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The effect of arsenite on spatial learning: Involvement of autophagy and apoptosis

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ABSTRACT

Spatial learning plays a major role in one's information recording. Arsenic is one of ubiquitous environmental toxins with known neurological effects. However, studies investigating the effects of arsenic on spatial learning and related mechanisms are limited. This study was planned toexaminethe effects of bilateral intra-hippocampal infusion of different concentrations of sodium arsenite (5, 10 and 100 nM, 5 μl/side) on spatial learning in Wistar rats. Moreover, we evaluated levels of LC3-II, Atg7 and Atg12 as reliable biomarkers of autophagy and caspase-3 and Bax/Bcl-2 ratio as indicators of apoptosis in the hippocampus. Interestingly, low concentrations of sodium arsenite (5 and 10 nM) significantly increased spatial acquisition but pre-training administration of sodium arsenite100 nM did not significantly alter spatial learning. LC3-II levels were significantly increased in groups treated with sodium arsenite 5 and 10 nM and decreased in the group receiving arsenite 100 nM compared to the control group. Atg7 and Atg12 levels were obviously higher in all groups treated with sodium arsenite compared to control. However, caspase-3 cleavage and Bax/Bcl-2 ratio were notably greater in 100 nM, and lesser in 5 nM arsenite group in comparison with control animals.

The results of this study showed that the low concentrations of sodium arsenite could facilitate spatial learning. This facilitation could be attributed to neuronal autophagy induced by low concentrations of sodium arsenite. These findings may help to clarify the regulatory pathways for apoptosis and autophagy balance due to sodium arsenite.

1. Introduction

Arsenic is one of the environmental toxicants ubiquitously found in water, air and soil. The primary source of human exposure is through drinking contaminated water (Martinez-Finley et al., 2009). Arsenic and its derivatives are thoroughly studied for their carcinogenic effects focusing on increased risk of skin, bladder, liver and kidney tumors (Diaz-Villasenor et al., 2006). In 2003, Rodriguez et al. reviewed the effects of arsenic on nervous system, declaring adverse effects of arsenic on neurobehavioral development (Rodriguez et al., 2003). Deficits in learning and memory following acute or chronic arsenic exposure have been reported in many human and animal studies. However, their cellular and molecular mechanisms are not understood completely (O'Bryant et al., 2011; Tsai et al., 2003). Luo et al. (2009) reported that long-term exposure to high concentration of arsenic in drinking water resulted in significant delay in acquisition of spatial memory but it had not significant effect on spatial memory retention (Luo et al., 2009). On the other hand, Jiang et al. (2014) and Jing et al. (2012) indicated impairment of both acquisition and retention of spatial memory due to long-term exposure to high dose of arsenic

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(Jiang et al., 2014; Jing et al., 2012). Then again, our recent study showed the dual effect of sodium arsenite on contextual and tone memory of Aβ-injected rats in pavlovian fear conditioning model. We found that low dose (1 and 5 nM) of sodium arsenite attenuated memory deficit induced by amyloid beta while high dose (10 and 100 nM) increased memory loss in these rats. Arsenite at these ultra-low concentrations caused a marked increase in Nrf2 and CREB phosphorylation and a significant decrease in caspase-3 and NF-κB amount (Nassireslami et al., 2016). This dual effect of sodium arsenite may be observed in spatial learning; however, it has not yet been studied. Additionally, low doses of arsenite undergo enzymatic methylation in the liver to the extent of 80% after oral administration (Vahter, 1981). It is worth mentioning that methylated arsenicals excreted more rapidly than inorganic arsenic in the urine (Rodriguez et al., 2001). Moreover methylated and inorganic arsenite have different effects on neuronal cells. It has been reported that in contrast to arsenite, methylated arsenicals did not cover cell viability of astrocytes (Dringen et al., 2016). So, regarding to the above description and the properties of low dose of sodium arsenite, we aimed at investigating the effects of local and direct intra-hippocampal injection of low and high doses of sodium arsenite on spatial memory.

