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Alteration of glutamate/GABA balance during acute alcohol intoxication in rats: Effect of Xingnaojing injection

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ABSTRACT

Ethnopharmacological relevance: Xingnaojing Injection (XNJI) is a modern Chinese formula came from famous Chinese medicine *An Gong Niu Huang* Pill. XNJI has been used for treatment of cerebral diseases and stroke in China, and is approved by the State Food and Drug Administration of China for the treatment of acute alcohol intoxication (AAI). XNJI belongs to the ethnopharmacological family of medicines. In this study, we investigated the mechanisms of the XNJI effect on AAI.

Aim of the study: To investigate the effects of XNJI on glutamate, gamma-aminobutyric acid (GABA) and related receptor in lateral hypothalamic area (LHA) of AAI rat.

Material and methods: Adult male Sprague-Dawley rats were implanted with microdialysis probes in LHA. Rats were randomly divided into control, model, 1.36 mg/kg XNJI, 0.68 mg/kg XNJI and 0.34 mg/kg XNJI groups. During microdialysis, baseline samples were collected from 1 h to 2.5 h; thereafter, the rats were given an intraperitoneal injection of 52% ethanol, 5.2 g/kg, or saline for control group. Twenty minutes later, three doses of XNJI was given by unilateral injection respectively, while saline for control and model groups, and samples were collected for the next 4 h. The extracellular glutamate and GABA levels were measured in the LHA by a high performance liquid chromatography coupled with fluorescence detector (HPLC-FLU). The expression levels of related receptors N-methyl-D-aspartate receptor (NR) subunit NR_{2A}, NR_{2B} and $GABA_A$ were analyzed by reverse transcription polymerase chain reaction (RT-PCR).

Results: Ethanol (5.2 g/kg) significantly decreased the extracellular levels of glutamate and increased extracellular GABA in LHA. On the other hand ethanol significantly decreased NR_{2A} and NR_{2B} mRNAs expression, and increase $GABA_A$ mRNA expression. XNJI could increase the extracellular level of glutamate and decrease that of GABA; moreover, induced an increase in NR_{2A} and NR_{2B} mRNA expression, and a decrease in $GABA_A$ mRNA expression in LHA.

Conclusions: The current changes in glutamate, GABA and mRNA expressions of related receptors in LHA after injection of XNJI suggest that changes in these neurotransmitters and receptors as a potential mechanism of action for AAI.

1. Introduction

Acute alcohol intoxication (AAI) is a clinically harmful condition that usually follows the ingestion of a large amount of alcohol. Clinical manifestations are heterogeneous and involve different organs and apparatuses, with behavioral, cardiac, gastrointestinal, pulmonary, neurological, and metabolic effects (Vonghia et al., 2008). According to different studies, up to 77% (Cherpitel, 2007; Browne et al., 2013) of subjects admitted in accident and emergency departments presented an alcohol-related injury. It has been shown that injuries are especially associated with alcohol drinking. A recent report has shown that alcohol was involved in 62.9% of violence-related injuries in 2012 (Cherpitel et al., 2012).

Ethanol (EtOH) is a water-soluble compound that rapidly crosses cell membranes, resulting in ready equilibration between intra- and extra- cellular concentrations (Marco and Kelen, 1990). EtOH produces intoxication through actions on the central nervous

Abbreviations: AAI, acute alcohol intoxication; GABA, gamma-aminobutyric acid; GC, gas chromatography; HPLC-FLU, high performance liquid chromatography coupled with fluorescence detector; LHA, lateral hypothalamic area; NR, N-methyl-D-aspartate receptor; RT-PCR, reverse transcription polymerase chain reaction; XNJI, Xingnaojing Injection

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system at concentrations ranging from low to ~100 mM. A number of proteins involved in synaptic transmission are altered by EtOH effects within this concentration range. The target proteins include, but are not limited to, ion channels, neurotransmitter receptors, and intracellular signaling proteins (Lovinger and Roberto, 2013). Studies have already shown that certain neurotransmitter systems such as glutamate and gamma-aminobutyric acid (GABA) are very sensitive to the actions of ethanol (Radel and Goldman, 2001). Acute alcohol administration inhibits glutamatergic transmission via effects at N-methyl-d-aspartate (NMDA) receptors (NR), however facilitates GABAergic transmission in central amygdala via both pre- and post synaptic mechanisms (Roberto et al., 2012).

Due to the long history and widespread use of alcohol as a recreational beverage, the clinical manifestations of AAI are usually not taken seriously; however, the adverse effects of alcohol at sufficiently high levels can cause coma and respiratory depression (Jung and Namkoong, 2014). The treatment of an intoxicated patient entails supportive and symptomatic care, in which wake-promoting is an important step. Xingnaojing Injection (XNJI), is an effective traditional Chinese medicine (TCM) and was extracted and purified by modern biotechnology according to a famous Chinese traditional medicine named *An Gong Niu Huang Wan* (a classic traditional prescription, first documented in *Wen Bing Tiao Bian*, a classical work of TCM written in the Ming dynasty). Modern pharmacological studies confirmed that the XNJI can directly act on the central nervous system through blood brain barrier, reduce brain injury and enhance functional recovery in different clinical trials and animal models of injury (Shen et al., 2003; Wei and Li, 2009; Guo et al., 2010; Xu et al., 2010). It is now well documented that XNJI has obvious promoting revival effect (Bai et al., 1998; Chen and Xie, 2014) and antioxidation effect for AAI (Zhang et al., 2012; Wen et al., 2013). Yang et al. (2009) systemic reviewed the effectiveness of XNJI in the treatment of AAI, which showed that XNJI plus western medical therapy was superior to western medical therapy, the curative efficacy of XNJ and naloxone were similar. Despite the demonstrated benefits of XNJI in treating AAI, little is known about the underling mechanisms.

