Subchronic Early Life Arsenic Exposure at Low Doses Impaired the Biogenic Amine Neurotransmitter and Nitric Oxide Levels in Different Brain Regions of Rats

Lalit P Chandravanshi1,2* and Devendra K Patel3

1Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, 221005, India
2Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research, Post Box No. 80, MG Marg, Lucknow, 226 001, India
3Analytical Chemistry Division, CSIR-Indian Institute of Toxicology Research, Post Box No. 80, MG Marg, Lucknow, 226 001, India

Abstract

Current approaches to risk assessment assume the neurotoxic response for arsenic at the low doses while the mechanism of arsenic-induced developmental neurotoxicity is unknown. Because of this, our previous studies point out that arsenic-induced cholinergic and dopaminergic dysfunctions with associated behavior and also observed the persistent effects were more pronounced in dopaminergic system in early life exposed rats. In continuation of previous studies, rats were exposed to arsenic in drinking water at low doses (2 or 4 mg/kg) during early life. Here, we investigated that alteration in the levels of biogenic amines and their metabolites (norepinephrine (NE), epinephrine (EPN), dopamine (DA), 3, 4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT) by using reversed phase high performance liquid chromatography in different brain regions of arsenic exposed rats on PD60 as compared to controls and furthermore determine persistent effect of arsenic after withdrawal of exposure. Levels of nitric oxide (NO) were also declines in brain regions of arsenic-treated rats in different brain regions of early life exposed rats. Arsenic has been found to modulate the monoaminergic and nitricergic systems during the critical periods of brain development; however, these modifications get more compromised in corpus striatum than frontal cortex and hippocampus even after withdrawal of exposure on PD90 as compared to controls. Modification at the level of biogenic amines and NO in certain brain regions would provide opportunities for the development of therapeutics tools for minimizing developmental neurotoxicity.

Keywords: Biogenic amines; Nitric oxide; Early life; Corpus striatum; High-performance liquid chromatography

Introduction

Arsenic is a heavy metalloid that can exist in biological systems in many different inorganic and organic compounds with several oxidation states, the most common being the trivalent and pentavalent forms [1]. The majority of the epidemiological studies of arsenic are associated with exposure to inorganic arsenic compounds through drinking water. Pentavalent arsenic has been reported in the surface water and trivalent arsenic in the ground water, which suggests this, is the most common reason for the humans generally being exposed to trivalent arsenic [2]. Arsenic is a major global health alarm due to its wide distribution and adverse health effects on the human body. The people of the large portions of the world are being exposed to arsenic through drinking water or ingestion of food materials and causing serious health effects involving the dermal, gastrointestinal, nervous, hepatic, renal, cardiovascular, respiratory, hematopoietic and ophthalmic systems [3-8]. In contrast to other physiological systems of the body, the nervous system appears to be the most vulnerable and the main target for arsenic-induced neurotoxicity [9]. Central nervous system and the peripheral nervous system both are commonly affected by the arsenic exposure [10], however, the effects of arsenic exposure on the peripheral nervous system are more prominent in adults while the arsenic significantly affects the central nervous system during the crucial phase of brain development.

It has been proved that arsenic can easily cross the placental barrier to the fetus [11] and even moderate exposure to arsenic during pregnancy and early life is linked with various adverse health outcomes, including neurodevelopmental problems in the fetus [12,13] and children's [14,15]. Further, the various human population-based studies found that arsenic concentrations in urine were inversely associated with verbal IQ, performance IQ and processing speed problems in children's [16-20]. Exposure occurred during gestation is associated with adverse effects on the fetus including premature and low birth weight of infants, spontaneous abortion [21,22], increased risk of birth defects [23] and reduced fetal size even at low to moderate levels of arsenic.

