Neuroscience 329 (2016) 122–133

NMDA RECEPTORS ARE INVOLVED IN THE ANTIDEPRESSANT-LIKE EFFECTS OF CAPSAICIN FOLLOWING AMPHETAMINE WITHDRAWAL IN MALE MICE

SHAYAN AMIRI, [a,b†] **SAKINEH ALIJANPOUR,** [c†] **FATEMEH TIRGAR,** [d] **ARYA HAJ-MIRZAIAN,** [a,e] **HOSSEIN AMINI-KHOEI,** [a,e] **MARYAM RAHIMI-BALAEI,** [f] **MOJGAN RASTEGAR,** [b] **MARZIEH GHADERI,** [d] **MAHMOUD GHAZI-KHANSARI** [a,e] **AND MOHAMMAD-REZA ZARRINDAST** [a,g,h*]

[a] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

[b] *Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada*

[c] *Department of Biology, Faculty of Science, Gonbad Kavous University, Gonbad Kavous, Iran*

[d] *Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[e] *Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

[f] *Department of Human Anatomy and Cell Science, College of Medicine, Faculty of Health Sciences, University of Manitoba, Canada*

[g] *School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran*

[h] *Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran*

Abstract—Amphetamine withdrawal (AW) is accompanied by diminished pleasure and depression which plays a key role in drug relapse and addictive behaviors. There is no efficient treatment for AW-induced depression and underpinning mechanisms were not well determined. Considering both transient receptor potential cation channel, subfamily V, member 1 (TRPV1) and *N*-Methyl-D-aspartate (NMDA) receptors contribute to pathophysiology of mood and addictive disorders, in this study, we investigated the role of TRPV1 and NMDA receptors in mediating depressive-like behaviors following AW in male mice. Results revealed that administration of capsaicin, TRPV1 agonist, (100 μg/mouse, i.c.v.) and MK-801, NMDA receptor antagonist (0.005 mg/kg, i.p.) reversed AW-induced depressive-like behaviors in forced swimming test (FST) and splash test with no effect on animals' locomotion. Co-administration of sub-effective doses of MK-801 (0.001 mg/kg, i.p.) and capsaicin (10 μg/mouse, i.c.v) exerted antidepressant-like effects in behavioral tests. Capsazepine, TRPV1 antagonist, (100 μg/mouse, i.c.v) and NMDA, NMDA receptor agonist (7.5 mg/kg, i.p.) abolished the effects of capsaicin and MK-801, respectively. None of aforementioned treatments had any effect on behavior of control animals. Collectively, our findings showed that activation of TRPV1 and blockade of NMDA receptors produced antidepressant-like effects in male mice following AW, and these receptors are involved in AW-induced depressive-like behaviors. Further, we found that rapid antidepressant-like effects of capsaicin in FST and splash test are partly mediated by NMDA receptors. © **2016 Published by Elsevier Ltd on behalf of IBRO.**

Key words: amphetamine withdrawal, depression, TRPV1, NMDA receptors, FST, splash test.

INTRODUCTION

Several lines of evidence indicate that withdrawal from psychostimulants induces behavioral and neurochemical alterations (Renoir et al., 2012; Che et al., 2013). Withdrawal from stimulants is accompanied by dysphoric state which contributes to drug-seeking behavior and relapse, and causes treatment failure in individuals with stimulant dependence (Koob et al., 1998; Oleson et al., 2014). Recent evidence suggests that applying animal models of psychostimulants withdrawal provided appropriate tools for understanding underlying mechanisms involved in mood disorders namely depression (Barr and Markou, 2005). Amphetamine (AMPH)-style drugs are potent psychostimulants which their acute abstinence is associated with depressive-like behaviors such as motivational impairment and behavioral despair (Cryan and Holmes, 2005). In animal studies, it has been proposed that depressive-like behaviors following AMPH withdrawal (AW) are associated with abnormal neurotransmission in the regions of the brain relevant to depression (Sulzer et al., 2005). In this regard, it has been accepted that acute phase of psychostimulant withdrawal is associated with abnormal glutamatergic neurotransmission in several brain areas that may be associated with negative affect observed in early recovery state (Kalivas and Volkow, 2005; D'Souza and Markou, 2010). Further, recent studies have shown that chronic administration of stimulants (such as AMPH) enhances hippocampal glutamatergic

*Correspondence to: M. R. Zarrindast, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran. Tel/fax: +98 21 66402569.
E-mail address: zarinmr@ams.ac.ir (M.-R. Zarrindast).
† Authors contributed equally in this work.

