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Frankincense upregulates the hippocampal calcium/calmodulin kinase II-α during development of the rat brain and improves memory performance

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ABSTRACT

Background: Frankincense is an oleo gum resin derived from trees of genus *Boswellia.* It has favorable effects on memory formation. However, the probable underlying molecular mechanisms have not been assessed. Frankincense exerts some of its effects via activation of protein kinases. Calcium/calmodulin kinaseII (CaMKII) and CaMKIV are crucial mediators of learning and memory. We studied the effect of maternal injection of the aqueous extract of frankincense during gestation and lactation periods on spatial memory performance and the mRNA expression levels of the hippocampal CaMKIIand CaMKIV in the offspring rats.

Methods: Aqueous extract of Frankincense (50 and 100 mg/kg) or tap water was gavaged to distinct female rats during gestation and lactation periods. Memory performance was assessed in groups of male offspring using Morris water maze. In other groups of the offspring (with no memory test), the hippocampi of the juvenile rats were removed 30 days after labor. A real-time PCR method was used to measure the mRNA levels of CaMKII and CaMKIV.

Results: Frankincense improved spatial memory retrieval in the offspring rats in a dose-dependent manner. The mRNA expression of hippocampal CaMKIV was unchanged between groups. However, the mRNA expression of hippocampal CaMKII was dose-dependently upregulated in the rats, whose mothers had received frankincense.

Conclusions: Due to the crucial role of the CaMKII in memory formation, the results provide a molecular basis for the effect of administration of frankincense to mother rats on improvement of the memory in the offspring.

1. Introduction

Learning and memory are crucial cognitive capabilities of the animals that permit them to alter their behavior in response to new experiences. There are some natural products, which can expand memory formation (Shojaii et al., 2016; Singh et al., 2013). Frankincense is an oleo gum resin derived from trees of genus *Boswellia*. These trees are native to the Arabic peninsula, Ethiopia, India, and Somalia. In traditional medicine, it is introduced as a memory improving compound (Mahboubi et al., 2016). Accordingly, various experimental studies have been conducted during recent years to clarify the potential effects of frankincense on memory performance (Farshchi et al., 2010; Hosseini-Sharifabad et al., 2016a; Mahmoudi et al., 2011). These studies have confirmed the potential improving effects of frankincense on learning and memory. For example, frankincense was shown to facilitate acquisition of spatial memory (Farshchi et al., 2010) and improve spatial memory retrieval in the Morris water maze (MWM) (Mahmoudi et al., 2011). Meanwhile, no significant side effects or toxicity have been reported from frankincense (Ammon, 1996). However, the potential mechanisms of this activity have not been well studied. Some interesting studies have shown that maternal injection of frankincense improves memory of the offspring. For example, two month old male Wistar rats whose mothers were given orally aqueous extract of the *Boswellia serrata* (100 mg/kg/day) during gestation for 3 weeks showed a significant increase in power of learning at post-learning stage and short-term and long-term memory in an active avoidance task (Hosseini Sharifabad et al., 2004). It is claimed that frankincense induces developmental changes in brain such as increase in the volume of the neurons, as well as number of dendritic spines and consequently improves memory formation.

The neurons of the hippocampal CA_3 area in the rats whose mothers had received frankincense during gestation had significantly more dendritic segments than the controls. The dendritic branching density was also higher in experimental rats relative to that found in the control

Abbreviations: AD, Alzheimer's disease; CaMK, calcium/calmodulin protein kinase; cDNA, complementary deoxyribonucleic acid; LTP, long-term potentiation; MWM, Morris water maze; PCR, polymerase chain reaction

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rats (Hosseini Sharifabad and Esfandiary, 2007). Also, the volume of cellular layer of dentate gyrus and CA_3 and individual volume of their neurons increased following maternal administration of frankincense during lactation (Hosseini Sharifabad and Esfandiari, 2012). Nonetheless, the underlying molecular changes are not studied.

It was reported that frankincense exerts some of its effects via activation of protein kinases (Altmann et al., 2002; Poeckel et al., 2006). Calcium/calmodulin kinase II (CaMKII) and CaMKIV are two serine/threonine-specific protein kinases that are regulated by the Ca^{2+}/calmodulin complex. They are involved in many signaling cascades and are thought to be crucial mediators of learning and memory (Kang et al., 2001a; Yamauchi, 2005). CaMKII activity increased during memory formation (Cammarota et al., 1998). Spatial learning was impaired in CaMKII-α mutant mice (Silva et al., 1992a). These mice were also deficient in their ability to produce LTP (Silva et al., 1992b). Meanwhile, CaMKIV KO mice displayed severe impairment in contextual fear memory, but showed normal memory in the standard passive avoidance task (Song et al., 2015).

Here, we have evaluated the memory performance in the rats whose mothers were treated with frankincense during gestation and lactation periods and measured the mRNAs expression of the hippocampal CaMKII and CaMKIV.