The accumulation property of arsenic in the brain is the prime reason for its prolonged persistence in the brain. The presence of arsenic in the brain causes various dysfunctions of the nervous system through several mechanisms and alters the development of the nervous system. Oxidative stress destroyed the balance between pro-oxidant and antioxidant system and it has been one of the main mechanisms in the neurotoxicity and also a loss of brain functions. In our previous studies on early life exposure to arsenic at different concentrations, we reported a behavioural cholinergic and dopaminergic modification with biochemical alterations, such as apoptosis, oxidative stress and antioxidant enzymatic activity of brain tissue [24,25]. Exposure to arsenic in vitro experiments on Murine neuralblasta cell line caused the enhancement of the reactive oxygen species leading to neuronal cell apoptosis through JNK/ERK-mediated mitochondria-dependent and...
GRP 78/CHOP-regulated pathways [26]. Moreover, some studies have also confirmed the neuronal apoptosis contributing to the development of many human diseases including neurodegenerative diseases [27,28].

A lot of information regarding the disturbance on biogenic amines in adults by arsenic at high doses has been reported [29-31], while the information about the effect of arsenic on biogenic amine at low doses during crucial period of brain development is scanty. However, mechanisms underlying the developmental neurotoxicity of arsenic are indistinct and the research on neurobiological consequences of arsenic exposure is important to understand the mechanism of developmental neurotoxicity. Impairment of neurotransmitters functions in the central nervous system (CNS) is closely related to neurodegenerative disease, learning, and memory impairment. Therefore, studying the impairment of biogenic amine and their metabolites in the brain of rats is extremely considerable to understand the developmental neurotoxicity of arsenic. Developmental exposure to arsenic affects the release, synthesis, uptake of neurotransmitter and receptor expression could be deleterious and lead to long-term behavioral deficits [32-34]. Levels of some neurotransmitters and the activity of enzymes related to neurotransmitter metabolism, such as cholinesterase and dopamine b-hydroxylase, in the brain of rodents were found to be altered after exposure to arsenic [34-37].

NO may also regulate the neurotransmitter release and the intercellular neuronal signaling which is involved in neuronal plasticity, learning and memory [38-40]. Impairment in NO-related function may also contribute to the cognitive deficits in children and adolescents exposed to arsenic [17,41]. Rios et al. [35] have reported the decreased NO markers associated with the morphological changes in the brain of rats exposed to arsenic. Several nitrergic markers deficit has been observed in blood and brain tissue of rats and rabbits that may be playing a significant role in the arsenic-induced neurotoxicity [42,43]. However, an impact of arsenic exposure on the biogenic amines and NO levels are not understood during crucial periods of brain development. Moreover, it is not understood whether these changes are transient or persistent after the withdrawal of arsenic exposure. Therefore, we aimed to assess the association between impairment of these functions and arsenic exposure at low doses during the early life crucial period of brain development. The present study may be useful in disclosing the mechanisms underlying the developmental neurotoxicity of arsenic and suggesting the occurrence of developmental neurotoxicity is associated with changes of biogenic amines and their metabolites and NO in CNS.

Materials and Methods

Animals and treatment

Early life exposure of rats to sodium arsenite: Male Wistar rats (21 days old) were obtained from the central animal breeding colony of CSIR-Indian Institute of Toxicology Research (CSIR-ITR), Lucknow, India. All the animals were maintained on a commercial pellet diet and water ad libitum, housed in plastic cages with bedding at a temperature (22 ± 2°C) controlled room with a 12/12 h light/dark cycle. All experimental procedures involving animals were carried out strictly in accordance with the international care guidelines for animals approved by the Ministry of Environment and Forests (Government of India), New Delhi, India. Further, male rats were randomly divided into three groups on PD22. Group I rats were treated with normal saline (p.o.) identically as in groups II and III and served as controls. Group II and Group III rats were treated daily with one dose per day sodium arsenite 2 or 4 mg/kg body weight, p.o. respectively from PD22 to PD59. On PD60, control and treated rats were sacrificed and brains were taken out from skull quickly and washed in ice-cold saline. The frontal cortex corpus striatum and hippocampus regions of the brain were carefully separated, following the method as described by Glowinski et al. [44]. The brain regions were kept frozen at -80°C for the estimation of biogenic amines and their metabolites and levels of NO.

