SEX DIFFERENCES IN THE RESPONSE OF ACETYLCHOLINESTERASE TO SUB-CHRONIC LOW-LEVEL INORGANIC MERCURY EXPOSURE

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ABSTRACT

To evaluate the direct inorganic mercury-induced neurotoxicity and to study the mechanism underlying these effects, male and female rats were exposed to mercury chloride (0.5, 1.0 and 1.5mg/kg) for 12 weeks. The activity of acetylcholinesterase (AcChE) in plasma, erythrocyte, brain and erythrocyte membrane were determined: AcChE activity in all the compartments was inhibited to varying extents more profoundly in female. However, at highest dose of mercury; plasma AcChE activity was inhibited to the tune of 52% in the male and 84% in female animals. In the erythrocyte, the highest inhibition of 88% was achieved in the female rats. Erythrocyte membrane AcChE inhibition ranged from 55% to 84% in male while, and 37% to 88% in female. The lowest inhibition of 34% was obtained in the brain AcChE of the male animals. We observed negative associations between tissue mercury levels and AcChE activities in all the compartments except for brain where no significant relationship was found. The results call for further insight into the mechanisms of mercuric chloride-induced neurotoxicity.

INTRODUCTION

Mercury is one of the most naturally occurring harmful metals found in the environment. Therefore, every human being irrespective of age and location, is exposed to one form of mercury or another

Industrialization of developing countries exposed many people to mercury in the environment particularly from residential heating power and waste incinerator Mercury can cause biochemical damage to tissues and genes through diverse mechanisms.

Although inorganic mercury does not normally reach the placenta or cross the blood brain barrier, it has been found in the neonatal brain due to the absence of a fully formed blood-brain barrier (National Research Council 2000) or alteration of the cell membrane permeability (ATSDR 1999). The increased permeability of the blood-brain barrier may leads to inhibition of some enzymes. Recent experimental evidence however indicates that biochemical signals of the neurotransmission system similar to those involved as neurotoxic targets in the central nervous system are also present in more easily and ethically obtainable body fluids like blood, plasma, urine, saliva, cerebrospinal fluid and peripheral blood cells and can thus be used in assessing exposure and response of the central nervous system when functional damage may not yet be apparent (Castoldi et al. 1994; Manzo et al. 2001). Plasma and red cell acetylcholinesterase (AcChE) and neuropathy target esterase (NTE) are currently being investigated in humans as surrogate biomarkers of exposure and for detecting and predicting the neurotoxic effects of environmental toxicants (Castoldi et al. 1994; Manzo et al. 2001)

To the best of our knowledge, there are no studies concerning the direct inorganic mercury induced neurotoxicity and gender impact. Therefore, the present study was carried out to investigate: 1) the effect of inorganic mercury exposure on acetylcholinesterase (AcChE) activity in plasma, erythrocyte, erythrocyte membranes and brain; 2) the possible mechanism underlying the effects; and 3) the sex differences.

MATERIALS AND METHODS

Chemicals

Mercury chloride was products of Sigma-Aldrich, Missouri, USA. All other chemicals and kits used in this study were of purest grade available and obtained from British Drug House (BDH) Chemicals Limited, Poole, England.

Animals and treatment

Experimental protocols were conducted in accord with guidelines of the Institutional Animal Care and Use Committee and were approved by the Animal Ethical Committee of the Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria.

Sixty four male and female albino Sprague-Dawley rats (bred in the College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria) with a mean body weight of 150g were used for the experiment. Housed in an animal room with average controlled temperature (22±2°C) and a regular 12h light-dark cycle (06:00-18: 00h). They were allowed 14days to acclimatize before the commencement of mercury exposure. The animals maintained on a standard pellet diet.

Animals were divided into eight groups of eight animals each. While two groups served as control (male and female) and received distilled water, the remaining groups (three groups each for male and female) were exposed to mercury chloride (0.5, 1.0 and 1.5mg/kg) respectively for 12 weeks. At the end of mercury exposure, blood was collected from the animals into heparinized tubes by cardiac puncture under light anesthesia after an overnight fast. Liver, kidney and brain were also removed from the animals, weighed and placed on ice until required for biochemical analysis. An aliquot of the blood samples was used for mercury determination while the remaining blood samples were centrifuged to separate plasma and red blood cells. The red blood cells were washed twice with physiological saline before being used for AcChE assay.All samples were stored at -20°C until analysed.