2. Materials and methods

2.1. Laboratory animals

Totally 18 female and 9 male adult Wistar rats weighing 180–220 g and their offspring were used. Rats were obtained from the breeding colony of Department of Biology, University of Isfahan. Animals were housed in standard cages in a temperature (24 ± 1 ∘C) controlled room that was maintained on a 12:12 light cycle (light on at 07:00 a.m.). Animals had free access to food and water in their home cage. The ethical aspects of the project were in accordance with the guide for the care and use of laboratory animals (USA National Institute of Health publication No. 80–23, revised 1996) and approved by the graduate studies committee of Department of Biology, University of Isfahan. Each experimental group consisted of 6 male juvenile rats for either behavioral test or gene expression study of which one rat was chosen from apiece mother.

2.2. Preparation of the aqueous extract of frankincense

The aqueous extract of frankincense was prepared as previously described (Beheshti and Aghaie, 2016). In brief, an appropriate amount of frankincense was powdered and soaked in distilled water. After 24 h it was warm-heated on a 50 °C water bath for 60 min and filtered before injection. The doses of frankincense (50 and 100 mg/kg; P.O) were selected according to previous reports designating its effectiveness on improvement of memory (Beheshti and Aghaie, 2016; Beheshti and Karimi, 2016; Mahmoudi et al., 2011; Yassin et al., 2013).

2.3. Behavioral experiments

Two female rats and one male rat were placed in separate cages to mate. Following observation of the vaginal plaques (zero day), male rats were removed from the cage and the female rats were treated with the aqueous extract of frankincense (50 and 100 mg/kg; P.O) or tap water (1 ml/kg) for about 20 consecutive days, one day before labor. Three days after labor, the mother rats received again frankincense (50 and 100 mg/kg; P.O) or tap water for another 25 consecutive days (Fig. 1). Thirty days after labor, in three groups memory performance was evaluated using a MWM. In other groups, the juvenile rats were decapitated and their hippocampi were removed and frozen immediately in liquid nitrogen, then stored in a −70 °C freezer.

2.4. Morris water maze apparatus

The water maze used consisted of a dark circular pool (140 cm in diameter and 55 cm high) filled with water (24 °C) to a depth of 30 cm . A transparent Plexiglas platform (11 cm diameter) was positioned 1 cm below the water surface in the midpoint of one of the randomly designed north-east (NE), south-east (SE), south-west (SW) or north-west (NW) orthogonal quadrants (Fig. 2). The platform provided the only escape from the water. Many extra-maze cues such as a window, a door and pictures on the walls surrounded the room where the water maze was housed. These were kept in fixed points with respect to the swimming pool to let the rat to find the escape platform hidden below the water surface. The place of the animal was monitored by a camera that was fixed above the midpoint of the pool.

The camera signal was digitized and fed to a computerized tracking system that monitored and stored the position of the rat. The time required reaching the platform (latency) and the time spent in the quadrants were recorded.

2.5. Training and testing procedure

The rats were adapted to the pool by allowing them to perform a 60 s swim without the platform, twenty-four hours prior to the start of training. A single training session was used, which consisted of eight trials with four different starting positions that were equally distributed around the perimeter of the maze. Each rat was placed in the water facing the wall of the tank at one of the four selected starting points (north, east, south and west) and allowed to swim and find the hidden platform placed in the SW quadrant (target quadrant) of the maze. Each of four starting positions was used twice in eight training sessions. During each trial, each rat was given 60 s to find the hidden platform. After finding the platform, the animals were permitted to remain there for 20 s, and were then sited in a holding cage for 30 s until the start of the next trial. After completion of training, the animals returned to their home cages. 24 h later, a retention test (probe trial) was performed. In the probe trial the hidden platform was removed and the animal was released from the north position and allowed to swim freely for 60 s. All of experiments were conducted between 9:00 and 15:00 h.

2.6. RNA extraction and complementary DNA (cDNA) synthesis

The mRNA expression assay was performed as previously described with some modifications (Beheshti et al., 2017). In brief, the frozen right hippocampi were pulverized completely and mixed with 200 µL chilled phosphate-buffered saline (in mmol/L: 137 NaCl, 2.7 KCl, 4.3 Na2HPO4.7H2O, and 1.4 KH2PO4), vortexed for 30 s and then divided into aliquots. Total cellular RNA was isolated using RNX-PLUS reagent (SinaClon, Iran). The RNA was treated with 1 U RNase-free DNase I (Thermo Fisher Scientific Inc, United States) to prevent DNA contamination. The integrity of the RNA samples was determined using denaturing agarose gel electrophoresis. The concentration and purity of the RNAs were determined by a Nano drop spectrophotometer. The mean absorbance ratio at 260/280 nm was 1.88 ± 0.02 and at 260/230 nm was 1.9 ± 0.02. The reverse transcription reaction was performed with a cDNA synthesis kit (Takara, Japan) using Oligo-dT primer, MULV reverse transcriptase and 500 ng total RNA as template, according to the manufacturer's instructions.

2.7. Real-time PCR

CaMKII and CaMKIV were chosen as target genes and GAPDH was used as an internal reference gene. All primers were designed using the NCBI primer design tool (Table 1). The specificity of the primers for their target sequences was checked on the NCBI website (www.ncbi.nlm.nih.gov/blast). The SYBR Green I real-time PCR assay was carried out in a final reaction volume of 10 µL with 5 µL SYBR Green I Master