To understand whether these changes were transient or persistent after withdrawal of arsenic exposure, early life exposed rats were left for 30 days and monitored daily. Neurotoxic effects of arsenic on biogenic amines and their metabolites and levels of NO were studied on PD60 and PD90.

Estimation of biogenic amines and their metabolites in brain regions of rats

To determine the levels of DA, NE, EPN, 5-HT, DOPAC and HVA in different brain regions of rats, reversed-phase high-performance liquid chromatography coupled with an electrochemical detector (HPLC-ECD) was carried out following the method described by Kim et al. [45] with some modifications. The HPLC system (Waters, Melford, USA) was integrated with a high-pressure isotropic pump (515 HPLC Pump), a sample injector valve, C-18 reverse phase column (250 × 4 mm, particle size 5 µm) and an electrochemical detector (464 Pulsed electrochemical detector). The frozen brain tissue sample was weighed and transferred into the tube and added the pre-chilled homogenize buffer solution (0.1 M perchloric acid and 3,4-dihydroxy benzylamine, an internal standard at a final concentration of 25 ng/ml) at a volume of 1 ml/100 mg sample. Brain tissues were homogenized and kept on ice for 15 min. The homogenate was centrifuged at maximal speed (36,000 xg) at 4°C for 10 min and the supernatant was collected and transferred to a new sample tube. The supernatant was filtered through nylon filters (0.25 mm, Millipore, USA) and used for the estimation of biogenic amines and their metabolites.

20 µl volume of the sample was injected into a reverse-phase analytical column with a mobile phase (pH 4.2) containing sodium dihydrogen phosphate (0.15 M), ethylene diaminetetraacetic acid (0.25 mM), sodium octyl sulfate (1.75 mM) and 4% methanol at the flow rate of 1.5 ml/min. The concentrations of biogenic amines and their metabolites in different brain regions of rats were determined by using an electrochemical detector coupled with an integrator. Chromatograms were analyzed with the help of Empower2 software (Waters, Melford, USA) and results were expressed in ng/g tissue weight.

Estimation of NO levels in brain regions of rats

NO level in different brain regions of rats was analyzed using NO assay kit procured commercially (Calbiochem, USA). The assay principle is based on the conversion of nitrate to nitrite by the enzymatic action of nitrate reductase. Nitrate is converted into a fluorescent compound 1H-naphthotriazole after the addition of 2,3-diaminonaphthalene-1(H)-naphthotriazole after the addition of 2,3-diaminonaphthalene and sodium hydroxide. The emitted fluorescence was measured spectrophotometrically (excitation 430 nm/ emission 450 nm) using a multwell plate reader (Biotek Synergy HT, USA) [46]. The measured values were expressed in pmoles nitrate/mg tissue weight.

Protein estimation

Protein content in samples was measured following the method described by Lowry et al. [47] using bovine serum albumin as a reference standard.
Statistical analysis
In the present study Graph Pad prism software has been used to analyze data of biogenic amine and their metabolites and levels of NO. One way analysis of variance (ANOVA) followed by Newman–Keuls test has been used to analyzed the data for posthoc comparisons. Values up to p<0.05 have been considered significant.

Results
Effects on biogenic amines and their metabolites in brain regions of rats on PD60 and PD90
The levels of biogenic amines and their metabolites showed dose dependent changes in the frontal cortex, corpus striatum and hippocampus of arsenic exposed rats as compared to controls. In comparison to controls, the administration of both doses of arsenic has increased the levels of DA (16%, p<0.05; 39%, p<0.05), EPN (46%, p<0.05; 59%, p<0.01), NE (18%, p<0.05; 39%, p<0.01) and decreased the level of DOPAC (19%, p<0.05; 29%, p<0.01), HVA (20%, p<0.05; 36%, p<0.01) on PD60 (Table 1). The arsenic exposed rats showed a trend of recovery in the levels of DA (6%, p<0.05; 14%, p<0.05), NE(5%, p>0.05; 8%, p>0.05), EPN (11%, p>0.05; 15%, p>0.05), DOPAC (6%, p<0.05; 10%, p>0.05), and HVA (9%, p<0.05; 15%; p>0.05) in frontal cortex even at 30 days after withdrawal of arsenic exposure in comparison to respective controls (Table 1). No significant change was observed in the level of 5-HT in frontal cortex of arsenic-treated rats as compared to controls.

