Neuroscience Letters 597 (2015) 143–148

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

The tegmental–accumbal dopaminergic system mediates the anxiolytic effect of acupuncture during ethanol withdrawal

ZhengLin Zhao[a,1], Sang Chan Kim[b,1], RongJie Zhao[a,b,*], YiYan Wu[a], Jie Zhang[a], HongFeng Liu[a], Young Woo Kim[b], XiaoDong Zhu[a], ChangHong Gu[a], Chul Won Lee[b], Bong Hyo Lee[b], Eun Young Jang[b], Hae Li Ko[b], Chae Ha Yang[b]

[a] Department of Pharmacology, Mudanjiang Medical University, Mudanjiang 157011, China
[b] College of Oriental Medicine, Daegu Haany University, Daegu 706-060, South Korea

HIGHLIGHTS

- Acupuncture elevated accumbal dopamine levels during ethanol withdrawal.
- Acupuncture improved ventral tegmental TH mRNA levels during ethanol withdrawal.
- Acupuncture increased accumbal TH protein levels during ethanol withdrawal.
- Acupuncture decreased ventral tegmental BDNF expressions during ethanol withdrawal.
- Concurrent SCH23390 and eticlopride blocked the anxiolytic effect of acupuncture.

ARTICLE INFO

Article history:
Received 1 February 2015
Received in revised form 20 April 2015
Accepted 27 April 2015
Available online 30 April 2015

Keywords:
Ethanol withdrawal
Acupuncture
Dopamine
Tyrosine hydroxylase
BDNF

ABSTRACT

This study investigated the involvement of the mesolimbic dopamine (DA) system in the anxiolytic effects of acupuncture during ethanol withdrawal (EW). Rats were intraperitoneally treated with 3 g/kg/day of ethanol for 28 days and experienced 3 days of withdrawal. During EW, the rats were bilaterally treated with acupuncture at acupoints HT7 (Shenmen) or PC6 (Neiguan) or at a non-acupoint (tail) once daily for 1 min over 3 days. High-performance liquid chromatographic (HPLC) analysis showed that EW significantly decreased both DA and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the nucleus accumbens shell (NaccSh); however, these processes were inhibited by acupuncture at HT7 but not at PC6. Real-time polymerase chain reaction and western blot assays also revealed that acupuncture at HT7 prevented the EW-induced reductions in tyrosine hydroxylase mRNA expression in the ventral tegmental area (VTA) and tyrosine hydroxylase protein expression in the NaccSh. A prior intra-NaccSh infusion of a cocktail of the selective DA1 receptor antagonist SCH23390 and the selective DA2 receptor antagonist eticlopride blocked the anxiolytic effect of acupuncture at HT7 in elevated plus maze tests. In addition, acupuncture at HT7 suppressed EW-induced increased BDNF levels in the VTA. These findings suggest that acupuncture at HT7 improves the VTA–Nacc DAergic function via inhibition of BDNF expression in the VTA, thereby exerting anxiolytic effects during EW.

1. Introduction

Ethanol withdrawal (EW)-induced anxiety, a major contributing factor to alcoholic relapse, derives from a reduction in the functioning of the reward system. The dopamine (DA) projection from the ventral tegmental area (VTA) to the nucleus accumbens (Nacc) is a pivotal structure in the mediation of the rewarding effects of ethanol. Acute ethanol increases the activity of this projection responsible for the rewarding effect of ethanol [1], while EW results in a decreased level of DA in this pathway [2]. Clinical and preclinical evidence indicates that a decline in VTA–Nacc DAergic activity is involved in EW-induced anxiety. Higher levels of sensitivity of presynaptic DA2 receptors (Rs) (autoreceptors) are positively associated with high levels of anxiety in humans [3], and the DA2R antagonist tiapride can treat anxiety in alcoholic patients

* Corresponding author at: Department of Pharmacology, Mudanjiang Medical University, 3 Tongxiang Street, Aimin District, Mudanjiang 157011, China. Tel.: +86 453 698 4682; fax: +86 453 618 9377.
E-mail address: zhao_rongjie@yahoo.com (R. Zhao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2015.04.045
0304-3940/

[4]. Rodents undergoing EW exhibit a reduction in both the firing of DA neurons in the VTA and in the release of extracellular DA in the Nacc [2,5]. Additionally, the DA system stabilizer aripiprazole attenuates anxiety during EW [6], and the intra-Nacc infusion of the non-selective DARs agonist apomorphine inhibits morphine and nicotine withdrawal-induced anxiety [7].

