Progress in Neuropsychopharmacology & Biological Psychiatry 79 (2017) 426–433

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Mitochondrial impairments contribute to spatial learning and memory dysfunction induced by chronic tramadol administration in rat: Protective effect of physical exercise

Hajar Mehdizadeh[a], Jalal Pourahmad[b], Ghorban Taghizadeh[c,d], Nasim Vousooghi[a,e,f], Ali Yoonessi[a], Parvaneh Naserzadeh[b], Ladan Behzadfar[g], Mohammad Reza Rouini[h], Mohammad Sharifzadeh[a,i,*]

[a] Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Department of Occupational Therapy, Faculty of Rehabilitation, Iran University of Medical Sciences, Tehran, Iran
[d] Rehabilitation Research Center, Faculty of Rehabilitation, Iran University of Medical Sciences, Tehran, Iran
[e] Genetics Laboratory, Iranian National Center for Addiction Studies (INCAS), Tehran University of Medical Sciences, Tehran, Iran
[f] Research Center for Cognitive and Behavioral Sciences, Tehran University of Medical Sciences, Tehran, Iran
[g] Department of Pharmacology and Toxicology, Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[h] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[i] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Tramadol
Neurotoxicity
Spatial memory
Physical exercise
Mitochondria
ROS
Cytochrome *c*

ABSTRACT

Despite the worldwide use of tramadol, few studies have been conducted about its effects on memory and mitochondrial function, and controversial results have been reported. Recently, there has been an increasing interest in physical exercise as a protective approach to neuronal and cognitive impairments. Therefore, the aim of this study was to investigate the effects of physical exercise on spatial learning and memory and brain mitochondrial function in tramadol-treated rats. After completion of 2-week (short-term) and 4-week (long-term) treadmill exercise regimens, male Wistar rats received tramadol (20, 40, 80 mg/kg/day) intraperitoneally for 30 days. Then spatial learning and memory was assessed by Morris water maze test (MWM). Moreover, brain mitochondrial function was evaluated by determination of mitochondrial reactive oxygen species (ROS) level, mitochondrial membrane potential (MMP), mitochondrial swelling and cytochrome *c* release from mitochondria. Chronic administration of tramadol impaired spatial learning and memory as well as brain mitochondrial function as indicated by increased ROS level, MMP collapse, increased mitochondrial swelling and cytochrome *c* release from mitochondria. Conversely, treadmill exercise significantly attenuated the impairments of spatial learning and memory and brain mitochondrial dysfunction induced by tramadol. The results revealed that chronic tramadol treatment caused memory impairments through induction of brain mitochondrial dysfunction. Furthermore, pre-exposure to physical exercise markedly mitigated these impairments through its positive effects on brain mitochondrial function.

1. Introduction

Tramadol, an atypical opioid, is a common analgesic used worldwide for relieving moderate to severe pain (Minami et al., 2015; Sawynok et al., 2013; Szkutnik-Fiedler et al., 2012). Chronic administration of tramadol is prescribed to treat chronic cancer and non-cancer pain such as osteoarthritis, neuropathic pain and low-back pain, etc. (Babul et al., 2004; Leppert, 2009). Analgesic effects of tramadol are mediated by a mixed central mechanism including agonistic effects on μ-opioid receptor and inhibition of serotonin and norepinephrine reuptake (Filip et al., 2004; Jesse et al., 2010; Lagard et al., 2016; Minami et al., 2015). Tramadol is clinically interested because of its lower

Abbreviations: MWM, Morris water maze test; ETC, electron transport chain; MMP, mitochondrial membrane potential; ATP, adenosine triphosphate; ROS, reactive oxygen species; HEPES, 4-2-hydroxyethyl-1-piperazineethanesul-fonic acid; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; Rh 123, Rhodamine 123; EGTA, Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid; DCFH, non-fluorescent dichlorofluorescin; DCF, highly fluorescent dichlorofluorescin

* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran.
E-mail address: msharifzadeh@sina.tums.ac.ir (M. Sharifzadeh).

http://dx.doi.org/10.1016/j.pnpbp.2017.07.022
Received 8 March 2017; Received in revised form 6 July 2017; Accepted 26 July 2017
Available online 27 July 2017
0278-5846/

incidence of dependence, tolerance and other side effects compared to other opioids like morphine (Faria et al., 2016; Pinho et al., 2013). However, recently research studies have shown increased fetal intoxications, respiratory depression and abuse of tramadol (Costa et al., 2013; Pinho et al., 2013; Ryan and Isbister, 2015). Despite the wide use of tramadol, few studies have been conducted on its behavioral effects especially learning and memory, and controversial results have been reported. Szkutnik-Fiedler et al. (2016) investigated the effects of acute (single injection) and chronic (14 days) administration of tramadol (5 mg/kg) on spatial memory function. They reported that both acute and chronic administration of tramadol enhanced spatial memory as indicated by decreased escape latency in Morris water maze test (MWM) (Szkutnik-Fiedler et al., 2016). Enhancement of cognitive function 30 min after oral administration of tramadol (50 mg/70 kg) has been reported in the elderly with chronic osteoarthritis (Freye and Levy, 2006). However, Ng et al. (2006) did not find any significant effects of patient-controlled administration of tramadol (20 mg) on short-term visual memory after lower abdominal surgery (Ng et al., 2006). Conversely, Zakaryaee et al. (2012) found impairments of memory and other cognitive functions following chronic administration of tramadol (Zakaryaee et al., 2012). Moreover, memory impairments have been reported following chronic administration of other opioids in many studies (Li et al., 2001; Pan et al., 2016; Pu et al., 2002; Yang et al., 2013; Zhou et al., 2015). Thus further research is needed to determine the effects of tramadol on learning and memory.

Although opioids are effective for pain treatment, their neurotoxic effects have been shown in previous studies. It has been reported that neurotoxic effects of opioids are often mediated by mitochondrial dysfunction, which results in oxidative stress and apoptosis (Cunha-Oliveira et al., 2008; Faria et al., 2016). So far, however, too little attention has been paid to the neurotoxic effects of tramadol. Atici et al. (2004) reported that chronic tramadol administration resulted in rat hippocampal apoptosis as evident by histologic marker of apoptosis, red neurons (Atici et al., 2004). A recent proteomic study also showed that chronic exposure to tramadol caused down-regulation of mitochondrial electron transport chain (ETC) proteins, decreased activity of antioxidant enzymes, increased lipid peroxidation and mitochondrial swelling in zebrafish brain (Zhuo et al., 2012). Moreover, inhibition of activities of rat brain mitochondrial complexes (I, II and IV) due to chronic administration of tramadol has also been reported (Mohamed et al., 2015). However, a recent in-vitro study revealed that tramadol exposure did not significantly alter the mitochondrial membrane potential (MMP), cytochrome *c* release and apoptosis in SH-SY5Y cells (Faria et al., 2016). Thus, more experimental investigations need to be done to determine the effects of tramadol on brain mitochondrial function. In addition, mitochondria play an important role in learning and memory through calcium regulation, adenosine triphosphate (ATP) generation and redox signaling. Further, brain mitochondrial dysfunction has been suggested as a potential mechanism underlying memory dysfunction in different pathological conditions (Mattson, 2007; Mattson et al., 2008). Therefore, it can be suggested that mitochondrial dysfunction induced by chronic tramadol administration may cause cognitive impairments. However, this hypothesis should be investigated directly.