A significant increase in the levels of DA (29% p<0.05; 64%, p<0.01), HVA (22%, p<0.05; 49%, p<0.01) and decrease in the levels of NE (22%, p<0.05; 35%, p<0.05), EPN (24%, p<0.05; 35%, p<0.05) and DOPAC (25%, p<0.05; 36%, p<0.01) were observed in corpus striatum of rats exposed to arsenic (2.0 or 4.0 mg/kg body weight) from PD22-59 on PD60 in comparison to controls (Table 2). A trend of recovery in the levels of DA (19%, p<0.05; 37%, p<0.05), NE(14%, p<0.05; 24%, p<0.05), EPN (16%, p<0.05; 24%, p<0.05), DOPAC(5%, p<0.05; 14%, p<0.05) and HVA (21%, p<0.05; 29%; p<0.05) were seen in the corpus striatum of arsenic-treated rats. The changes were found to persist at the higher dose 30 days after withdrawal of exposure on PD90 in comparison to respective controls (Table 2). The level of 5-HT was not significantly impaired in corpus striatum of arsenic-treated rats.

Exposure with arsenic to rats caused a significant increase in the levels of DA (19%, p<0.05; 28%, p<0.05), NE (17%, p<0.05; 35%, p<0.01), EPN (23%, p<0.05; 59%, p<0.05) and decrease in the levels of DOPAC (26%, p<0.05; 32%, p<0.05) and HVA (26%, p<0.05; 33%, p<0.05) in hippocampus on PD60 as compared to controls (Table 1). The animals were appeared to be recovered from the toxic effects of arsenic 30 days after withdrawal of exposure as no significant variation in the levels of NE (2%, p>0.05; 4%; p>0.05), EPN (10%, p>0.05; 16%, p>0.05), DOPAC (5%, p>0.05; 12%, p>0.05), and HVA (2%, p>0.05; 6%; p>0.05) were observed. The levels of DA (9%, p>0.05; 19%, p>0.05) was found to be significantly remained to persist at the higher dose of arsenic in the hippocampus of arsenic exposed rats 30 days after withdrawal of exposure on PD90 in comparison to respective controls (Table 3). The hippocampal level of 5-HT was not significantly altered in arsenic exposed rats on PD60 and 30 days after withdrawal of arsenic exposure as compared to controls.

Effect on the level of NO in different brain regions of rats on PD60 and PD90
A significant decrease in the levels of NO in frontal cortex (1.57-
Table 2: Early life exposure (PD22 – PD59) to sodium arsenite and its effect on biogenic amines and their metabolites in corpus striatum of rats on PD60 and PD90.

| Age/ Biogenic amine(s)/ Metabolite(s) | Control | Treatment groups |
|--------------------------------------|---------|-----------------|
|                                      |         | ARS I (2 mg/kg) | ARS II (4 mg/kg) |
| Postnatal day 60                     |         |                 |                 |
| Dopamine                             | 5360 ± 456.30 | 6944 ± 319.80* | 8800 ± 818.10** |
| Norepinephrine                       | 595.90 ± 61.87 | 460.10 ± 25.47* | 386.40 ± 9.69*  |
| Epinephrine                          | 363.80 ± 16.31 | 274.80 ± 9.79*  | 234.90 ± 25.73* |
| Serotonin                            | 779.20 ± 86.87 | 860.50 ± 125.20 | 907.80 ± 9.24   |
| DOPAC                                | 3216 ± 183.90 | 2380 ± 151.60** | 2033 ± 152.70** |
| Homovanillic acid                    | 594.80 ± 44.90 | 731.40 ± 34.01* | 889.40 ± 46.94**|
| Postnatal day 90                     |         |                 |                 |
| Dopamine                             | 5068 ± 272     | 6064 ± 277.90   | 6986 ± 481.10*  |
| Norepinephrine                       | 403.70 ± 26.10 | 344.70 ± 10.93  | 304.40 ± 10.83* |
| Epinephrine                          | 311.10 ± 40.26 | 258.70 ± 12.33  | 234.70 ± 10.56* |
| Serotonin                            | 663.50 ± 64.17 | 689.50 ± 97.66  | 710.10 ± 167.50 |
| DOPAC                                | 4484 ± 187.40 | 4236 ± 65.44    | 3818 ± 139.20*  |
| Homovanillic acid                    | 648.70 ± 37.55 | 790.20 ± 57.85  | 842.80 ± 58.57* |