Acupuncture has long been used in traditional Chinese medicine (TCM) for the treatment of alcoholism. Acupuncture can restore the biochemical imbalance in the central nervous system induced by withdrawal from abused drugs to treat the withdrawal syndrome. Acupuncture at Zusanli reduces EW-induced behavioral symptoms by reducing c-Fos expression in the brain [8], and acupuncture at HT7 (Shenmen) alleviates nicotine withdrawal-induced anxiety via inhibiting amygdaloid CRF mRNA expression [9]. In a previous study, we demonstrated that acupuncture at HT7 ameliorated EW-induced anxiety by normalizing amygdaloid catecholamines [10]. To extend these findings, the present study evaluated the possible involvement of VTA–Nacc DA in the anxiolytic effects of acupuncture during EW.

2. Materials and methods

2.1. Animals and experimental design

Adult male Sprague–Dawley rats (250–270 g) were obtained from the Laboratory Animal Center at Mudanjiang Medical University (Mudanjiang, China). The rats were given food and water ad libitum and maintained on a 12 h light, 12 h dark cycle throughout the course of the study. All animal procedures were performed in accordance with the National Institutes of Health guidelines concerning the care and use of laboratory animals and were approved by the Animal Care and Use Committee of Mudanjiang Medical University.

The rats were treated with 3 g/kg/day ethanol (20% w/v) or saline by intraperitoneal injection for 28 days. After the final dose of ethanol, the rats underwent EW for 3 days, and during which time they were received acupuncture at acupoints HT7 or PC6 (Neiguan) or at a non-acupoint (tail) once daily for 1 min over 3 days.

The acupuncture stimulation involved the insertion of stainless steel needles (0.2 mm in diameter) into HT7 or PC6 acupoints or into tail non-acupoints with a depth of 2–3 mm; these needles were manipulated using the reduction and reinforcement method (for details see [10,11]). The sham acupuncture treatment involved treating two groups of rats with ethanol (ethanol-treated control rats) or saline (saline-treated control rats) and holding them for 1 min without the insertion of acupuncture needles to match the immobilization experienced by the acupuncture-treated rats.

Ten minutes after the acupuncture or sham treatment, the rats were euthanized and decapitated, and the entire brain was removed and stored at −80 °C. Tissue samples of the Nacc shell (NaccSh) and VTA were punched out from the stored brains according to their coordinates (NaccSh: anterior–posterior (AP) = −1.7 mm, medial–lateral (ML) = 1.0 mm, dorsal–ventral (DV) = −7.2 mm; VTA: AP = −6.0 mm, ML = −0.7 mm, DV = −7.8 mm, based on the Paxinos and Watson rat brain atlas) [12] for high-performance liquid chromatographic (HPLC), real-time polymerase chain reaction (PCR) and western blot analysis, respectively.