Mercury determination

A portion of the frozen organs (≈200mg) and whole blood (0.2ml) were digested in nitric and sulphuric acid mixture. Total mercury was determined using Inductively-coupled plasma spectrometry (ICP-MS) in central analytical laboratory, Stellenbosch University. Results are expressed as μg Hg/ml for blood and μg Hg/g wet weight for the organs.

Plasma and red cell acetylcholinesterase (AcChE)

The activity of plasma and erythrocyte acetylcholinesterase (AcChE) was determined using the colorimetric method described by – . Briefly, 0.10 ml of Buffered Ellman's reagent, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) (DTNB, 10 mmol/l, $NaHCO_3$, 17.85 mmol/l in phosphate buffer 100 mmol/l, pH 7.0) and 0.02 ml of acetylthiocholine solution (acetylthiocholine iodide, 75 mmol/l) were added to 3 ml phosphate buffer in a cuvette and incubated at 25 ◦C for 10 min. A 0.02 ml of diluted saline suspension of red cells (plasma) was then added and absorbance monitored at 30 s interval for 5 min at 410 nm. A Jenway 6405 UV/Visible spectrophotometer (Jenway Ltd. Felsted, Dunmow, Essex, UK) was used for all measurements. Enzyme activity was expressed as U/l of packed red cells (plasma).

Brain acetylcholinesterase (BrAcChE)

A 10% brain homogenate was prepared by homogenizing 0.2 g of the tissue in 0.8 ml of phosphate buffer with 3 up and down strokes at 1100 rpm using a power-driven, Teflon pestle in a glass homogenizing cup maintained at 4oC (Model RQ-127A; RPM 8000; Clearance 2.5 mm; REMI Motors Ltd; Mumbai, India). The homogenate was then centrifuged at 5000 rpm at 4oC after which AcChE was determined in 0.02ml of the supernatant as described for erythrocytes.

Isolation of erythrocyte ghost and determination of its AcChE activity

Erythrocyte membranes were prepared according to the method described by . Briefly, blood samples were centrifuged at 5000rpm for 15 minutes at 4°C. Plasma and buffy coat were removed by careful suction and the cells were resuspended in isotonic Tris-HCl buffer. After mixing by inversion, the samples were recentrifuged at 5000rpm for 15 minutes at 4°C. The supernatant was again removed by careful suction and a few red cells were sacrificed to remove any remaining buffy layer. This washing procedure was repeated twice. The washed cells were then suspended in isotonic Tris-HCl buffer pH 7.6 to an approximate hematocrit of 50% and were kept on ice. The samples were mixed gently by inversion for about 1 minute before membrane preparation. 5ml aliquots of the 50% cell suspensions were transferred to 50ml polyethylene tubes. Thirty ml of hypotonic Tris-HCl buffer pH 7.6 were added to the cell suspension for osmotic lysis. After allowing the tubes to stand for about 10 minutes, they were centrifuged at 20,000rpm for 20 minutes at 4°C. The supernatants were discarded and the pellets resuspended in 10 ml Tris-HCl and centrifuged for 20 minutes at 20,000rpm at 4°C. The pellets were washed four times until the membranes were colourless. Finally, the resultant pellets were rinsed twice with 100μl cold Tris-HCl buffer and poured into Eppendorf tubes. The membrane suspensions were kept frozen in this latter buffer at -20°C. AcChE was determined in the membrane suspensions as described for erythrocytes.

Statistical Analysis

The results are expressed as mean ± SEM. Statistical differences in the means were determined using One-way analysis of variance (ANOVA) followed by Turkey's test (Turkey honest significant difference (THSD)) with p<0.05 considered significant. The associations among the parameters and their magnitudes were tested for by using Multiple Linear Regression analysis.

