SUBCHRONIC TREATMENT WITH MERCURIC CHLORIDE SUPPRESSES IMMUNE RESPONSE, ELICITS BEHAVIORAL DEFICITS AND INCREASES BRAIN SEROTONIN AND DOPAMINE METABOLISM IN RATS

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ABSTRACT

In view of the neurotoxic effects of mercuric chloride the following study was designed to explore its effects on brain serotonin and dopamine metabolism. Mercuric chloride injected at a dose of 1 mg/ml/kg increased locomotor activity, decreased latency to move, corner sittings and food intake. A decrease of 33% of immune response was observed in mercuric chloride treated rats as evident from anti nuclear antibody (ANA) positive test. Neurochemical analysis revealed that mercuric chloride administration increased brain tryptophan concentrations 5-Hydroxyindoleacetic acid (5-HIAA) and dihydroxyphenyl acetic acid, while 5-Hydroxytryptamine (5-HT) and Dopamine (DA) were decreased. The results also showed an increase in brain 5-HT and DA turnover rate in mercuric chloride injected rats. These increases in 5-HT and DA metabolism suggest that mercuric chloride may tend to contribute to anorexia and hyperactivity induced in drug administered rats.

Keywords: Mercuric chloride, ANA, 5-HT metabolism, DA metabolism.

INTRODUCTION

An important goal of the present study was to elucidate the behavioral and neurochemical effects produced due to mercuric chloride exposure in experimental animals. Previous studies have documented that mercuric chloride used for the treatment of rheumatoid arthritis (RA) had a role in eliciting autoimmune disorders in susceptible mice and rats (Goldman *et al*., 1991; Enestrom and Hultman, 1995). Mercuric intoxication from skin ointments containing mercuric ammonium chloride can produce severe neuroasthenia (Pelclova *et al*., 2002). Reports from clinical studies have shown that calomel based teething powder can cause Pink disease (Weinstein and Bernstein, 2003) and Young's Syndrome (Hendry *et al*., 1993). Toxicity of mercuric ions lead to accelerated apoptosis (Szasz *et al*., 2002). Studies on experimental animals show that exposure to mercuric chloride also produces neurotoxic effects (Aschener *et al*., 1994; Gyori *et al*., 1994; Szasz *et al*., 2002 and Berntssen *et al*., 2003)

A role of serotonin has been well documented in various physiological functions and (Haleem *et al*., 1988; Blundell, 1984 and 1991) neuropsychiatric disorders (Haleem, 1996: Haleem *et al*., 2002 and Totterdell, 2006).

The present study was designed to investigate mercuric chloride induced behavioral changes and its relation with brain 5-HT and DA metabolism in rats.

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MATERIALS AND METHODS

Locally bred female Albino Wistar rats weighing 230-240 g purchased from Aga Khan University Hospital, were caged individually and exposed to standard rat diet and tap water one week before start of the experiment. The animals were randomly assigned as controls and tests. The controls received injection of saline while the tests were administered with 1 mg/ml/kg of mercuric chloride (s/c) on alternate days. The treatment continued for 10 days. Food intake and body weights were monitored daily. Open field was monitored for 5 minutes after the 5^{th} injection on the 10^{th} day. Animals were decapitated on 11^{th} day by cervical dislocation and plasma was collected for ANA detection. All the experiments were carried out keeping in view the ethical conditions approved by Local Animal Care Committee.

Liver samples were collected for determining liver tryptophan concentrations. Brain was dissected out and stored at -70^{o}C for neurochemical analysis by HPLC-EC method as described earlier (Haleem and Perveen, 1994).

Drug

Mercuric chloride was purchased from BDH (England). It was prepared by dissolving it in saline.

Open field activity

The activity of saline and mercuric chloride injected rats were monitored on the 10^{th} day, i.e. after the 5^{th} injection,

in an open-field apparatus consisting of a square area measuring 76x76 cm with 42 cm high walls. To determine the activity, the rat was placed in the central square and the number of squares crossed with all four paws was scored for 5 minutes. Activities of saline and mercuric chloride treated rats were recorded in a balanced design to avoid order effect

Screening of ANA

The immuno SLE Latex Kit (Immunostics, Inc., New Jersey) was used in the detection of ANA. An agglutination of latex particles suspension in no longer than one minute inferred a positive result.

STATISTICAL ANALYSIS

Food intake data were analyzed by two way 2-5 ANOVA. Student t-test was used to compute the analysis of locomotor activity, liver and brain tryptophan, 5-HT, 5-HIAA, 5-HT turnover rate, DA, DOPAC, and DA turn over ratio of saline and mercuric chloride injected rats.

RESULTS

Fig. 1 shows the effects of mercuric chloride on 24 h cumulative food intake in experimental models. Statistical analysis by two way 2-5-ANOVA showed significant ($F=55.44$, d.f.1,20, $P<0.01$) effects of mercuric chloride administration, while day effect ($F=0.42$, d.f. 4,50 $P>0.05$) was not significant. The interaction between the two values was also not significant ($F=1.026$, d.f. 4, 50, $P>0.05$). Post hoc analysis showed that food intake was decreased significantly ($P<0.01$) following all the five injections of mercuric chloride.

