Toxicology 310 (2013) 61–72

Contents lists available at SciVerse ScienceDirect

Toxicology

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

Roles of 3,4-methylenedioxymethamphetamine (MDMA)-induced alteration of connexin43 and intracellular Ca^{2+} oscillation in its cardiotoxicity

Luo Zhuo[a,b,1], Quan Liu[a,c,1], Liang Liu[a,b,1], Ting-yi Sun[a], Rong-shuai Wang[a], Guo-qiang Qu[a], Qian Liu[a], Yan Liu[a], Liang Ren[a,*]

[a] Department of Forensic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China
[b] Institute of Evidence Law and Forensic Science of China University of Political Science and Law, Beijing 10046, China
[c] Hubei University of Police, Wuhan, Hubei 430034, China

ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form 16 May 2013
Accepted 28 May 2013
Available online 6 June 2013

Keywords:
3,4-Methylenedioxymethamphetamine
Connexins43
N-cadherin
Phosphorylation
Calcium oscillation

ABSTRACT

Although it is well known that 3,4-methylenedioxymethamphetamine (MDMA) can cause various cardiovascular abnormalities and even sudden death from cardiac arrhythmia, whether it has any effect on myocardial gap junctions, which might be one of the targets mediating MDMA-induced cardiotoxicity, remains unclear.

Objective: To test the hypothesis that MDMA may affect the myocardial gap junction protein connexin43 (Cx43) and induce cardiac dysrhythmia.

Method: (1) *In vivo* study: adult rats were treated with a single dose MDMA administration (20 mg/kg, i.p.). Electrocardiogram detection and immunohistochemical analysis were performed to evaluate cardiac function and expression of Cx43, respectively; (2) *in vitro* study: cultured ventricular myocytes of neonatal rats were treated with MDMA (10, 100, 1000 μmol/L) for 1 h. Western blotting and real-time quantitative polymerase chain reaction (RT-qPCR) were performed to investigate the total Cx43 mRNA expression. Immunofluorescent analysis was used to evaluate the amount of junctional Cx43. The phosphorylation status of Cx43 at site Ser368 and intracellular Ca^{2+} oscillation were also studied.

Results: Obvious changes in electrocardiographic patterns were found in rats following MDMA administration. They were characterized by prolonged QRS duration associated with increased amplitude of QRS complex. The heart rates in treated rats were significantly decreased compared to the rats in the control group. The immunohistochemical findings revealed a significant decrease in Cx43 expression. The *in vitro* study also showed a marked decline in total Cx43 protein associated with reduction of Cx43 mRNA, whereas the phosphorylated Cx43 at Ser368 was increased. Decrease of junctional Cx43 was found correlated with reduction in N-cadherin induced by high concentration of MDMA. Additionally, confocal microscopy findings revealed alteration of intracellular calcium oscillation patterns characterized by high frequency and increasing influx Ca^{2+}.

Conclusions: MDMA reduces expression of cardiac gap junction protein Cx43. The increase of phosphorylation status of Cx43 at Ser368 induced by MDMA is attributed, at least in part, to the Ca^{2+}-dependent regulation of protein kinase C (PKC) activity. Our findings provide first evidence of MDMA-mediated changes in those cardiac gap junctions that may underlie MDMA-induced cardiac arrhythmia.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA or 'Ecstasy') is a commonly abused illicit drug that is popular among the young adults at rave clubs. Since it produces euphoria and intimacy, the abuse of MDMA has now reached epidemic proportions worldwide (Landry, 2002; Reid et al., 2007; Schifano et al., 2006). Previous studies have reported that MDMA affects both central nervous and cardiovascular systems. In the central nervous system, MDMA produces deficits in serotonin neurons and causes neurotoxic syndromes, such as hyperthermia, treading or head waving (Gold et al., 1988; Slikker et al., 1989; Spanos and Yamamoto, 1989), changes of mood and consciousness, and hallucinations (Jones and Dargan, 2000).

Recent results suggest that use of MDMA also plays a role in the development of heart diseases (Turillazzi et al., 2010). Acute administration of MDMA induces tachycardia and increases myocardial

* Corresponding author. Tel.: +86 27 83692644; fax: +86 26 83692638.
E-mail address: zorrocool_1@163.com (L. Ren).
[1] These authors contributed equally to this research.