RESULTS

Exposure of the animals to inorganic mercury resulted to increase in mercury concentrations in blood and brain of both sexes although only in the blood of female animals was the building up found to be dose-dependent (Table 1). In male animals, exposure to an inorganic mercury at highest dose of 1.5mg/kg resulted in a mercury content of blood (0.041μg Hg/ml) and of brain (0.1200μg Hg/g). Whereas in female, at same dose the mercury content of blood and brain was 0.060μg Hg/ml and 0.090μg Hg/g respectively

Inorganic mercury exposure resulted in a significant inhibition (p < 0.05) of the activity of AcChE in the plasma, erythrocyte, and erythrocyte membranes with the exception of brain where no significant inhibition was observed. The inhibition of AcChE in plasma at the highest inorganic mercury dose amounted to 52% in male and 84% in female (dose dependently) at significant level of p < 0.001 compared with control (Figure 1). It was also observed that the plasma AcChE activity in the male controls was significantly (p < 0.0001) lower than that of their female counterparts. There was a significant decrease in the activity of AcChE in the erythrocyte of both sexes (p < 0.001). The lowest activity was observed in the female animal at highest mercury dose. When compared with activity of control, AcChE activity in the female animals amounted to 87% inhibition. While in the male counterpart, the highest inhibition was 67% (figure 2).

The AcChE activity of the erythrocyte membrane of the animals are shown in figure 3. In both sexes, mercury exposure resulted in significant decrease of the activity of AcChE of the erythrocyte membrane in the animals (p < 0.05). Mercury-exposure resulted in 75% and 84% decreases in AcChE of the erythrocyte membrane of male rats with 1.0mg/ml and 1.5mg/ml Hg respectively. In mercury-exposed female rats; 88 and 67% reduction resulted from exposure 1.0mg/ml and 1.5mg/ml mercury doses respectively. Depicted in figure 4 is the AcChE activity of the brain of the animals. At highest dose of mercury, a significant decrease in the activity of AcChE in both sexes with 34% inhibition in male. While, 88% inhibition was observed in female at 1.0mg/ml mercury dose.

Table 2 shows the relationship between tissue mercury levels and AcChE activities in various compartments of male and female animals. A significant negative correlation was observed between tissue mercury levels and AcChE activity in both sexes in all the compartments except brain. No relationship was observed between brain mercury levels and brain AcChE activity in both sexes.

Table 1: Mercury concentrations in the tissues of the animals.

Mercury dose(mg/kg body weight)	Sex	Brain Mercury µg/g	Blood mercury µg/ml
	Male	0.050±0.000[a]	0.004±0.000[a]
Control	Female	0.055±0.002[a]	0.005±0.001[a]
	Male	0.055±0.004[a]	0.035±0.010[c]
0.5	Female	0.058±0.004[a]	0.020±0.004[b]
	Male	0.060±0.007[a]	0.022±0.005[b]
1.0	Female	0.061±0.007[a]	0.037±0.010[c]
	Male	0.120±0.016[b]	0.041±0.007[c]
1.5	Female	0.090±0.012[b*]	0.067±0.016[d]

Each value represents the mean±S.E.M. of 8 rats. Values within a row with different alphabets are significantly different from each