Our previous study results showed that XNJI can shorten the time of loss of the righting reflex, improve the activity of alcohol dehydrogenase and aldehyde dehydrogenase and promote the metabolism of ethanol significantly in AAI mice (Wei et al., 2013). We hypothesized that XNJI may be responsible for the changes in glutamate and GABA during AAI. To test this hypothesis we carried out microdialysis studies to determine XNJI's effects on these neurotransmitters in the lateral hypothalamic area (LHA), major brain region central to the AAI process (Morganstern et al., 2010; Chen et al., 2013). Further we defined the effects of XNJI on NR_{2A}, NR_{2B} and $GABA_A$ mRNA expressions in LHA to determine the potential mechanism of action for AAI.

2. Materials and methods

2.1. Chemicals, reagents and drugs

Glutamate, GABA and *o*-phthalaldehyde (OPA, contain β-mercaptoethanol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The RNAiso™ Plus, PrimeScript™ RT Master Mix, SYBR Premix Ex TaqTM II and Diethypyro carbonate (DEPC) were purchased from TaKaRa (Dalian, China). XNJI was purchased from Jiminkexin Pharmaceutical Company (Wuxi, China). XNJI and its constituents combination (1 mL XNJI contains 7.5 mg artificial musk, 1 mg synthetic borneol, 30 mg *Curcuma aromatic* Salisb and 30 mg *Gardenia jasminoides* Ellis). To ensure the quality and stability of the XNJI, we use a gas chromatography (GC) to assay the volatile components in musk and borneol, the high performance liquid chromatography (HPLC) to assay non-volatile components in *Curcuma aromatic* Salisb and *Gardenia jasminoides* Ellis. The protocol conditions were as follows:

For volatile components assay in musk and borneol: the chromatographic separation was cut out on a 3% SE-54 fused silica capillary column (15 m × 0.32 mm, 0.4 μm) with flame ionization detector (FID) as the detector, the detector temperature was 300 °C and the injector temperature was 250 °C, the column temperature was programmed as maintaining 60 °C for 15 min and then raising to 170 °C at a rate of 45 °C/min and maintaining for 12 min. The concentrations of the four volatile components were: muscone (0.087 mg/ml), borneol (1.11 mg/ml), isoborneol (0.054 mg/ml) and camphor (0.078 mg/ml). The chemical structure of each component and the results of GC are shown in Fig. 1.

For non-volatile components assay in *Curcuma aromatic* Salisb and *Gardenia jasminoides* Ellis: the chromatographic separation of jasminoidin was performed on a phenomenex column(250 × 4 mm, 4 μm)with isocratic elution of acetonitrile–water (17:83) at a flow rate of 1.0 ml/min and the detection wave length was 238 nm; The chromatographic separation of three kinds of curcumin was performed on the same column with isocratic elution of isopropyl alcohol-methanol-0.4% acetic acid (26:27:47) at a flow rate of 0.5 ml/ml and the detection wave length was 425 nm. The concentrations of the four non-volatile components were: jasminoidin (2.83 μg/ml), curcumin (0.36 μg/ml), demethoxycurcumin (0.14 μg/ml) and bisdemethoxycurcumin (0.50 μg/ml). The chemical structure of each component and the results of HPLC are shown in Fig. 2.

2.2. Animals

Male Sprague-Dawley rats (Guangdong Medical Experimental Animal Center, Guangzhou, China) weighing from 350 g to 400 g were used. Rats were housed in a temperature and humidity controlled animal facility maintained on a 12 h light/dark cycle with access to food and water ad libitum. Experiments were conducted during the light phase. All animal experimental protocols and procedures were performed strictly within national regulations and guidelines and approved by the Animal Experimentation Committee at the Guangzhou University of Chinese Medicine.

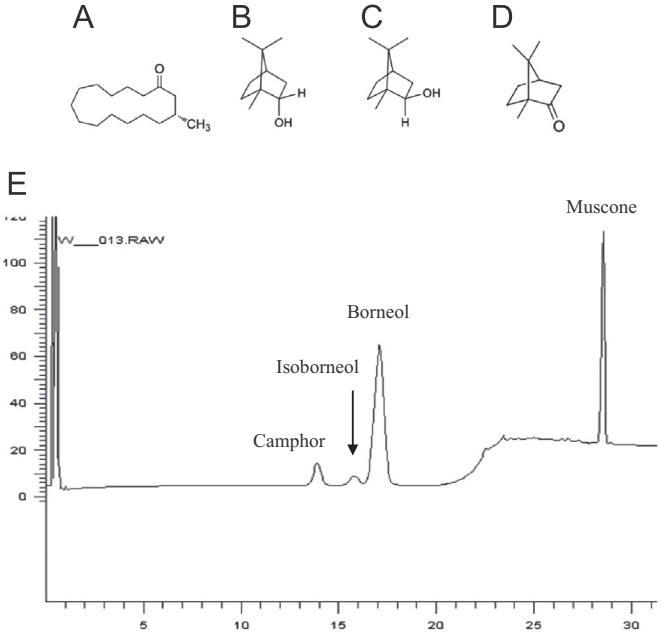

Fig. 1. Chemical structures and GC analysis of XNJI. (A) muscone ($C_{16}H_{30}O$, molecular weight: 238.40); (B) borneol ($C_{10}H_{18}O$, molecular weight: 154.25); (C) isoborneol ($C_{10}H_{18}O$, molecular weight: 154.25); (D) camphor ($C_{10}H_{16}O$, molecular weight: 152.23); (E) GC analysis of XNJI.