In recent years, there has been an increasing interest in physical exercise as a preventive non-pharmacological approach to neuronal and cognitive impairments. The various beneficial effects of physical exercise on different cognitive functions including learning and memory (Griesbach et al., 2009; Heyn et al., 2004; Rolland et al., 2008; Rovio et al., 2005; Saadati et al., 2015; van Praag, 2009), recovery after brain injury (Gobbo and O'Mara, 2005; Luo et al., 2007), decreasing consequences of aging and neurodegenerative disorders at both behavioral and cellular levels (Cotman and Berchtold, 2007; Griesbach et al., 2009; Heyn et al., 2004; Lambert et al., 2005; Rolland et al., 2008; Rovio et al., 2005; van Praag et al., 2005) have been reported in both human and experimental animals. Physical exercise exerts these neuroprotective effects through different mechanisms including mitochondrial-dependent ones. Improved function of mitochondrial ETC and increased activity of mitochondrial antioxidant enzymes, which prevents reactive oxygen species (ROS) overproduction, are among beneficial effects reported for physical exercise (Bayod et al., 2011; Navarro and Boveris, 2007; Navarro et al., 2002). Previous studies have shown the protective effects of physical exercise on neurotoxicity and memory impairments induced by chronic administration of opioids like morphine (Alaei et al., 2006; Mokhtari-Zaer et al., 2014). However, no research has been found to survey the effects of physical exercise on tramadol-induced memory and mitochondrial alterations. Thus the current study was designed to examine whether chronic tramadol administration could affect spatial learning and memory as well as brain mitochondrial function. The study is also aimed at investigating the effects of physical exercise on spatial learning and memory and brain mitochondrial alterations induced by chronic tramadol treatment.

2. Materials and methods

2.1. Animals

Male Wistar rats (180–200 g) were obtained from Faculty of Pharmacy, Tehran University of Medical Sciences. Rats were housed in groups (four per cage) and maintained on a 12 h light/dark cycle in humidity- and temperature-controlled environments with free access to food and water. Experimental procedures were approved by Animal Ethics Committee of Tehran University of Medical Sciences. Rats were randomly assigned into 12 groups with 8 rats in each group. Tramadol groups (groups 1–3) received 20, 40 or 80 mg/kg/day of tramadol hydrochloride intraperitoneally for 30 days. Exercise groups including group 4 (short-term (2 weeks) treadmill exercise) and group 5 (long-term (4 weeks) treadmill exercise) were forced to run on a treadmill for 2 and 4 weeks (30 min/day, 5 days/week), respectively. Tramadol + exercise groups (groups 6–11) underwent the same physical exercise as the exercise groups and then received tramadol hydrochloride with the same dose and duration as tramadol groups. Rats in control group (group 12) were put on treadmill without running for the same time as exercise group and then received saline intraperitoneally for 30 days.

2.2. Drugs

Tris–HCl, rotenone, sodium succinate, sucrose, 4-2-hydroxyethyl-1-piperazineethanesul-fonic acid (HEPES), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), KCl, Na_2HPO_4, $MgCl_2$, Rhodamine 123 (Rh 123), Coomassie blue, Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), xylazine and Ketamine were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Physical exercise procedure

Physical exercise was started with an adaptation period in which rats were allowed to familiarize with treadmill by running on it (at a speed of 8 m/min, with no inclination) for 10 min/day in 3 consecutive days. The treadmill included a metal bar grid at the beginning of each lane which delivered electrical stimulation causing tingling sensation in order to encourage rats to run. Rats were placed on the treadmill facing away from the metal grid and were taught to run in the direction opposite to the movement of treadmill belt to avoid electrical stimulation. After familiarization, the exercise program was conducted which consisted of running for 30 min/day, 5 days/week at a speed of 2 m/min for the first 5 min, 5 m/min for the next 5 min and 8 m/min for the final 20 min with no inclination. Animals were observed during each session of treadmill running in order to ensure continuous running and monitor the signs of pain, stress and exhaustion (Shafiee et al., 2016; Taghizadeh et al., 2016). Previous studies have shown the effectiveness of this exercise protocol (in both short- and long-term form) in