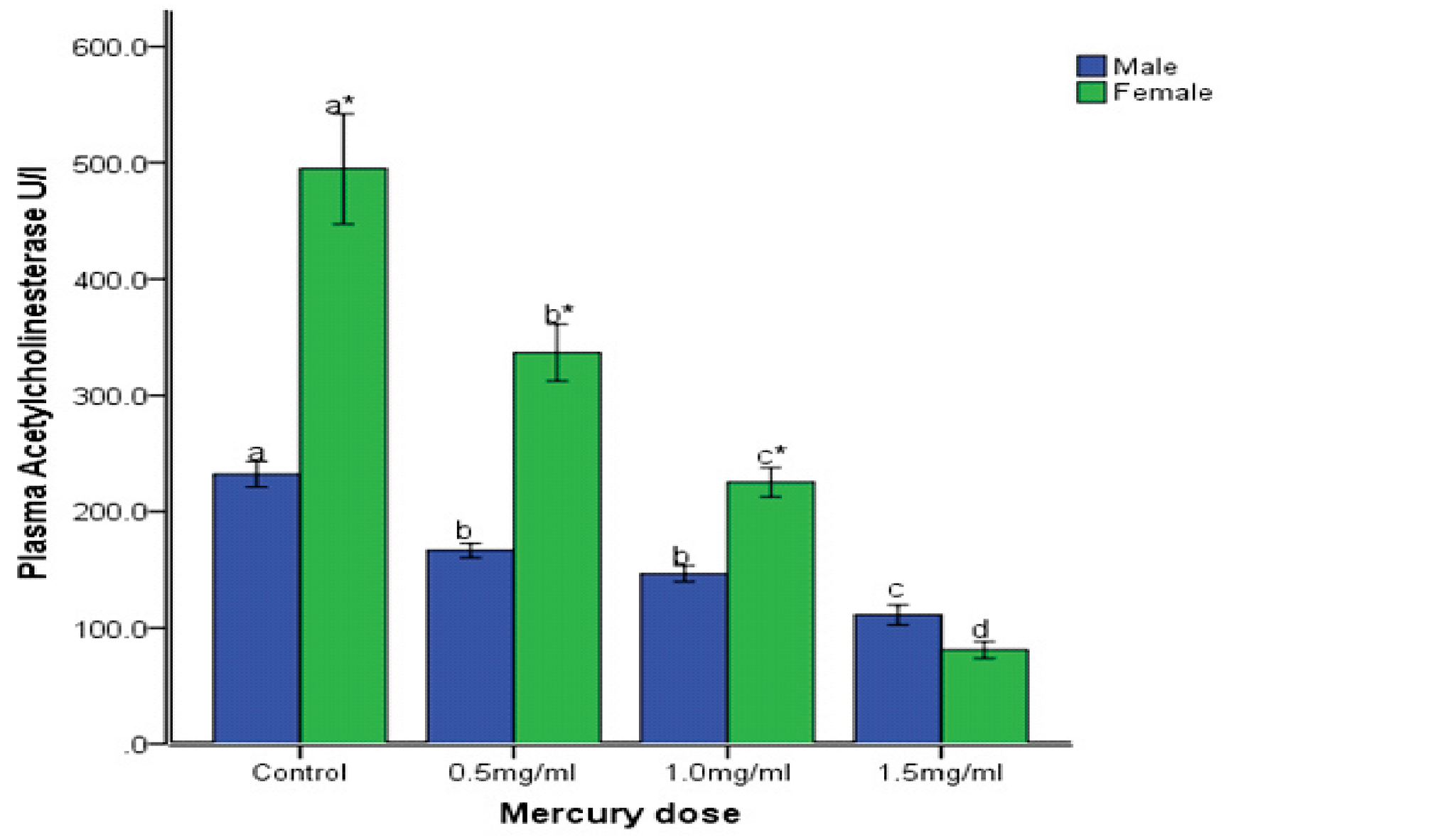

Figure1: Effects of inorganic mercury on plasma acetylcholinesterase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

