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INORGANIC COMPOUNDS

Effects of arsenic exposure on D-serine metabolism in the hippocampus of offspring mice at different developmental stages

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Abstract
The main purpose of this study was to verify the hypothesis that cognitive dysfunctions induced by arsenic exposure were related to the changes of D-serine metabolism in the hippocampus of offspring mice. Mother mice and their offsprings were exposed to 0, 15, 30 or 60 mg/L sodium arsenite ($NaAsO_2$) through drinking water from the first day of gestation until the end of lactation. D-serine levels in the hippocampus of mice of postnatal day (PND) 10, 20 and 40 were examined by high-performance liquid chromatography. Expressions of serine racemase (SR), D-amino acid oxidase (DAAO), alanine–serine–cysteine transporter-1 (asc-1) and subunits of *N*-methyl-D-aspartate receptors (NMDARs) in the hippocampus of mice were measured by Western blot and Real-time RT-PCR. Results showed that arsenic exposure significantly decreased D-serine levels of mice exposed to 60 mg/L $NaAsO_2$. Exposure to 60 mg/L $NaAsO_2$ could inhibit both mRNA and protein expression of SR, whereas increase in the protein expression of DAAO, only enhances the mRNA levels of DAAO of PND 20 mice. In addition, arsenic exposure could upregulate protein expression of asc-1. The mRNA and protein levels of NR1, NR2A and NR2B in the hippocampus of mice were down-regulated by arsenic. Findings from this study suggested that SR might play an important role in the reduction of D-serine levels caused by arsenic exposure, which might further influence the levels of NMDAR subunits especially on PND20, and then might disturb the function of NMDARs and cause the deficits of learning and memory ability of offspring mice.

Keywords Arsenic · D-serine metabolism · NMDARs · Learning and memory · Hippocampus

Introduction

Arsenic poisoning due to contaminated water affects millions of people worldwide (Naujokas et al. 2013). It is well known that chronic exposure to inorganic arsenic is related to a greater risk of diseases, such as vascular diseases, skin lesion and neurotoxicity (Argos et al. 2011; Newman et al. 2016; Su et al. 2019). Epidemiological data of the cross-sectional studies disclosed an association between chronic consumption of arsenic-contaminated water and cognitive deficits in school-aged children from different parts of the world (Tsai et al. 2003; Rosado et al. 2007; Wasserman et al. 2014, 2018). These findings raised concern over neurotoxicity induced by low arsenic levels in drinking water. However, the cellular and molecular mechanisms underlying arsenic-induced neurotoxicity remain poorly understood.

To date, long-term potentiation (LTP) is thought to be the important neurobiological basis and a synaptic model of memory (Bliss and Collingridge 1993; Yasuda et al. 2003). It has been accepted that calcium influx into postsynaptic neurons via *N*-methyl-D-aspartate receptors (NMDARs), are essential for LTP, as well as learning and memory (Schotanus and Chergui 2008; Rebola et al. 2010; Cercato et al. 2014). Upon stimulation, NMDARs activate multiple biochemical pathways that transduce signals into the postsynaptic neurons (Sheng and Kim 2002). In addition to glutamate, NMDARs require the binding of a coagonist (glycine or D-serine) for channel opening (Traynelis et al. 2010).

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D-serine is present in the brain at a high concentration of up to one-third that of L-serine, and D-serine is heterogeneously distributed in the brain with a pattern resembling that of NMDARs (Schell et al. 1995). Several studies demonstrated that endogenous D-serine is the dominant coagonist for NMDAR-dependent processes ranging from LTP, synaptic transmission and neurotoxicity (Shleper et al. 2005; Panatier et al. 2006; Henneberger et al. 2010; Wolosker 2011). Data in our previous study showed that exposure to arsenic could affect the levels of D-serine (Wang et al. 2012). These results suggested a possible involvement of D-serine to the impaired cognitive functions induced by arsenic exposure.

D-serine is synthesized endogenously in the mammalian brain through the conversion of L- to D-serine by the enzyme serine racemase (SR) (Wolosker et al. 1999). The change of SR levels is the premise and basis of the dysfunction of D-serine (Wolosker 2011; Ishiwata et al. 2015). On the other hand, D-serine is degraded in the brain by D-amino acid oxidase (DAAO) (Sasabe et al. 2012). Data disclosed that LTP mediated by NMDARs increased obviously in the hippocampus of the mutant mice lacking DAAO (Ohide et al. 2011). Furthermore, studies also demonstrated that the extracellular D-serine levels are regulated by two types of transporters, the Na^+-dependent and Na^+-independent alanine–serine–cysteine transporter, such as ASCT1, ASCT2 and alanine–serine–cysteine transporter-1 (asc-1) (Ribeiro et al. 2002; Rosenberg et al. 2013; Wang et al. 2017). ASCT1 and ASCT2 are widely expressed, but exhibit low affinity for D-serine (Ribeiro et al. 2002). Conversely, asc-1 is restricted to neurons and displays high affinity for D-serine (Helboe et al. 2003). Furthermore, data exhibited that acute asc-1 inhibition decreased the tonic release of D-serine and asc-1 activity is required for optimal NMDAR activation and synaptic plasticity (Sason et al. 2017).

Taken together, D-serine is very important in the activation of NMDAR and LTP and D-serine level is mainly affected by SR, DAAO and asc-1. Until now, few studies have focused on the effects of arsenic exposure on D-serine metabolism in the developmental brain. The present study aimed to verify the hypothesis that cognitive dysfunctions induced by arsenic exposure were related to the changes of D-serine metabolism in the hippocampus of mice at the early life.

Materials and methods

Animals

Albino mice, weighing 25 ± 2 g, were obtained from the animal laboratory of China Medical University. Animal room was kept at a temperature of 20 ± 2 °C with a 12-h light/dark cycle and a relative humidity of 50–60%. Free access to food and water was allowed at all the time. Mice were housed in the sterilized plastic cages with wood shaving bedding. This study protocol has been approved by the Scientific Research Committee of China Medical University and was conducted in accordance with the Chinese National Guidelines for the protection of laboratory animal in animal experiments.

