Effect of sodium arsenite on adrenocortical activity in immature female rats: evidence of dose dependent response

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Abstract: The effect of sodium arsenite on adrenal weight, adrenal 5-ene-3β-hydroxysteroid dehydrogenase activity along with the serum level of corticosterone in immature female rats aged 35 days were investigated. Sodium arsenite was administered orally in the doses of 0.2 and 0.4 ppm/(100g body weight. rat. day) for a duration of 28 days. The experiments indicated in both cases significant stimulation in the activities of adrenal 5-ene-3-β-HSD elevation of adrenal weights and serum levels of corticosterone were observed but no body growth of these animals. It was considered that arsenite has an adverse effect on adrenocortical activities. Furthermore, the altered adrenocortical activities were evident when arsenic levels in feeding water is within the range in drinking water at wide areas of West Bengal in India. Hence the data of our experiments may have some potential implications in the field of environmental toxicology.

Key words: arsenic; adrenal corticosteroidogenesis; corticosterone

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Introduction

Arsenic, a metalloid, is a non essential trace element, has been claimed to be of clinical benefit in the treatment of syphilis, amoebiasis and other tropical diseases(Klaassen, 1990; Kowaguchi, 1981). Beside its clinical importance, arsenic is reported to be an important water pollutant found drastically in the ground water at several districts of West Bengal, India and creating an epidemic' Arsenic Dermatosis'(Saha, 1991; Mazumdar, 1988). A plethora of endocrine and gonadal effects has been reported to be associated with arsenic treatment like severe metabolic disorder (Mahaffey, 1981), inhibition in spermatogenesis(Sukla, 1984) as well as testicular steroidogenesis(Sarkar, 1991), pronounced structural changes in thymus of pregnant and new born mice (Skal'naia, 1991), chronic exposure of arsenic exhibits interference with reproduction (abortion, low birth weight) and reduced milk production (Donald, 1995). Unfortunately, there is a lack of information concerning the effects of arsenic on adrenocortical activities when arsenic levels in water is within the range which is noted in drinking water at wide areas of West Bengal in India. Recently we have shown that arsenic administration in mature rats results in inhibition in plasma levels of ovarian steroids and gonadotrophins along with diminution in the activities of ovarian 5-ene-3β and 17β-hydroxysteroid dehydrogenase (Chattopadhyay, 1998). The present work is a continuation of our study(Chattopadhyay, 1998) on the adverse effects of arsenic on adrenal gland. Sexually immature animals were selected in the experiment as the hypothalamic pituitary axis is more sensitive in immature than that of mature animals(Swerdlofs, 1968). The possibility of an indirect action of arsenic at the levels of the adrenocortical zone is also discussed. Hence the data of our experiments have potential implication in environmental toxicology.

1 Materials and methods

1.1 Animals and treatment

Twenty four immature female Wistar strain rats of 22—27g were used for the present experiment. Animals were acclimatized to standard laboratory condition for a week before conducting experiments and were provided with food and water *ad libitum*. Animals were placed in normal light-dark cycle (12L:12D) at the animal house with $26 \pm 3°C$ surrounding temperature. Animals were divided into 3 groups equally.

1.2 Arsenic treatment

Sodium arsenite was purchased from Loba Chemical Company (Bombay, India). It was dissolved in sterile distilled water. 8 animals of treated groups were subjected to intragastric application orally with sodium arsenite by a canula at a dose of 0.2 ppm. Dissolved in 0.5 ml distilled water/(rat. day) for 28 days and 8 animals of other treated group were fed with the same at a dose of 0.4 ppm dissolved in 0.5 ml distilled water/(rat. day) for 28 days. Rest of the 8 animals of control group were received the same amount of distilled and sterile water only. Treatment was started when the rats were 35 days old and at the age of 64 days, all animals a were sacrificed by instant decapitation after 24 hours of the last oral application of sodium arsenite. Body weights of the animals were noted. The intact adrenal glands were dissected out, weighed and placed in crude ice for the determination of the activity of adrenal 5-ene-3β-HSD. Blood was sucked from dorsal aorta by a sterilized syringe and serum samples were collected and stored at $-20℃$ for estimation of serum corticosterone.

1.3 Assay of adrenal 5-ene-3β-hydroxysteroid dehydrogenase(5-ene-3β-HSD)

Adrenal 5-ene-3β-HSD was measured by the following procedure of Rubin *et al*. (Rubin, 1961). Adrenal tissue was homogenized in chilling state(4℃) in a medium consisting of equal parts of 0.9% saline and 0.1 mol/L sodium phosphate buffer(pH 7.4) to give a tissue concentration of 4 mg/ml. 1.6 ml of this homogenate was incubated with 6 mg NAD in 0.2 ml phosphate buffer and 500 mg DHEA in 0.1 ml propylene glycol in a shaking incubator at 37℃ for 90 min. After the incubation was completed, the mixture was immediately acidified with 0.1ml of 3 mol/L acetate buffer(pH 5.0) and androstenedione was extracted with 10 ml ethyl acetate. The extract was allowed to dry by evaporation. The residue was dissolved in ethanol and the absorption was determined in a spectrophotometer at 240 nm.