2.2. HPLC analysis of DA and its metabolites in the NaccSh

The frozen NaccSh tissues were weighed, sonicated in 0.1 mol/L $HClO_4$, and centrifuged at 26,000 × *g* and 4 °C for 15 min. Then, a 20 μl supernatant aliquot was injected directly into the HPLC with an electrochemical detector (Coulochem II; ESA, Bedford, MA, USA). The HPLC system consisted of a C-18 reverse-phase column (5 μm ODS; Altex, Ann Arbor, MI, USA) and an electrochemical transducer with a glassy carbon electrode set at 350 mV. The mobile phase consisted of 0.163 mol/L citric acid, 0.02 mmol/L EDTA, 0.69 mmol/L sodium octanesulfonic acid as an ion-pairing reagent, 4% (v/v) acetonitrile, and 1.7% (v/v) tetrahydrofurane, and was tittered to pH 3.0 by H_3PO_4. Peaks and values of Peaks and values of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) in samples were identified and calculated by comparing their retention times and peak heights with those of standards (Sigma–Aldrich, St. Louis, MO). Results are expressed as ng/g wet tissue.

2.3. Real-time PCR analysis

Total RNA was extracted from the NaccSh and VTA tissues using Trizol (Invitrogen), and cDNA was synthesized by reverse transcription using an oligo (dT) primer. Then, real-time PCR was performed with a Light Cycler 1.5 (Roche) using a Light Cycler DNA Master SYBR green-I kit, according to the manufacturer's instructions. The primers (synthesized by Bioneer Corporation, Daejeon, Republic of Korea) for tyrosine hydroxylase (TH) are 5′-ATGCCCACCCCCAGCGCCCC-3′ (sense) and 5′-GACACTTTTCTTGGGAACCA-3′ (antisense), for β-actin are 5′-GTCGTACCACTGGCATTGTG-3′ (sense) and 5′-GCCATCTCTTGCTCGAAGTC-3′ (antisense). The housekeeping gene β-actin was used as the endogenous reference, and the relative expression levels of TH mRNA were calculated by the formula: ΔCT = CT (TH) - CT (β-actin), ΔΔCT = ΔCT (treated) - ΔCT (saline), and expressed as $2^{-\Delta\Delta CT}$.

2.4. Western blotting

The frozen NaccSh and VTA tissues were homogenized in lysis buffer [20 mM Tris, 5 mM EDTA, 1% Nonidet P-40 (vol/vol), protease inhibitors], incubated for 20 min on ice, and centrifuged (19,000 × *g* for 20 min at 4 °C). The supernatants were resolved by electrophoresis on a 12% SDS-polyacrylamide gel, and the proteins were transferred on NC membrane (Schleicher & Schuell GmbH, Dassel, Germany). The membrane was incubated with anti-mouse TH antibody, anti-rabbit polyclonal BDNF antibody or anti-goat β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), washed with TBST (10 mM Tris-Cl pH 7.5, 150 mM NaCl, 0.05% Tween 20) and incubated for 1 h with the appropriate peroxidase-conjugated secondary antibodies. Bands corresponding to TH, BDNF, and β-actin were visualized using enhanced chemiluminescence western blotting detection reagents (Amersham Biosciences, Piscataway, NJ, USA).

2.5. Elevated plus maze (EPM) test

To determine whether the anxiolytic effects of acupuncture treatment were mediated through accumbal DAergic receptors, a cocktail of the selective DA1R antagonist SCH23390 (0.6 μg/200 nL/side, Tocris Bioscience, Ellisville, MO, USA) and the selective DA2R antagonist eticlopride (0.7 μg/200 nL/side, Tocris Bioscience) was dissolved in a modified Ringer's solution (MRS: 150 mM NaCl, 3.0 mM KCl, 1.4 mM $CaCl_2$, and 0.8 mM $MgCl_2$ in 10 mM phosphate buffer at pH 7.1) [13], bilaterally delivered into the NaccSh using motorized syringe pumps over a period of 60 s at 5 min before the third acupuncture treatment (Sage Instruments, Boston). For the intra-NaccSh infusion of the DAR antagonists, stainless steel guide cannulae (15 mm; 23 gauge) were bilaterally implanted into the rats in a stereotaxic instrument under anesthesia (sodium pentobarbital, 50 mg/kg, i.p.) with the cannula tips 2 mm above the NaccSh. Five minutes after the third acupuncture treatment, the rats were tested in the EPM (Shanghai Yishu Co.; Shanghai,