Experimental procedures

After 1-week adaptation, female mice were mated with healthy male mice. Gestation was determined by checking vaginal plug twice daily. Conception was estimated by vaginal plug. Experimental procedures are shown in Fig. 1. Twenty-four pregnant mice were randomly divided into four groups, six mice in each group. Pregnant mice were fed separately (one per cage) and exposed to 0, 15, 30 or 60 mg/L sodium arsenite ($NaAsO_2$, Invitrogen, USA, dissolved in distilled water, and newly made every 24 h) through drinking water from the first day of gestation until the end of lactation. The day of birth was designated as postnatal day (PND) 1. On PND 21, pups were weaned and housed in a colony room, and permitted free access to food and drinking water with $NaAsO_2$. Pregnant mice and their offspring in control drank distilled water. $NaAsO_2$ addition did not affect the consumption of drinking water compared to the control. Mice taken one per litter were decapitated under deep ethylether on PND 10, 20 and 40. The hippocampal tissues were dissected rapidly, and stored at − 80 °C for further analysis.

Reagents and laboratory wares

All glasses and plastic wares were washed with detergent and nitric acid, and rinsed with redistilled water. Water used in present study was doubly distilled. All reagents used are of analytical grade and methanol was of chromatographic grade for D-serine analysis.

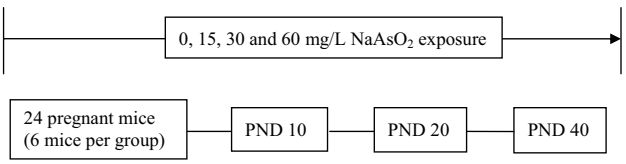

Fig. 1 The experimental process. *PND* postnatal day. Twenty-four pregnant mice were divided into four groups and given 0, 15, 30 and 60 mg/L of $NaAsO_2$. Feed pregnant mice separately (a cage to raise a mouse) from the first day of gestation. The mice born in each group were subdivided into three groups, PND 10, PND 20 and PND40, and six mice from different litter in each group at different developmental stages were sacrificed for experimental analysis

Morris water maze

Method reported by Yu et al. (2014) was followed and modified. PND 40 mice were used to detect the effects of arsenic exposure on the spatial learning and memory ability by Morris water maze. Briefly, a circular tank (120 cm diameter × 50 cm height) with surrounding black-painted walls, was filled with water (24 ± 1 °C) to a depth of 40 cm and the water was made opaque by milk powder to prevent visualization of the platform (10 cm in diameter), which was submerged 2 cm below the water surface. The pool was divided into four quadrants. At beginning, the mice were individually placed into the pool facing the wall. Each mouse was randomly started from a different position on each trial to find the hidden escape platform that remained in the middle of the same quadrant throughout training. If the mouse failed to reach the platform in 90 s, it was gently guided to the platform. Once on the platform, the mouse was left there for 15 s. Escape latencies from the three quadrants were recorded as their final performances. After 5 consecutive days of place trial, the mice were given one probe trial of 90 s for which the platform was removed from the pool to assess short-term memory. All trails were recorded on a video and analyzed using tracking system (Stoelting Co., USA).

High-performance liquid chromatography

The hippocampal tissues of mice were individually weighed and homogenized in ice-cold sodium chloride (1:9 w/v) and centrifuged at 5000×*g* for 25 min at 4 °C. The supernatants were filtered using a 0.22 μm filter, and stored at − 40 °C until ready for analysis. The standard of D-serine was purchased from Sigma Company (USA).

Levels of D-serine in the hippocampal tissues of mice were measured by high-performance liquid chromatography (Waters Corporation, Milford, MA, USA). Briefly, pre-column derivatization with o-phthaladehyde was used. Elution was carried out at room temperature with a Waters C18 column (4.6 × 150 mm, 5 μm) and a mobile phase of 0.1 M potassium acetate (pH 5.89)-methanol at a flow rate of 1 mL/min. Fluorescence detector conditions were excitation 250 nm with detection at emission 410 nm.

Quantitative real-time RT-PCR

Method described by Yu et al. (2014) was followed. Total RNA was extracted from the hippocampal tissues of mice using Trizol Reagent (Invitrogen, CA, USA). First strand of cDNA was synthesized from the total RNA using the PrimeScript RT reagent Kit (Takara, Tokyo, Japan) and the random primers of SR, DAAO, asc-1, NR1, NR2A, NR2B and GAPDH (as the house-keeping gene). Thereafter, the cDNA was served as templates for real-time PCR amplification using the SYBR Premix Ex Taq II (Takara, Tokyo, Japan) and ABI 7500 real-time PCR System (Applied Biosystems, CA, USA). To amplify a fragment of SR, DAAO, asc-1, NR1, NR2A, NR2B and GAPDH, the following primer pairs detailed in Table 1, were used. Amplification was conducted for 40 cycles of 5 s at 95 °C and 34 s at 60 °C. Results were analyzed using the comparative Ct method. RNA abundance were expressed as $2^{-\Delta\Delta Ct}$ for the target mRNA relative to those of the GAPDH gene (as the internal control), and presented as fold change vs contralateral control samples.

Western bolt analysis

The hippocampal tissues of mice were homogenized, and then the lysates were centrifuged at 4 °C, 12,000×*g* for 20 min. Protein concentrations were measured with a BCA protein assay kit (Pierce, Rockford, IL, USA). Fifty or thirty micrograms of total protein were resolved by 10% or 7%