Abbreviations: AW, amphetamine withdrawal; FST, forced swimming test; MK-801, dizocilpine; NMDA, *N*-Methyl-D-aspartate; OFT, open-field test; TRPV1, transient receptor potential cation channel, subfamily V, member 1.

http://dx.doi.org/10.1016/j.neuroscience.2016.05.003
0306-4522/

activity which induces hippocampal plasticity through *N*-Methyl-D-aspartate (NMDA) receptors (D'Souza and Markou, 2010; Underhill et al., 2014). Increase in sensitivity to glutamate following AW plays a crucial role in behavioral difficulties observed after drug cessation (Kalivas and Volkow, 2005; Koltunowska et al., 2013). Evidence suggests that impairment of dopaminergic system activity contribute to difficulties with self-care and motivation following AW (Rossetti et al., 1992). It is well-established that serotonergic drugs are inefficient for treatment of depression in acute phase of AW (Zorick et al., 2011). In this context, emerging lines of research suggest that glutamatergic system can be an appropriate therapeutic target in moderating dysphoric state following AW (D'Souza and Markou 2010; Iijima et al., 2013; Fuller et al., 2015). Although it has been well documented that glutamatergic system plays an important role in AMPH-induced behavioral abnormalities, underlying mechanisms by which acute AMPH abstinence exerts its depressant effects is not clear.

Evidence indicates that transient receptor potential cation channel, subfamily V, member 1 (TRPV1) is involved in pathophysiology of mood disorders (Di Marzo et al., 2008; Terzian et al., 2009; Ho et al., 2012). In this regard, both TRPV1 agonists and antagonist have been reported to have antidepressant effects by different mechanisms including involvement of NMDA receptors (Manna and Umathe, 2012; Abdelhamid et al., 2014). Furthermore, it has been reported that TRPV1 plays a role in pathophysiology of addiction (Wescott et al., 2013; Martins et al., 2014). Expression of TRPV1 undergoes alterations following stimulant administration in the brain, (Tian et al., 2010) and also these channels have been shown to have a regulatory role in morphine-induced reward (Nguyen et al., 2014). Since both NMDA and TRPV1 receptors are implicated in behavioral abnormalities relevant to depression and addiction, their roles in mediating depressive-like behaviors following AW have remained elusive.

Considering that dysphoria following AMPH abstinence is involved in drug seeking and relapse, we aimed to investigate the effects of capsaicin, a selective TRPV1 receptor agonist, on depressive-like behaviors following AW in male mice. In this regard, we challenged the hypothesis that TRPV1 and NMDA receptors play a role in depressive-like behaviors following acute AW in male adult mice.

EXPERIMENTAL PROCEDURES

Animals

Male NMRI mice (Pasteur Institute, Tehran, Iran), weighing 25–30 g, were used. Animals were housed under standard conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle, and free access to food and water). All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS).

Stereotaxic surgery

Animals were anesthetized by a ketamine (100 mg/kg) and xylazine (10 mg/kg) subsequently submitted to a stereotaxic frame. The animals were implanted with a 22-gauge stainless-steel guide cannula placed above the right lateral cerebral ventricle (stereotaxic coordinates were: AP, −0.9 mm to the bregma; L, 1.4 mm lateral to the midline; V, −2.5 mm below the top of the skull). The cannula was fixed to the skull using one screw and dental cement. A stylet was inserted into the guide cannula to keep it open prior to injections. Implantation was performed at least five days before initiation of the chronic AMPH treatment.

Drugs

Following drugs were used: D-amphetamine sulfate and MK-801 (Sigma-Aldrich, St. Louis, MO, USA), Capsaicin (Fluka, Switzerland), Capsazepine (Tocris, UK), *N*-Methyl-D-aspartate or NMDA (Tocris, UK). Capsaicin and capsazepine were dissolved in Tween 80: DMSO: saline in 1:2:7 ratios and administered i.c.v 15 min before the examination. MK-801 and NMDA (intraperitoneally, i.p.) were dissolved in saline and administered 15 min before the test. D-amphetamine sulfate was dissolved in saline and administered for five consecutive days (i.p.) in the volume of 10-ml/kg of mouse weight.

Open-field test (OFT)

The OFT was used to evaluate the locomotion of animals in response to AW and different treatments according to the criteria described by Amiri et al. (2015a). The open-field apparatus was white opaque Plexiglas (50 cm × 50 cm × 30 cm), which was dimly illuminated. Each mouse was placed gently on the center square (30 cm × 30 cm), and behaviors were recorded by a camera for 5 min and were analyzed by Ethovision software version 8.5 (Noldus, Netherlands). The surface of the apparatus was cleaned with 70% ethanol after each experiment. The distance moved (horizontal activity) and the number of rearings (vertical activity) were evaluated.

Forced swimming test (FST)

We used FST to evaluate the immobility time of animals in response to an acute inescapable stress challenge reflecting behavioral despair (Cryan and Holmes, 2005; Haj-Mirzaian et al., 2015). In brief, mice were separately placed in an open cylinder-shaped flask (diameter: 10 cm, height: 25 cm), containing 19 cm water at 23 ± 1 °C. Mice were permitted to swim for 6 min and the immobility time was recorded throughout the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling and stayed floating motionless in the water, making only those movements necessary to keep its head above water.