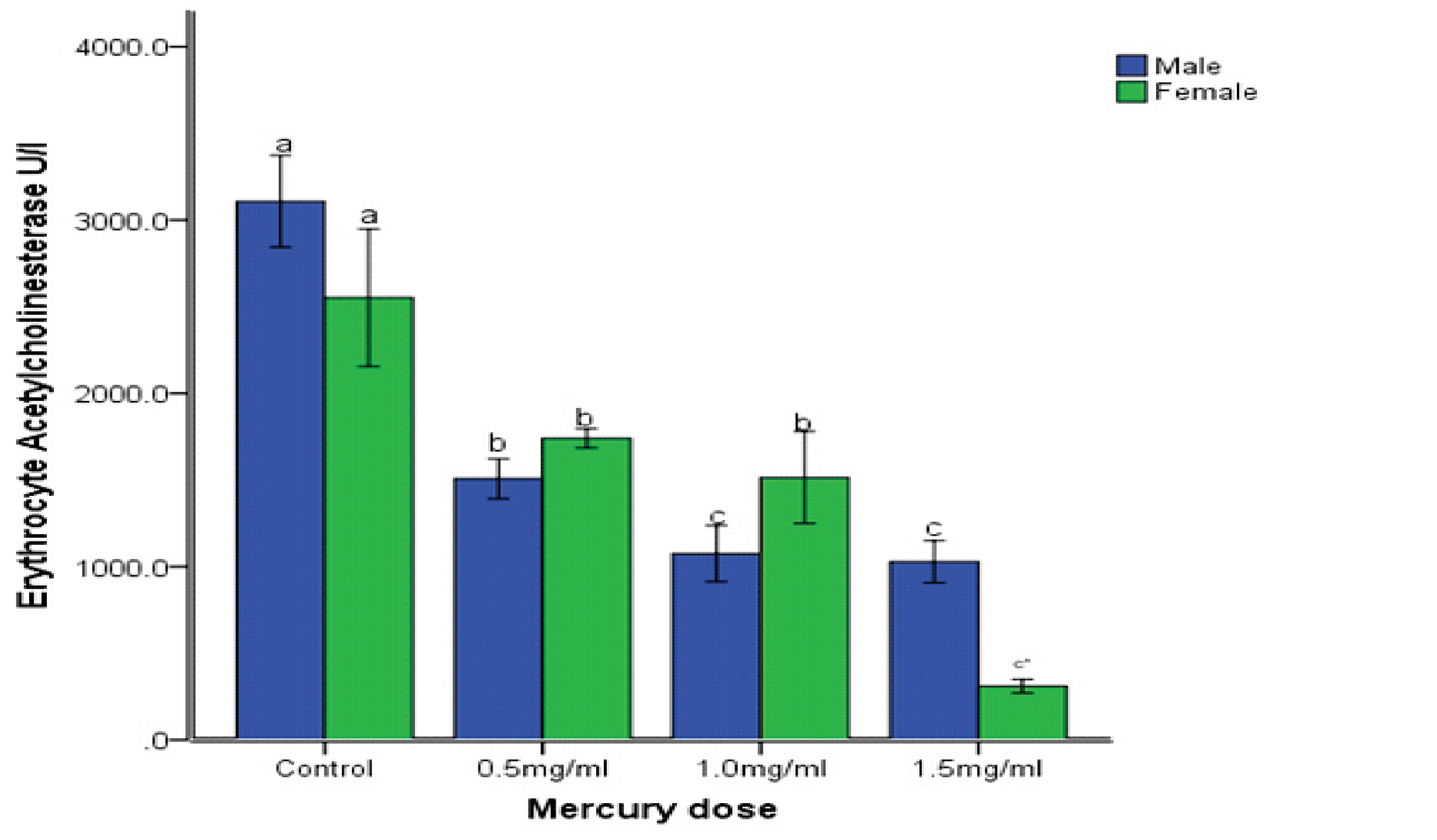

Figure 2: Effects of inorganic mercury on erythrocyte acetylcholinesterase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