Table 1 The sequence of primer pairs for PCR analysis

Gene	Primer sequences (5′–3′)	Length (bp)
SR	Sense: GTAGGAGGAGGAGGAATGGTT Antisense: TTCGGTGACAGTGAAGACATC	243
DAAO	Sense: GGTGGCAAGAGGAGTGGAT Antisense: GATGATGTACGGAGAGTTGTAGATA	181
asc-1	Sense: ACCAATGCCTTCGCCTTCT Antisense: GCTCCTCCGTGACATAGTTGA	115
NR1	Sense: CACAGAAGTGCGATCTGGTGAC Antisense: GGCATTGCTGCGGGAGT	191
NR2A	Sense: CTCTGATAATCCTTTCCTCCAC Antisense: GACCGAAGATAGCTGTCATTTACT	123
NR2B	Sense: TCCATCAGCAGAGGTATCTACAG Antisense: CCGTTGACTCCAGACAGGTT	161
GAPDH	Sense: CAATGTGTCCGTCGTGGATCT Antisense: GTCCTCAGTGTAGCCCAAGATG	124

sodium dodecyl sulphate polyacrylamide gel electrophoresis and blotted onto the polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA), and subsequently probed with rabbit antibodies against SR (1:200), DAAO (1:100), asc-1 (1:100) and β-actin (1:2000) or goat antibodies against NR1 (1:500), NR2A (1:500) and NR2B (1:500) (Santa Cruz Biotech, Santa Cruz, CA, USA) at 4 °C overnight. Expression of specific protein was detected by 1:5000 horseradish peroxidase-labeled secondary antibody. Intensity of each band was assessed semi-quantitatively by densitometry using an image analyzing software (Gel-Pro analyzer v4.0), and normalized by the intensity of β-actin. Six separate experiments for each group were examined.

Statistical analysis

SPSS for Windows, version 22.0 (IBM, USA) was used for statistical analysis. Results were expressed as mean ± SD. Mean differences among groups were assessed by one-way ANOVA followed by Student–Newman–Keuls test (SNK) for multiple comparison. Statistical significance was defined as $P < 0.05$.

Results

Effects of arsenic exposure on mice body weights and brain weights

The body and brain weights of mice in control and $NaAsO_2$-exposed groups are shown in Table 2. Body weights of PND 20 mice in 60 mg/L $NaAsO_2$-exposed group decreased significantly than those of the control and 15 mg/L $NaAsO_2$-exposed group. Body and brain weights of PND 40 mice in 60 mg/L $NaAsO_2$-exposed group were significantly lower than those in control and other $NaAsO_2$-exposed groups. There were no differences of the body and brain weights of PND 10 mice among groups.

Effects of arsenic exposure on learning and memory ability in PND 40 mice

The effect of arsenic exposure on spatial learning in PND 40 mice is shown in Fig. 2. The results showed that there was no difference in the escape latency among groups on the first 3 days of training. On the fourth day of training, the escape latency in 60 mg/L $NaAsO_2$-exposed group was significantly longer than that in the control. On the fifth day of training, compared with the control, the escape latency in $NaAsO_2$ exposed groups were significantly longer.

The effect of arsenic exposure on spatial memory in PND 40 mice is shown in Fig. 3. In the probe trail, the time spent in target quadrant (platform existed previously) in 60 mg/L $NaAsO_2$-exposed group was significantly shorter than that in the control.

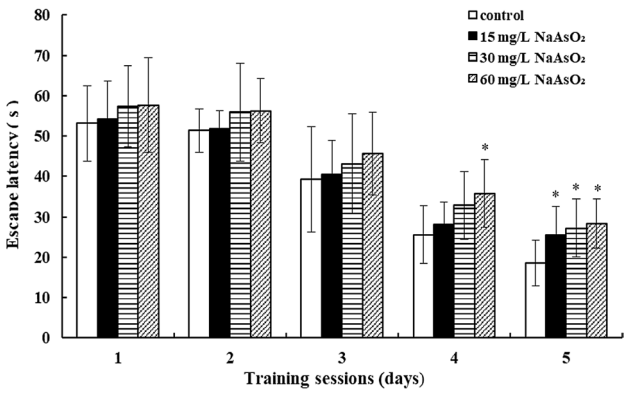

Fig. 2 Comparison of escape latency among groups in PND 40 mice. *PND* postnatal day. The place trail in Morris water maze was used to test the spatial learning ability in PND 40 mice. A 5-consecutive day training program was performed. The escape latency for each mouse was automatically recorded by the computer. Data were represented as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control

Table 2 Effects of arsenic exposure on mice body weights and brain weights (mean ± SD, g)

Group	PND 10		PND 20		PND 40	
	Body weights	Brain weights	Body weights	Brain weights	Body weights	Brain weights
Control	6.69 ± 0.96	0.31 ± 0.01	14.56 ± 1.66	0.40 ± 0.01	34.44 ± 2.54	0.47 ± 0.01
15 mg/L $NaAsO_2$	6.44 ± 0.98	0.31 ± 0.02	14.00 ± 1.98	0.40 ± 0.02	32.81 ± 1.83	0.45 ± 0.02
30 mg/L $NaAsO_2$	6.25 ± 0.89	0.30 ± 0.02	13.25 ± 1.22	0.39 ± 0.02	31.19 ± 2.24*	0.45 ± 0.02
60 mg/L $NaAsO_2$	5.50 ± 0.85	0.30 ± 0.02	11.94 ± 1.35*,#	0.39 ± 0.02	28.25 ± 1.87*#,△	0.41 ± 0.02*#,△

Data were represented as mean ± SD, $n = 6$

PND postnatal day

Significant difference was defined as $P < 0.05$, and, *compared with the control; #compared with 15 mg/L $NaAsO_2$ exposed group; △compared with 30 mg/L $NaAsO_2$-exposed group

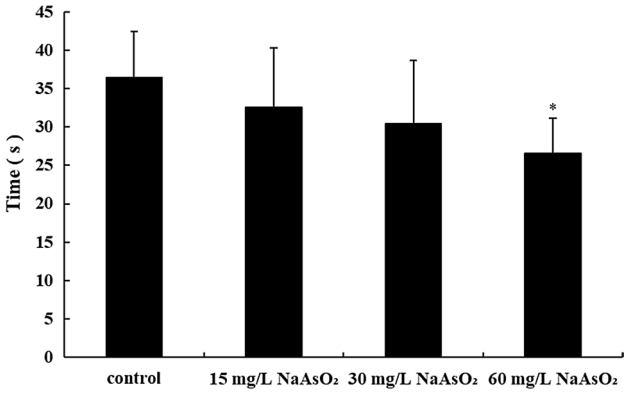

Fig. 3 Comparison of time spent in target quadrant (platform existed previously) among groups. Mice were given one probe trial after the place trail. Data were represented as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control

