma, B.L. McNaughton, C.M. Pennartz, Hippocampus leads ventral striatum in replay of place-reward information, PLoS Biol. 7 (2009) e1000173.
[19] T.H. Kang, Y. Murakami, H. Takayama, M. Kitajima, N. Aimi, H. Watanabe, K. Matsumoto, Protective effect of rhynchophylline and isorhynchophylline on in vitro ischemia-induced neuronal damage in the hippocampus: putative neurotransmitter receptors involved in their action, Life Sci. 76 (2004) 331–343.
[20] M.P. Kummer, T. Hammerschmidt, A. Martinez, D. Terwel, G. Eichele, A. Witten, S. Figura, M. Stoll, S. Schwartz, H.C. Pape, J.L. Schultze, D. Weinshenker, M.T. Heneka, Ear2 deletion causes early memory and learning deficits in APP/PS1 mice, J. Neurosci. 34 (2014) 8845–8854.
[21] L. Huang, Y. Liu, W. Jin, X. Ji, Z. Dong, Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCgamma-ERK signaling pathway in the developing brain, Brain Res. 1476 (2012) 164–171.
[22] Z. Liu, C. Lv, W. Zhao, Y. Song, D. Pei, T. Xu, NR2B-containing NMDA receptors expression and their relationship to apoptosis in hippocampus of Alzheimer's disease-like rats, Neurochem. Res. 37 (2012) 1420–1427.
[23] Y. Tizabi, B.H. Bhatti, K.F. Manaye, J.R. Das, L. Akinfiresoye, Antidepressant-like effects of low ketamine dose is associated with increased hippocampal AMPA/NMDA receptor density ratio in female Wistar-Kyoto rats, Neuroscience 213 (2012) 72–80.