Furthermore, it has been previously reported that sodium arsenite could induce both autophagic and apoptotic responses (Bolt et al., 2010, 2012; Keim et al., 2012). Autophagy and apoptosis are two major types of programmed cell death that are main mechanisms for cells survival (Salminen et al., 2013). Apoptosis, a process triggering a signaling cascade to definitive cellular death, have been thoroughly investigated and it is established that two protein families are involved in its regulation: B lymphoma 2 (Bcl-2) family members and cysteine-aspartic acid protease (caspase) family (Bolt et al., 2010; D'Amelio et al., 2012; Shin et al., 2011). Autophagy is considered as an evolutionarily cell survival process, which is responsible for degradation of long-lived proteins and removal of dysfunctional organelles (Ding et al., 2013). There are many pathways involved in the process of autophagosomes formation, fusion with lysosomes to form lysosomic vacuoles (i.e. autolysosomes) and their degradation (Yue et al., 2009). Two ubiquitin-like pathways are important in forming autophagic vesicle. The first is covalent conjugation of Atg12 and Atg5 by E1 ligase-like protein Atg7 which is essentially needed for progression of the second pathway that is conjugation of long chain 3 (LC3) protein to a phospholipid molecule leading to expansion of autophagic membrane. This conjugate converts soluble LC3-I to autophagic vesicle-associated LC3-II (Gozuacik and Kimchi, 2007). Measuring the conversion of LC3-I to LC3-II helps to quantify autophagic activity (Mizushima and Yoshimori, 2007).

It seems that autophagy and apoptosis function appositely. While apoptosis is a cellular suicidal program, autophagy can either be a cell survival mechanism as a homeostatic process or a stress-induced cell death pathway depending on variable context (Bolt et al., 2010; Kralova et al., 2012).

Therefore, in search for crosstalk between apoptosis and autophagy pathways and to see how sodium arsenite affects these processes, the levels of Bax, Bcl-2 and caspase-3, as major markers involved in apoptosis, and also LC3, Atg7 and Atg12, as major proteins playing important roles in autophagy, were assessed. The main purpose of our study was a better understanding of the local and direct effects of sodium arsenite on spatial learning and determining its related mechanisms in the brain.

2. Material and methods

2.1. Materials

Antibodies directed against Caspase-3, LC3, Bax, Bcl-2, Atg7, Atg12 and β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). Electrochemiluminescence (ECL) kit was obtained from Amersham Bioscience (Piscataway, NJ, USA). Sodium arsenite and all other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Animals

Male Wistar Rats (220 ± 20 g) were purchased from the animal house of the Tehran University of Medical Sciences. All animals were housed in cages (four/cage) with ad libitum access to food and water. They were kept on a 12 h-light/dark cycle at a constant temperature (20–22 °C). All animal experiments were performed in daylight and according to the guidelines of the Ethical Committee for the Care and Use of Laboratory Animals of Tehran University of Medical Sciences (code: 23516-151-02-92, 2013). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Stereotaxic surgery

Under anesthesia with intraperitoneal injection of 100 mg/kg ketamine and 25 mg/kg xylazine, animals were placed into stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Stereotaxic coordinates used for intra-hippocampal injection according to the atlas of Paxinos and Watson, were as follows: Anterior–posterior (AP), 3.8 mm posterior to the bregma; medial-lateral (ML), ± 2.2 mm lateral to the sagittal suture and dorsal–ventral (DV), 2.7 mm down from the skull surface. Guide cannulas (21-gauge) were inserted bilaterally into the dorsal hippocampus (CA1 region) and were attached to the skull surface using orthopedic cement (synment®). After cannulation, animals were maintained in their cages and handled daily for one week as a recovery period. Microinjection was performed using a 10 μl Hamilton microsyringe and an injection needle (27-gauge) attached to polyethylene tube (PE-10) 30 min before each training session.

2.4. Experimental design

In the present study, animals were assigned into four groups. All animals of these four groups underwent stereotaxic surgery and cannula implantation. Different concentrations of sodium arsenite (5, 10, 100 nM) were infused bilaterally (5 μl/side) via intra-hippocampal cannula. Control group received 5 μl/side normal saline via intra-hippocampal injection. Animals were trained for four consecutive days and all injections were made 30 min before training.

2.5. Behavioral tests

Morris water maze (MWM) includes a black-painted circular pool (136 cm diameter, 60 cm height), divided into four equal quadrants. A platform made of Plexiglas was located in north-west quadrant (target quadrant) and tank was filled with water (25 ± 2 °C) up to 1 cm above surface of platform. After 1 week recovery period of surgery, training was done for 4 consecutive days. One block of 4 trials was conducted on each day. Each trial was started with placing animals in one of starting points (north, east, south and west) of MWM. Animals were let to swim freely for 90 s to find the immersed platform. If a rat did not find the platform, it was manually guided to platform. Animals were allowed to rest 30 s between two trials. Directions of rats were recorded by a video camera linked to a computer, located above MWM center. Spatial learning and memory parameters were evaluated using EthoVision video tracking system (Noldus Information Technology, Wageningen, Netherlands). Escape latency (time to find the hidden platform), traveled distance (path length to reach the hidden platform), and swimming speed were measured for further analyses. Probe test was conducted on day 5 to evaluate time spent in target quadrant and hidden platform's proximities. In probe test, hidden platform was removed; all animals were placed in the same starting point opposite of target quadrant and allowed to swim for 90 s. The visible test was