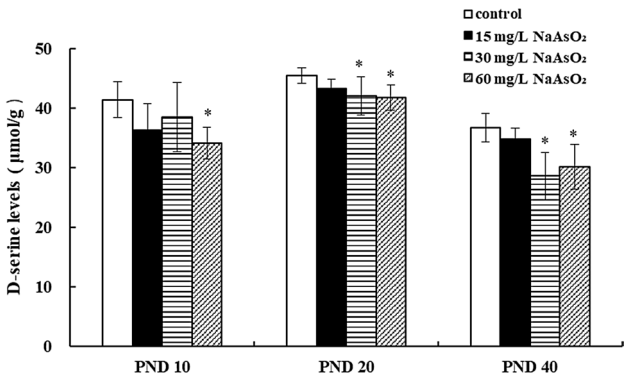

Fig. 4 Comparison of D-serine levels among groups at different developmental stages. *PND* postnatal day. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out and processed for measurement of D-serine levels via high performance liquid chromatography analysis. Data were represented as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control

Effects of arsenic exposure on D-serine levels at early developmental stages

Effects of arsenic exposure on D-serine levels at early developmental stages are shown in Fig. 4. D-serine levels of PND 10 mice in 60 mg/L $NaAsO_2$-exposed group were lower than those in the control. Furthermore, those of PND 20 and 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were also significantly lower than those in the control.

Effects of arsenic exposure on SR, DAAO and asc-1 levels at early developmental stages

Changes of arsenic exposure on expression of SR in the hippocampus during different developmental stages are shown in Fig. 5. Levels of SR protein of PND 10 mice in 60 mg/L $NaAsO_2$-exposed group were significantly lower than those in control and 15 mg/L $NaAsO_2$-exposed group. Those of PND 20 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were also significantly lower than those in control. In addition, those of PND 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups decreased significantly than those in control and 15 mg/L $NaAsO_2$ exposed groups. Levels of SR mRNA of PND 20 mice in 60 mg/L $NaAsO_2$-exposed group and those of PND 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups decreased significantly than those in the control. The differences of SR mRNA levels of PND 10 mice among groups failed to show any significance.

Alterations of DAAO expression in the hippocampus of mice affected by arsenic exposure during different developmental stages are shown in Fig. 6. Levels of DAAO protein of PND 10 and 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly higher than those in control. Otherwise, levels of DAAO mRNA and protein of PND 20 mice in 60 mg/L $NaAsO_2$-exposed group were significantly higher than those in the control. However, the differences of DAAO mRNA levels of PND 10 and 40 mice among groups were not significant.

Effects of arsenic exposure on asc-1 expression in the hippocampus of mice during different developmental stages are shown in Fig. 7. Levels of asc-1 protein of PND 10 mice in the $NaAsO_2$-exposed groups increased significantly than those in control. The differences of asc-1 protein levels of PND 20 and 40 mice among groups were not significant. Conversely, levels of asc-1 mRNA did not show any significant difference among groups during different developmental stages.

Effects of arsenic exposure on NMDAR subunits at early developmental stages

Changes of NR1, NR2A and NR2B expression in the hippocampus of mice induced by arsenic exposure at the early developmental stages are shown in Figs. 8, 9 and 10. As shown in Fig. 8, levels of NR1 protein of PND 10 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly lower than those in control, and those in 60 mg/L $NaAsO_2$ exposed group were significantly lower than those in 15 mg/L $NaAsO_2$ exposed group. Those of PND 20 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups decreased significantly than those in control and 15 mg/L $NaAsO_2$-exposed groups. However, the differences of NR1 protein levels of PND 40 mice among groups failed to show any significance. Levels of NR1 mRNA of PND 20 mice in $NaAsO_2$-exposed groups decreased significantly compared to the control. Otherwise, those of PND 40 mice in 60 mg/L $NaAsO_2$-exposed group were significantly lower than those in control. The

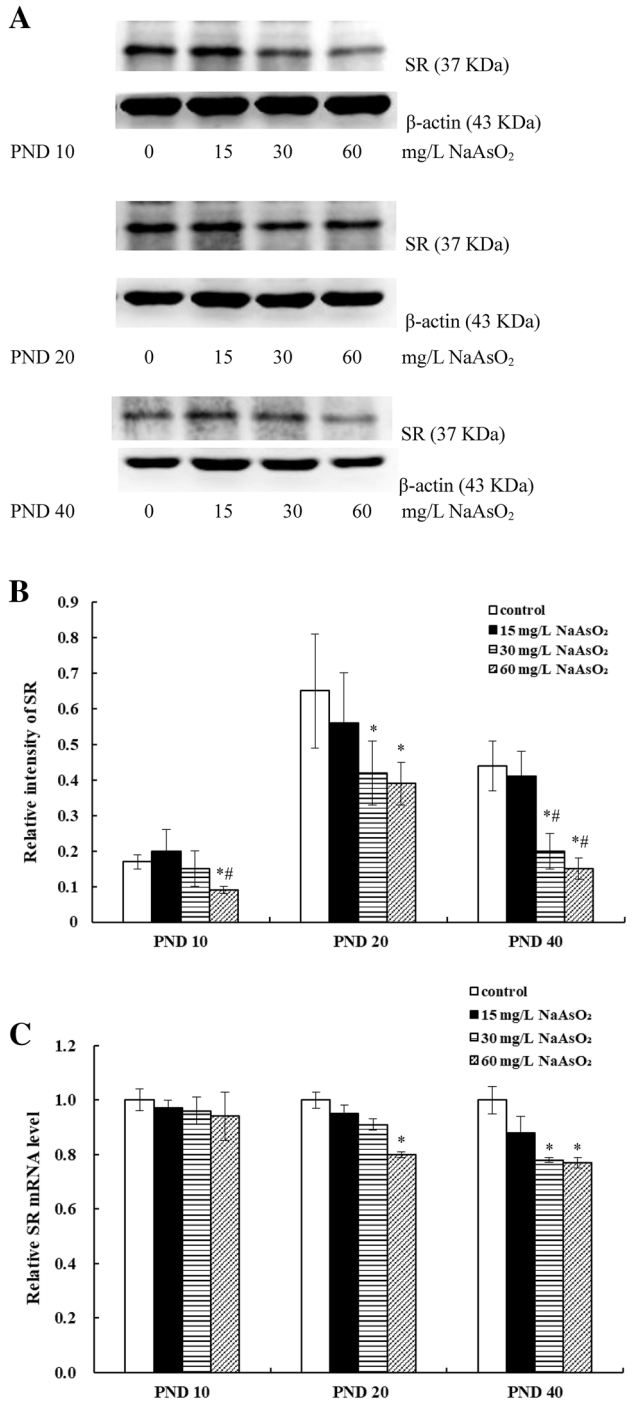

Fig. 5 Effects of arsenic exposure on SR expression at the early developmental stages. *SR* serine racemase. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (50 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly, they were transferred to PVDF membranes and immunoblotted for SR. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control; #compared with 15 mg/L $NaAsO_2$ exposed group