1.4 Assay of serum corticosterone

Serum corticosterone was determined by spectrofluorometer according to the methods of Glic *et al*. (Glic, 1964) and Silber(Silber, 1966).

1.5 Statistical analysis

Analysis of variance and student two tailed t test were used for statistical analysis of the data. Data presented as the mean $\pm$ SEM ($n = 8$) and differences were considered significant when $p < 0.001$.

2 Results

2.1 Body weights and adrenal weights

Body weights of arsenic treated animals in both groups did not differ from that of control (Table 1). Chronic treatment of immature rats with sodium arsenite for 28 days increased adrenal weight in mg/kg. Body weight significantly at both the treated doses in comparison to vehicle-treated controls(Table 1).

Table 1   Changes in body weight and adrenal weight in 64 days old female rats treated with sodium arsenite from age 35 days. Values are mean $\pm$ SEM($n = 8$)

Treatment	Body weight, g		Adrenal weight, mg/kg body weight
	Initial	Final	
Control	22 ± 2.5^a	53 ± 6.3^a	120 ± 21^a
Arsenic (0.2ppm)	27 ± 3.2^a	56 ± 8.5^a	180 ± 19^b
Arsenic (0.4 ppm)	25 ± 2.8^a	61 ± 5.7^a	260 ± 22^c

Notes: The same superscript in each vertical column does not differ from each other significantly. $p < 0.001$

2.2 Enzymatic study

Significant stimulation in the activities of adrenal 5-ene-3β-HSD was noticed after 28 days in both the arsenic treated groups in comparison to control group(Fig.1).

2.3   Hormone study

Significant elevation in serum corticosterone was observed after 28 days in both the arsenic treated groups in respect to control(Fig. 1).

3   Discussion

From the present experimental results it was demonstrated that arsenic treatment produces adrenal hypertrophy, stimulates adrenal 5 ene-3β-HSD activity and increases serum level of corticosterone. There is no effect of arsenic on body growth of these developing rats. This occurs when arsenic levels in water are within the
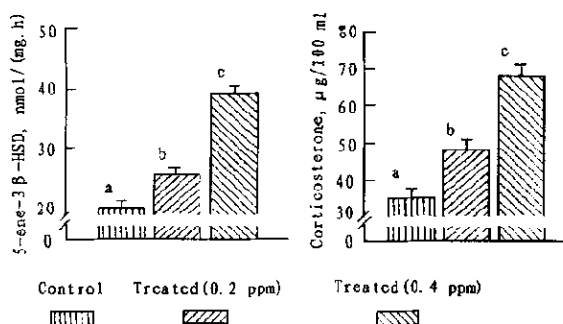

Fig. 1   Changes in adrenal 5-ene-3β-HSD activity and serum level of corticosterone after sodium arsenite treatment in immature female rats. The same superscript in each bar does not differ from each other significantly, $p < 0.001$

range which is noted in drinking water at wide areas of West Bengal in India.

After arsenic treatment the increased activity of 5 ene 3β-HSD in adrenal gland and serum level of corticosterone may be due to the effect of increased ACTH as corticosteroid synthesis is under the regulation of ACTH(Turner, 1976). But the mechanism by which arsenic increases ACTH secretion yet to be determined. In our earlier report(Chattopadhyay, 1998), we have already noted that arsenic treatment in female rats results inhibition in plasma levels of oestrogen and gonadotrophins. Previous investigations established that oestrogen is the inhibitor of adrenal 5-ene-3β-HSD activity(Kitay, 1963; Colby, 1974; Ghosh, 1981). So, due to low plasmal levels of oestrogen in arsenic treated rats, the activity of adrenal 5-ene-3β-HSD activity is elevated which results in elevation of serum corticosterone level. Moreover, elevated plasma levels of ACTH in arsenic treated rats may be due to low levels of gonadotrophin as ACTH production seems to be at the expense of gonadotrophins(Selye, 1950; Nowell, 1959). The elevated levels of ACTH in arsenic treated rats are also supported by increased weight of adrenal gland in these treated groups as ACTH regulates the enlargement of adrenal glands in developing animals(Stryer, 1995).

In conclusion, the results presented here provide evidences for the first time that arsenic treatment is associated with a elevation in the activities of adrenal cortex. In addition, our data suggest an effect of arsenic at the pituiary level. If arsenic would have acted only at the adrenal level, there would have decreased activity of 5-ene-3β-HSD and low level of serum corticosterone after 28 days of treatment, since the election in serum corticosterone by arsenic treatment may decrease ACTH secretion by negative feedback system in such chronic exposure. Moreover, our data have some applied value from the angle of environmental toxicology as adrenocortical activities are affected by chronic arsenic treatment at the dose which is noted in available samples of drinking water at wide areas of West Bengal, India. Though, the mechanism by which arsenic elevates adrenocortical activities in immature rats were not addressed by the present experiment, more information is necessary to better understand the effect of arsenic on the functional physiology of adrenocortical activities.

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