0300-483X/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tox.2013.05.013

oxygen consumption (Gesi et al., 2002) which may ultimately result in arrhythmias, heart failure (Badon et al., 2002; Green et al., 1995; Mas et al., 1999), and cardiac arrest (Dowling et al., 1987). As the use of MDMA is increasing, increasing attention being paid to sudden cardiac deaths due to acute MDMA intoxication with serious disturbances of cardiac rhythm (Henry et al., 1992; Mende et al., 2005; Milroy et al., 1996; Suarez and Riemersma, 1988). Experimental studies indicate that acute administration of MDMA in animals produces significant cardiotoxicity, a response that appears to be involved in a catecholominergic or non-catecholaminergic-dependent manner (O'Cain et al., 2000; Turillazzi et al., 2010). Metabolites of MDMA with redox activities were also shown to have a significant role in ecstasy-induced cardiotoxicity (Carvalho et al., 2004). Although there is growing evidence of MDMA-induced cardiac dysfunction, the molecular mechanism underlying the effects of MDMA on the cardiomyocytes remains elusive.

Cardiac gap junctions play a pivotal role in the velocity and the safety of impulse propagation in cardiac tissue, maintaining normal cardiac rhythm (Elfgang et al., 1995; Saez et al., 2003). A gap junction consists of six connexins. Four different gap junction proteins (connexin43, connexin40, connexin45 and connexin37) expressed in mammalian myocardium have been identified, of which connexin43 (Cx43) is considered to be the abundant connexin predominately found in adult ventricular working myocardium (Dhein, 1998). Alterations of Cx43 protein (Solan and Lampe, 2009), including protein expression, distribution patterns and phosphorylation status, are linked with arrhythmia (Delmar and Makita, 2012; Tribulova et al., 2008). However, the direct actions of MDMA on myocardial gap junction proteins have yet to be determined. Since acute MDMA administration led to arrhythmia (Badon et al., 2002) and triggered toxic effects in myocardium tissues (Turillazzi et al., 2010), we were interested in potential changes in Cx43 in myocardium induced by a single dose of MDMA (20 mg/kg) *in vivo*. Therefore, the aim of the present study was to test the hypothesis that MDMA alters expression of Cx43 in myocardial tissue, possibly accounting for the cardiac dysrhythmia caused by MDMA *in vivo*.

To better understand the mechanisms of a single application of MDMA on myocardial gap junction proteins, we conducted studies using *in vivo* and *in vitro* models to investigate the alterations in electrocardiogram (ECG), Cx43 expression, and phosphorylation status of this gap junction protein following a single exposure to MDMA. We discuss the correlated cellular and molecular mechanisms involved.

2. Materials and methods

MDMA was provided by the Ministry of Public Security of People's Republic of China (Beijing, China).

2.1. In vivo study

2.1.1. Animal care and handling

Eighteen male Sprague–Dawley rats weighing 200–250 g were used for investigating the cardiotoxicity of single dose MDMA administration. All animals were maintained on a 12-h light/dark cycle and had unlimited access to food and water. All animal protocols were performed in accordance with the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Huazhong University of Science and Technology animal welfare committee. The rats were divided into two groups randomly: one group of 9 rats was treated with a single dose of MDMA (20 mg/kg, i.p.). The nine rats in the control group were treated with saline i.p. All rats were sacrificed 24 h after injections.

2.1.2. Electrocardiogram

Rats were anesthetized with 10% chloral hydrate (0.3 mL/100 g of body weight, i.p., Sinopharm Inc., China). The electrocardiographic-patterns were recorded by 3 Lead-4 Channel physiological signal polygraph (Chengdu instrument factory, China). The leads were attached to the right front leg and to both hind legs of rats. The heart rate and ECG patterns were monitored and recorded right after the MDMA injection and after the saline injection in control rats. The polygraph parameter settings were as following: scanning speed, 80 ms/div; sensitivity, 500 μV; time constant, 0.2 s; filter frequency: 30 Hz.

2.1.3. Tissue preparation and immunohistochemical detection of Cx43

24 h after MDMA administration, the hearts of rats were immediately removed and washed by sterilized saline, subsequently fixed in 4% paraformaldehyde (Sigma–Aldrich, St. Louis, MO, USA) for 48 h, then embedded in paraffin and labeled. Paraffin-embedded tissue specimens of hearts were sectioned at 4 μm and incubated for 20 min in 3% hydrogen peroxide in phosphate buffer solution (PBS) to quench endogenous peroxidase activity. The immunohistochemical examination of the heart samples was performed with rabbit anti-total Cx43 antibody (Abcam, ab11370). The primary antibody was diluted in 1:1000 ratio, and incubated for 120 min at room temperature. The SABC kit (Abcam, ab64261) was utilized as the detection system. The positive reaction was visualized by 3,3-diaminobenzidine peroxidation. The sections were counterstained with Mayer's hematoxylin, dehydrated, cover slipped and observed under an Olympus optical microscope (Olympus, Tokyo, Japan; BX51TF with Micropublisher 5.0 RTV imaging system). Three randomly areas of each slides were selected as test areas at a magnification of 200×. Positive Cx43 labeling was represented by integral optical density (IOD). All observations were performed under the exactly same condition to avoid systematic difference.