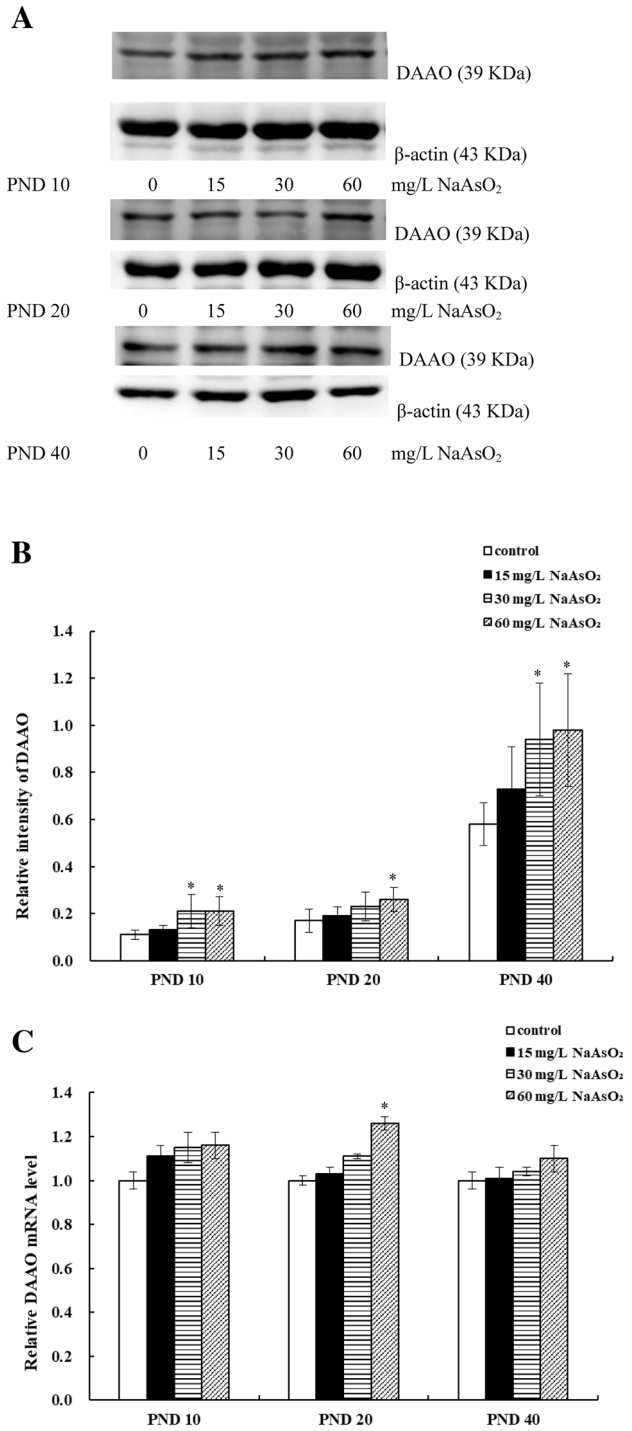

Fig. 6 Effects of arsenic exposure on DAAO expression at the early developmental stages. *DAAO* D-amino acid oxidase. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (50 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly they were transferred to PVDF membranes and immunoblotted for DAAO. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control

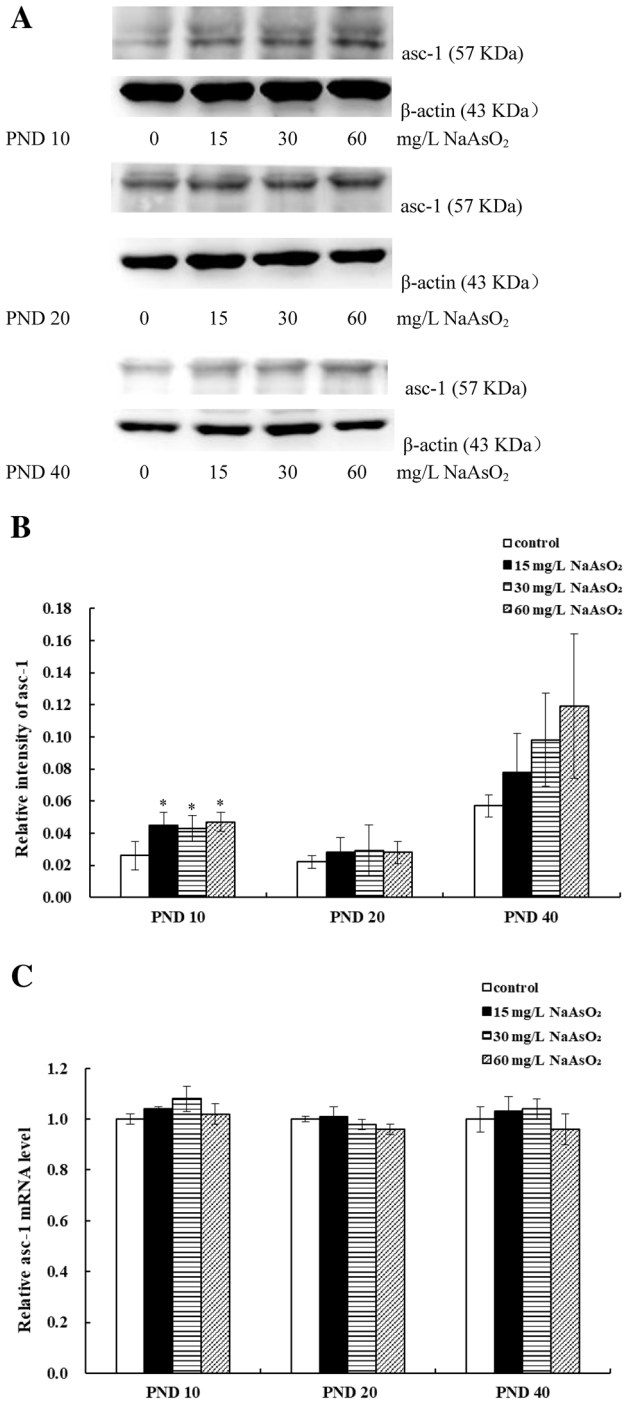

Fig. 7 Effects of arsenic exposure on asc-1 expression at the early developmental stages. *asc-1* alanine-serine-cysteine transporter-1. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (50 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly they were transferred to PVDF membranes and immunoblotted for asc-1. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control