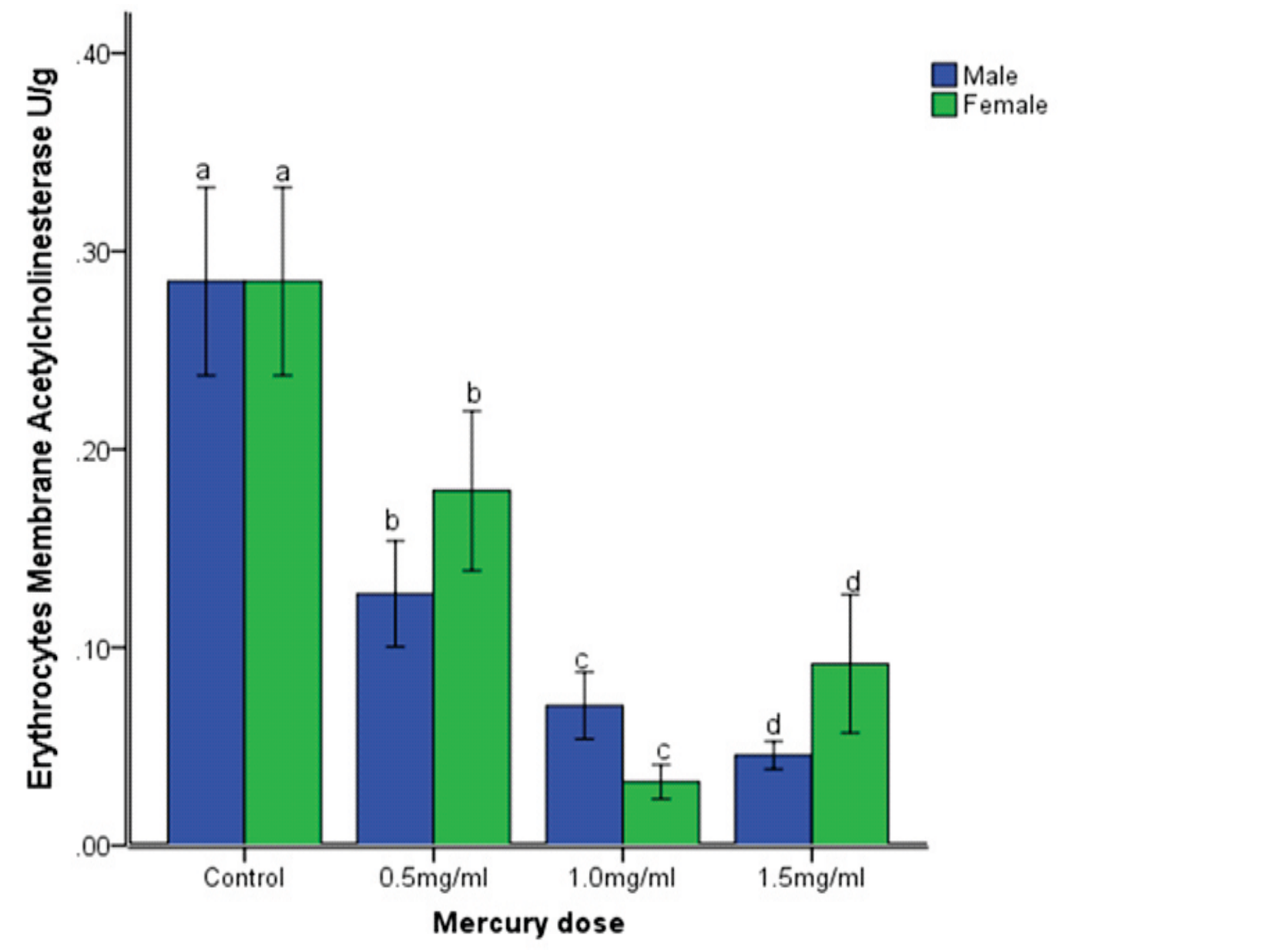

Figure 3: Effects of inorganic mercury on erythrocyte membrane acetylcholinesterase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