2.2. In vitro study

2.2.1. Isolation and culture of neonatal rat ventricular myocytes (NRVM)

Primary cultures of neonatal rat ventricular myocytes were prepared from 0- to 1-day-old Sprague–Dawley rats as described previously (Spector et al., 1998). Briefly, neonatal rats were anaesthetized in Metofane and then sterilized by swabbing with 75% alcohol. Hearts from neonatal rats were rapidly excised aseptically and atrial tissue and large vessels were trimmed off and discarded. The ventricles were rinsed in ice-cold Hank's Balanced Salt Solution (HBSS, without Ca^{2+} or Mg^{2+}, GIBCO, Grand island, NY, USA), washed to remove blood and debris, and then shredded into tissue fragments of about 1 mm^3, which were digested by stepwise digest solution (0.1% trypsin and 0.05% collagenase type II, GIBCO). Dissociation of the cells was obtained by gently stirring at 37 °C. Every ~10 min the dissociation medium was discarded and replaced by a fresh aliquot for 5–6 cycles till complete dissociation of the remaining fragments. Dulbecco's modified Eagle's medium (DMEM, with 4500 mg/L glucose, L-glutamine, and 25 mM HEPES, GIBCO) with 15% fetal bovine serum (FBS, GIBCO) was added into collected supernatant to terminate digestion. After centrifugation at 1000 rpm for 10 min at 4 °C, the cell pellet was washed and suspended in DMEM culture medium supplemented with 10% FBS. The cell suspension was transferred to culture flask. The fibroblast content of the cell suspension was reduced by using 90 min differential adhesion in order to enrich the culture with myocytes. After this pre-incubation, the myocytes remaining in suspension were counted with a hemocytometer. The cells were adjusted to a desirable concentration of 5×10^5 mL^{-1}. Aliquots of the cells were cultured in multi-well culture plates (Corning, Acton, MA, USA) and were maintained at 37 °C in a humidified incubator with 5% CO_2. To inhibit the growth of fibroblast, 5′-bromodeoxyuridine (5-BrdU, 0.1 mM, Sigma–Aldrich) was added into the medium. The culture medium was refreshed every 48 h. On day 6, the cells were incubated in serum free medium for 16–18 h and then treated with MDMA. The MDMA was dissolved in serum free cell culture medium and the final concentrations were 10 μM (μmol/L), 100 μM, and 1000 μM. Serum-free cell culture medium alone was added onto cells that served as our controls (0 μM). The cells were returned to the incubator for 1 h.

2.2.2. Protein extraction and quantitative Western blotting

Cultured NRVM exposed to MDMA for 1 h were scraped in ice-cold HBSS and then spun down (3000 rpm for 5 min) to pellet cells. Membrane proteins of harvested cells were extracted using the plasma membrane protein extraction kit (BioVison, Mountain View, CA, USA) as described in the manufacturer's protocol. Supernatants containing total membrane protein were quantified by using a BCA protein assay kit (Pierce, Rockford, IL, USA). Aliquots containing 10 μg of protein were mixed with Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) and β-mercaptoethanol (Amreso, Solon, OH, USA), and denatured at 100 °C for 10 min. Then samples were loaded onto 10% Tris–HCl Ready Gel® (Bio-Rad), electrophoresed and electrotransferred on to polyvinylidene fluoride (PVDF) membrane (0.2 μm, Invitrogen, Carlsbad, CA, USA). WesternBreeze® chemiluminescent Western blot immunodetection kit (Invitrogen) was applied for immunodetection of Western blots according to the manufacturer's instructions.

To investigate the effects of MDMA on total Cx43 expressions, rabbit anti-total Cx43 (Abcam, ab11370; dilution 1:2000) were used. Anti-Ser368 phosphorylated Cx43 (Abcam, ab30559; dilution 1:1000) was applied for investigating the phosphorylation status at the specific phosphorylation site Ser368. In order to clarify the possible mechanism on the alterations of Cx43 induced by MDMA, we also used rabbit anti-N-cadherin antibody (Abcam, ab18203, dilution 1:1000) to test the expression of N-cadherin in cultured NRVM. Besides, the amount of protein kinase-C, alpha (PKCα) and phosphorylated PKCα (Ser657) were immune-detected by using rabbit anti-PKCα antibody (Santa Cruz, CA, USA, sc-208; dilution: 1:2000) and rabbit anti-PKCα antibody (Santa Cruz, sc-12356; dilution: 1:2000) respectively. In the present experiments, rabbit anti-β-actin antibody (Proteintech, Chicago, IL, USA,

Download English Version:

https://daneshyari.com/en/article/2595631

Download Persian Version:

https://daneshyari.com/article/2595631

Daneshyari.com