Table 3: Early life exposure (PD22 – PD59) to sodium arsenite and its effect on biogenic amines and their metabolites in hippocampus of rats on PD60 and PD90.

| Age/ Biogenic amine(s)/ Metabolite(s) | Control | Treatment groups |
|--------------------------------------|---------|-----------------|
|                                      |         | ARS I (2 mg/kg) | ARS II (4 mg/kg) |
| Postnatal day 60                     |         |                 |                 |
| Dopamine                             | 450 ± 40.16     | 511.60 ± 7.64*  | 579.70 ± 36.54* |
| Norepinephrine                       | 197.90 ± 7.93  | 231 ± 12.18*    | 267.30 ± 3.67** |
| Epinephrine                          | 278.70 ± 23.17 | 344.40 ± 27.37* | 444.60 ± 40.43* |
| Serotonin                            | 396.40 ± 47.24 | 359 ± 42.24     | 347 ± 12.53     |
| DOPAC                                | 31.55 ± 0.83   | 23.14 ± 1.72*   | 21.38 ± 2.47*   |
| Homovanillic acid                    | 33.20 ± 2.35   | 27.52 ± 1.64*   | 24.83 ± 1.21*   |
| Postnatal day 90                     |         |                 |                 |
| Dopamine                             | 466.10 ± 66.77 | 508.30 ± 27.09  | 559.10 ± 37.91* |
| Norepinephrine                       | 224.10 ± 10.82 | 228.40 ± 11.5   | 233.90 ± 17.24  |
| Epinephrine                          | 267.30 ± 24.12 | 296.70 ± 14.15  | 310.10 ± 19.47  |
| Serotonin                            | 417.30 ± 24.93 | 403.30 ± 34.81  | 396.20 ± 63     |
| DOPAC                                | 45.96 ± 2.88   | 43.41 ± 2.39    | 40.12 ± 2.60    |
| Homovanillic acid                    | 27.63 ± 3.64   | 27.11 ± 3.34    | 25.96 ± 3.31    |

Discussion

Brain development during prenatal phase represents only one part of the lengthy nervous system developmental process. While the early life, childhood, and adolescence represent windows of remarkable brain changes and maturational processes. Biogenic amines are one of major groups of the neurotransmitters which play important roles in the CNS as major neurotransmitters, being involved in regulating the neurotransmission in the brain and the regulation of cognitive and motor functions, mood, emotional behaviour, including memory
Many investigators have reported the alteration in biogenic amines and their metabolites in various neurophysiological and neuropsychological diseases [4,50]. Many biogenic amines are essential for the shaping, wiring, brain growth events and the normal development of the nervous system during the critical period of the brain development [51]. Impairment in the levels of biogenic amines (DA, 5-HT, NE) in the brain during critical developmental phases of postnatal could be delayed postnatal maturation of the brain along with neurodevelopmental disturbances associated with cognitive deficits [52-54].