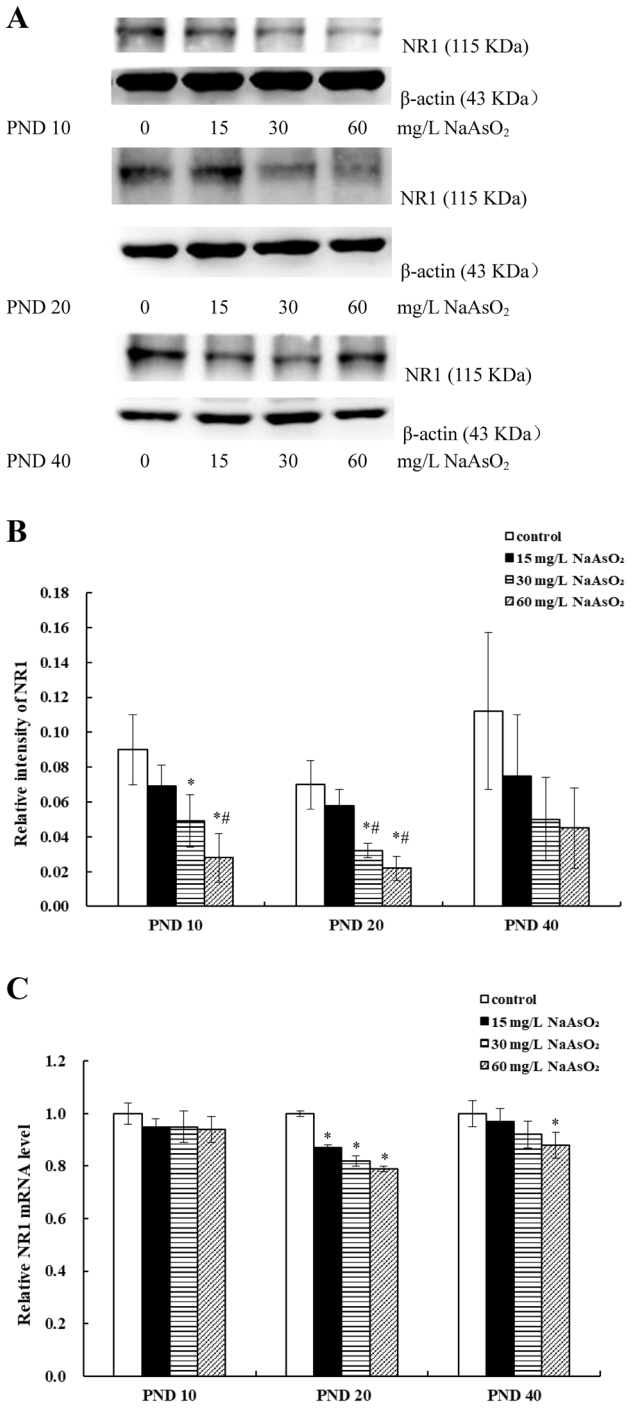

Fig. 8 Effects of arsenic exposure on NR1 expression at the early developmental stages. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (30 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly they were transferred to PVDF membranes and immunoblotted for NR1. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control; #compared with 15 mg/L $NaAsO_2$ exposed group