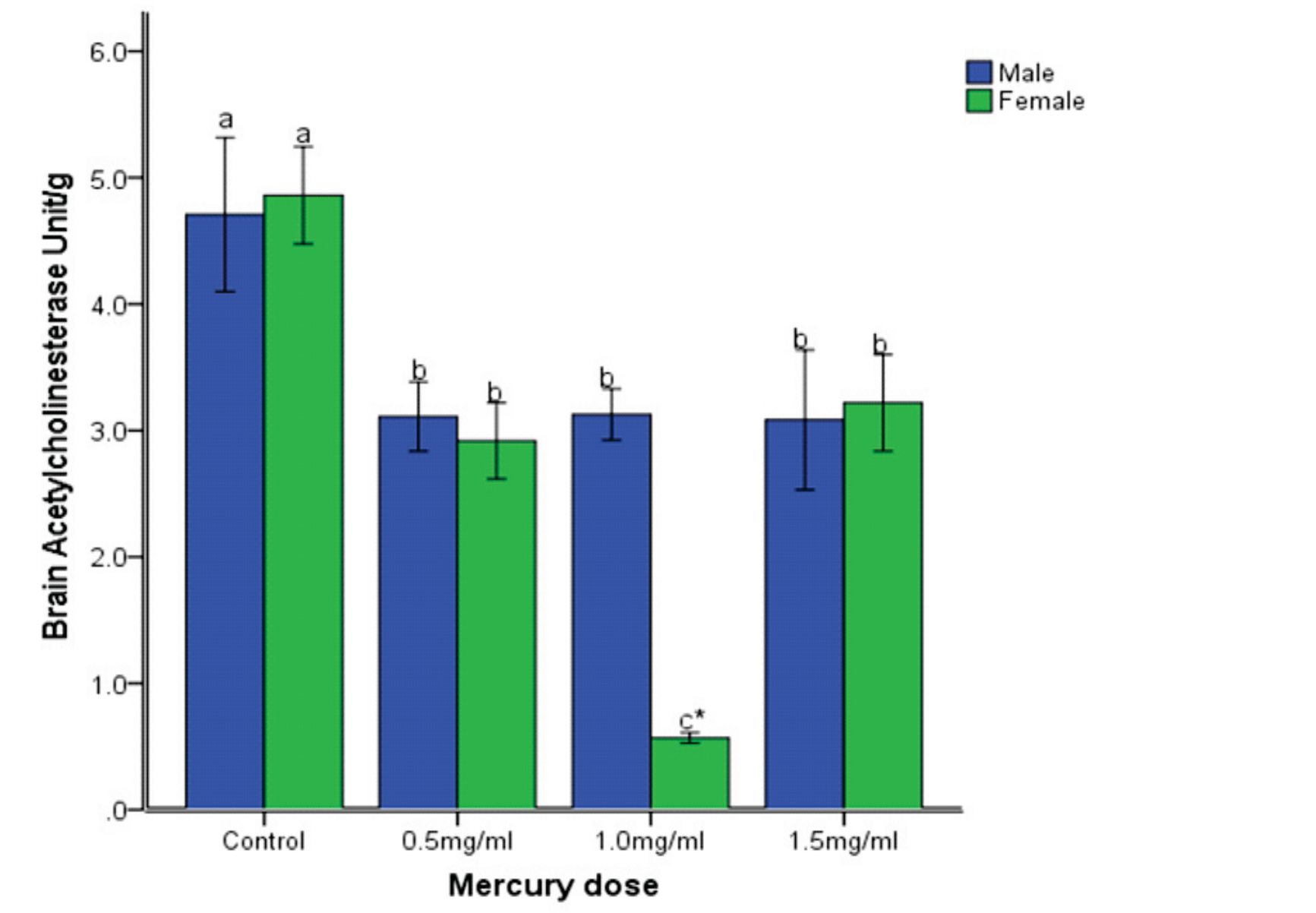

Figure 4: Effects of inorganic mercury on brain acetylcholinesterase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

Table 2: Associations between tissue mercury levels and acetylcholinesterase activity in various compartments of male and female animals

Tissue mercury		Plasma Acetylcholinesterase unit/L		Red Blood Cell Acetylcholinesterase unit/L		Erythrocytes Membrane Acetylcholinesterase unit/g		Brain Acetylcholinesterase unit/g	
		FŎǾŃŎMPŎŌ coefficient (r)	P value	Correlation coefficient (r)	P value	Correlation coefficient (r)	P value	Correlation coefficient (r)	P value
[illegible] mercury	Male	-0.527	0.002	-0.584	0.000	-0.409	0.02	-0.315	0.079
	Female	-0.581	0.000	-0.526	0.002	-0.472	0.006	-0.302	0.093
Brain Mercury	Male	-0.533	0.002	-0.392	0.026	-0.352	0.048	-0.086	0.642
	Female	-0.487	0.005	-0.428	0.14	-0.154	0.400	-0.016	0.931

CONCLUSION

In conclusion, the findings of this study indicate that AcChE is inhibited in all the compartments of both male and female rats and that the decrease in activity was negatively correlated with blood mercury levels

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