The present study was carried out to investigate the impact of arsenic on the levels of biogenic amines and their metabolites and levels of NO during critical periods of brain development of rats. The level of DA significantly higher was found in the arsenic exposed rats as compared to controls during the postnatal period of brain development in the frontal cortex, corpus striatum and hippocampus of rats at both doses of arsenic. Dose-dependent effect of arsenic on rodents has been observed at low and intermediate doses of arsenic produced hyperactivity whereas the high dose produced hypoactivity along with DA release as increase and decrease in extracellular DA levels [29,36,55], it could be a consequence of dysfunction of the dopaminergic neurotransmitter system. Overproduction of DA can lead to hyperactivity and linked with attention deficit hyperactivity disorder [56]. Weaned mice received to arsenic (20, 40, 60 or 100 ppm) showed a significant correlation between arsenic level and regional monoamine levels in the brain and also found the ACTH levels in plasma were significantly associated with NE concentration in pons and medulla (Delgado et al. 2000). Declined levels of DA while an increase in the levels of NE and 5-HT were reported in the whole brain of guinea pigs after the exposure to arsenic (25 mg/l) for 120 days [57].

Further, differential changes in the metabolites of DA (DOPAC and HVA) were observed in selected brain regions due to the arsenic exposure during crucial periods of brain development. Reduced levels of DOPAC were found in selected brain regions of early life arsenic exposed rats. HVA is another metabolite of DA was significantly impaired in frontal cortex and hippocampus of early life arsenic exposed rats. The hippocampal level of HVA was also imparted at both doses (2 or 4 mg/kg) in early life arsenic exposed rats. Interestingly, these changes were found to recover, but the levels of DA and their metabolites remained more persistent at a higher dose of arsenic in the corpus striatum of rats, after withdrawal of exposure as compared to controls. These findings showed that an intensity of these changes was more striking in the corpus striatum than the frontal cortex and hippocampus, after withdrawal of exposure. Alteration in the levels of DA and their metabolites (HVA, DOPAC), it might be suggested that the catalytic pathway of DA by catechol-o-methyltransferase might have been influenced by arsenic leading to increased DA degradation [58]. Rodriguez et al. [59] were reported the diminished level of DA, HVA and DOPAC in the corpus striatum of rats after the exposure to mining waste containing mainly arsenic. Hyper-locomotor activity at a low dose (3 mg/kg body weight) and while decreased motor activity in those treated with a high dose of arsenic trioxide (10 mg/kg body weight) was observed in mice [60].

In the present study, we observed increased levels of NE in the frontal cortex, hippocampus and decreased level in corpus striatum of early life arsenic exposed rats on PD60. The level of NE was increased may be due to amplifying the activity of the enzyme dopamine hydroxylase, catalyzing the conversion of DA to NE [61]. Alterations in the levels of EPN were also observed in this study, immediately after the arsenic exposure.

Several behavioral and physiological functions such as thermoregulation, mood, sleep, aggression, sexual behaviour, and appetite regulate by the 5-hydroxytryptamine (5-HT) [3, 27]. In our study decreased levels of 5-HT were observed in different brain regions of arsenic-treated rats at low doses, however, changes were insignificant. Our findings are in agreement, even at a high dose of arsenic with those of Tripathi et al. [33]. The differential changes in the level of biogenic amines and their metabolites in different brain regions were found might be due to the different duration of exposure and different sensitive period of exposure. These differential changes were more striking in the corpus striatum of the brain after the early life exposure to arsenic and intensity of these modifications were more in the high
The present study demonstrates that low doses of continuous exposure to arsenic during early life are associated with the disturbance in biogenic amine levels and their metabolites. Because of arsenic exposure, the reduction in the level of NO was found in the developing brain, which could modulate the several neurological risk factors. However, further studies are required to establish the arsenic-induced developmental neurotoxicity and by which mechanism changes were more compromised in the corpus striatum region even after withdrawal of arsenic exposure. The results of the present study will draw attention to these CNS effects of arsenic even at low doses, besides the more general damaging effects of arsenic reported at a higher dose of arsenic in other models. Further, the continuous exposure to arsenic during critical periods of brain development may be an important risk factor for increased vulnerability to neurodegenerative diseases.

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