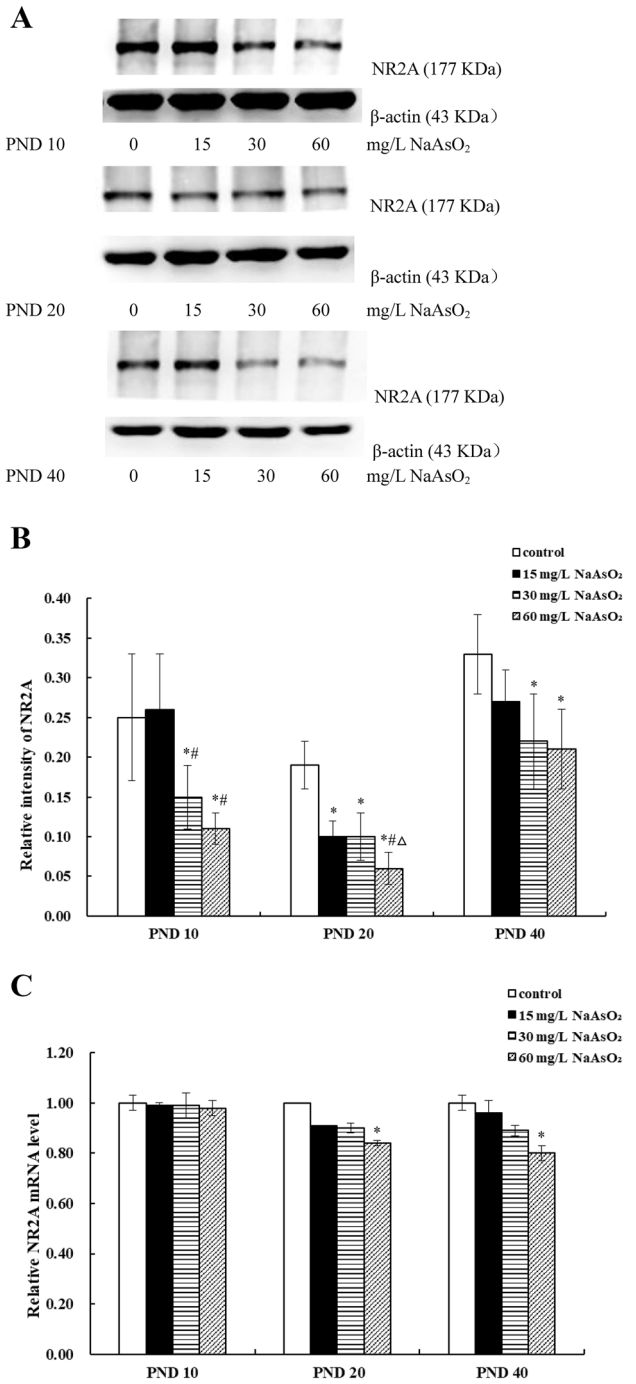

Fig. 9 Effects of arsenic exposure on NR2A expression at the early developmental stages. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (30 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly they were transferred to PVDF membranes and immunoblotted for NR2A. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n = 6$. Significant difference was defined as $P < 0.05$, and, *compared with the control; #compared with 15 mg/L $NaAsO_2$ exposed group; △compared with 30 mg/L $NaAsO_2$ exposed group

differences of NR1 mRNA levels of PND 10 mice among groups were not significant.

As shown in Fig. 9, levels of NR2A protein of PND 10 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly lower than those in control and 15 mg/L $NaAsO_2$-exposed groups. Otherwise, those of PND 20 mice in $NaAsO_2$-exposed groups decreased significantly than those in control, and those in 60 mg/L $NaAsO_2$-exposed group were significantly lower than those in 15 and 30 mg/L $NaAsO_2$-exposed groups. The levels of NR2A protein of PND 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly lower than those in control. In addition, levels of NR2A mRNA of PND 20 and 40 mice in 60 mg/L $NaAsO_2$-exposed groups decreased significantly compared to the control. However, the differences of NR2A mRNA levels of PND 10 mice among groups were not significant.

As shown in Fig. 10, levels of NR2B protein of PND 10 and 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly lower than those in control and 15 mg/L $NaAsO_2$-exposed group, of which the significant difference also existed between 30 and 60 mg/L $NaAsO_2$-exposed groups. Furthermore, those of PND 20 mice in $NaAsO_2$-exposed groups decreased significantly than those in the control, and those in 30 and 60 mg/L $NaAsO_2$-exposed groups were significantly lower than those in 15 mg/L $NaAsO_2$-exposed group. Levels of NR2B mRNA of PND 20 mice in $NaAsO_2$-exposed groups were significantly lower than those in control, but they did not differ significantly among the $NaAsO_2$ exposed groups. Those of PND 40 mice in 60 mg/L $NaAsO_2$ exposed group decreased significantly than those in the control. Conversely, those of PND 10 mice did not differ significantly among groups.

Discussion

Morris water test was used to examine the spatial learning and memory ability of PND 40 mice in this study to confirm the cognitive injury of the offspring mice induced by arsenic exposure at the early developmental stages. In the place trail, the escape latency of mice in $NaAsO_2$-exposed groups were significantly longer than that in control on the fifth day of training. In addition, in the probe trail, the time spent in target quadrant of mice in 60 mg/L $NaAsO_2$-exposed group was significantly shorter than that in control. Results of this study showed that arsenic exposure could lead to deficits in both spatial learning and memory ability of mice.

NMDARs are the key receptors in learning and memory, and D-serine as the endogenous coagonist of NMDARs, contribute to the activation of postsynaptic NMDARs (Oliet and Mothet 2009; Guercio and Panizzutti 2018). Results reported by our previous study (Wang et al. 2012) showed that arsenic exposure may affect glutamate-induced D-serine release from

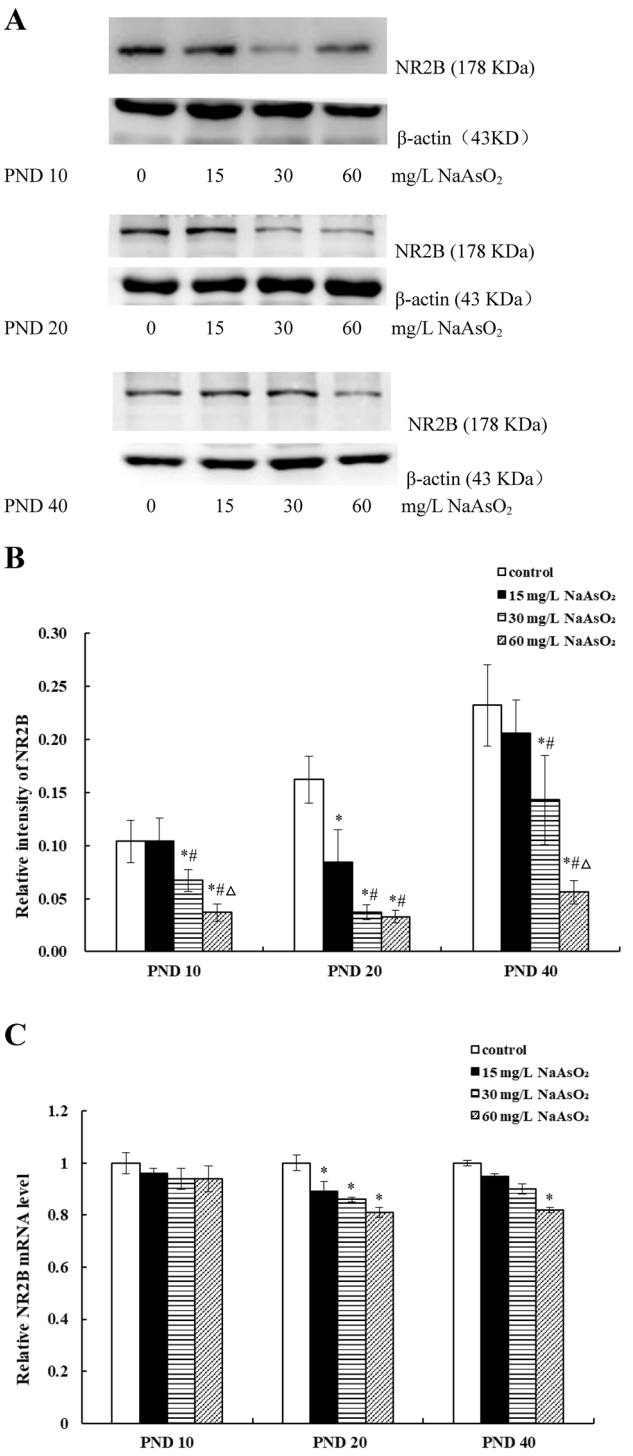

Fig. 10 Effects of arsenic exposure on NR2B expression at the early developmental stages. Mice were sacrificed on PND 10, 20 and 40, and their hippocampal tissues were immediately dissected out. Total proteins (30 μg/lane) in the hippocampus were collected and separated by SDS-PAGE; lastly, they were transferred to PVDF membranes and immunoblotted for NR2B. **a** Western blot analysis. Images were the representative results of six separate experiments for each group. **b** Densitometric analysis of Western blots. The relative intensity in arbitrary units compared to β-actin. **c** Quantitation of mRNA by Real-time RT-PCR. Gene expression were normalized to GAPDH and presented as fold change vs the control. Data were given as mean ± SD, $n=6$. Significant difference was defined as $P<0.05$, and, *compared with the control; #compared with 15 mg/L $NaAsO_2$-exposed group; △compared with 30 mg/L $NaAsO_2$-exposed group

astrocytes. Study demonstrated by Lin et al. (2016) disclosed that D-serine are associated with NMDARs in postsynaptic neurons and with glutamatergic synapse stability during synaptic development. Findings from this study showed that D-serine levels of mice exposed to 60 mg/L $NaAsO_2$ were lower than those in control at different developmental stages, suggesting that D-serine levels in the hippocampus reduced after arsenic exposure at the early stages of development. Therefore, the changes of D-serine levels might be related to the learning and memory impairment induced by arsenic exposure.

D-serine is synthesized by SR and degraded by DAAO in various brain regions (Wolosker 2011; Billard 2012; Van Horn et al. 2013; Coyle and Balu 2018; Liraz-Zaltsman et al. 2018; Choi et al. 2019). D-serine levels decrease by more than 85% in the brain of SR knock-out mice (Basu et al. 2009). The genetic deletion of SR impairs the connectivity and the functional plasticity of neuronal networks and has been related to cognitive impairments (Basu et al. 2009; Inoue et al. 2008, 2018). Hopkins et al. (2013) revealed that DAAO inhibition might increase NMDAR activity by regulating D-serine concentrations and further enhance NMDAR-related synaptic plasticity during phases of post-training memory consolidation to improve memory performance in hippocampal dependent behavioral tests. Data reported by Maucler et al. (2013) suggested that D-serine heteroexchange through ASC transporters is present in vivo and may constitute a key component in the regulation of D-serine extracellular concentration. Results of the present study demonstrated that exposure to 60 mg/L $NaAsO_2$ in the water could inhibit both mRNA and protein expression of SR, whereas increase the protein expression of DAAO, moreover, only enhance the mRNA levels of DAAO of PND 20 mice exposed to $NaAsO_2$. In addition, our results disclosed that arsenic exposure could only upregulate protein expression of asc-1 of PND 10 mice. As can be seen from the above, SR expressions were more sensitive to arsenic exposure not only at the transcriptional level, but also at the translational level, which might play an important role in the decrease of D-serine level caused by arsenic exposure. We will try to explore the exact mechanism in the following studies.

The most widely expressed NMDARs contain the obligate subunit NR1 and one of the NR2(A–D) and/or NR3 (Paoletti and Neyton 2007; Papadia and Hardingham 2007). NR1 is thought to form the ion channel and NR2 modulates channel activities (Stephenson et al. 2008). Studies reported by Luo et al. (2009, 2012) disclosed that

chronic exposure to arsenic could reduce gene and protein expression of NR2A in hippocampus, and impair the spatial learning ability of rats. Results of this study showed that NR1 and NR2B mRNA levels of PND 20 mice in $NaAsO_2$-exposed groups decreased significantly. On the other hand, those of PND 40 mice exposed to 60 mg/L $NaAsO_2$ were also significantly lower. Furthermore, NR2A mRNA levels of PND 20 and 40 mice exposed to 60 mg/L $NaAsO_2$ decreased significantly. These data indicated that different arsenic concentrations have differential effects on NMDAR subunits at the transcriptional level, which was more obvious in the hippocampus of PND 20 mice. Findings from this study showed that NR1 protein levels of PND 10 and 20 mice exposed to 30 and 60 mg/L $NaAsO_2$ were significantly lower. NR2A and NR2B protein levels of PND 20 mice exposed to $NaAsO_2$ decreased significantly, and those of PND 10 and 40 mice in 30 and 60 mg/L $NaAsO_2$-exposed groups were also significantly lower. Hence, our results were consistent with their results. Results of this study proposed that NR2A and NR2B in NMDARs might be the sensitive target site affected by arsenic at the translational level especially in PND 20 mice. Interestingly, all the mRNA levels of NMDAR subunits of PND 10 mice were not affected by arsenic exposure, while the intensity levels of these markers were affected. Levels of NMDAR subunits change during brain development, which are more abundant during the second to third week of postnatal development as neurons mature and become enriched at extrasynaptic sites (Roullet et al. 2010; Qiu et al. 2011). This study showed that gestational and postnatal exposure down-regulated NMDAR subunits especially on PND20 when these subunits are more predominant. Arsenic exposure might have no obvious effect on NMDAR subunits of PND 10 mice at the transcription level. However, changes of NMDAR subunits protein levels of PND 10 mice might be not affected by gene levels of these markers.

Conclusion

Results from this study showed that SR levels were more sensitive to arsenic exposure than the levels of DAAO and asc-1 not only at the transcriptional level, but also at the translational level, suggesting that SR might play an important role in the reduction of D-serine level caused by arsenic exposure. Furthermore, the abnormal of D-serine metabolism might further influence the gene and protein expression of NMDAR subunits especially on PND20, and then might disturb the function of NMDARs and result in the deficits of learning and memory ability of offspring mice. However, the exact underlying mechanisms need further research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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