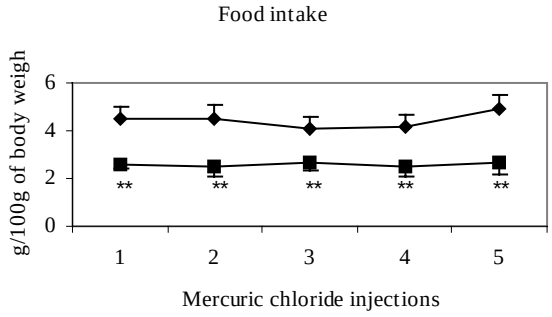

Fig. 1: Effects of mercuric chloride on 24 h cumulative food intake of rats. Values are: means ±SD (n=6). Significant difference by student's t-test from their (**P<0.01) respective saline-treated controls.

Fig. 2 shows the effects mercuric chloride in an openfield activity. Data computed using t-test revealed increased locomotor activity ($P<0.05$). However, latency to move ($P<0.01$) and corner sittings were decreased P (<0.01).

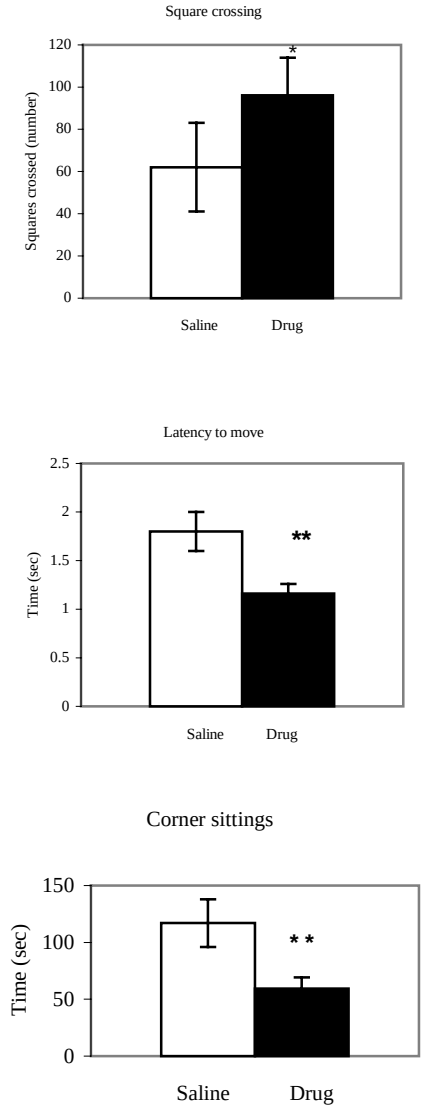

Fig. 2: Effects of mercuric chloride on different components of open field activity (square crossing, latency to move, corner sittings) in rats. Values are means+ SD (n=6). Significant differences by student t-test, *P<0.05 and **P<0.01 from their respective controls.

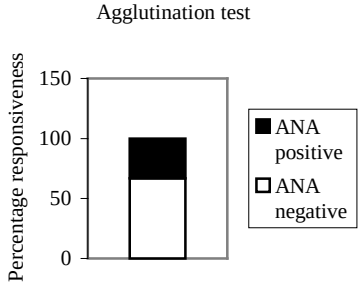

Fig. 3: Effects of mercuric chloride on agglutination test in drug injected rats. Values are percentage responsiveness. (n=6)

Fig. 3 shows the effects of mercuric chloride on ANA analysis. Percentage analysis showed that 33% of mercuric chloride injected animals were ANA positive.

Fig. 4 shows the effects of mercuric chloride on liver tryptophan and brain tryptophan. Data analysis by t-test revealed that mercuric chloride increased brain tryptophan concentrations ($P<0.01$), however, liver tryptophan concentrations were unaltered ($P>0.05$).

Fig. 5 shows the effects of mercuric chloride on 5-HT, 5-HIAA and 5-HIAA/5-HT. Data analysis by t-test revealed that mercuric chloride administration significantly decreased 5-HT ($P<0.01$) increased 5-HIAA ($P<0.05$) and 5-HT turnover ratio ($P<0.05$).

Fig. 6 shows the effects of mercuric chloride on DA, DOPAC and DOPAC/DA ratio. Student t test showed significant decreases of DA ($P<0.01$), significantly increased DOPAC concentrations ($P<0.01$) and increased DOPAC/DA ratio ($P<0.01$) in mercuric chloride injected rats.

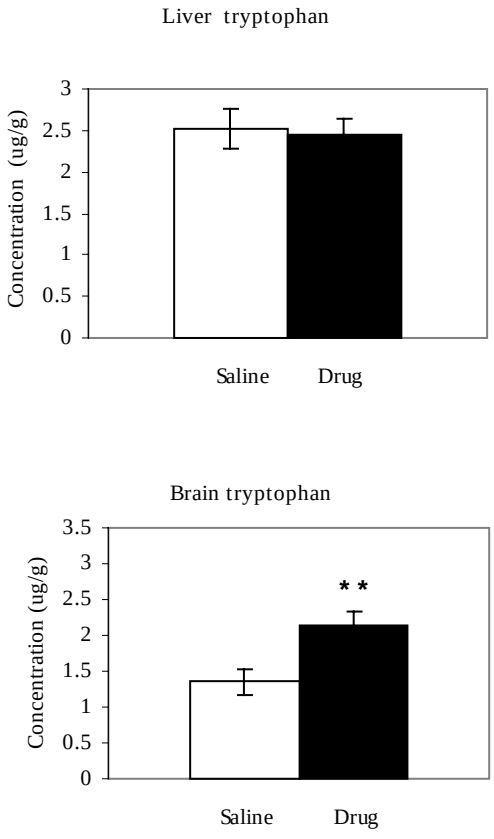

Fig. 4: Effects of mercuric chloride on liver and brain tryptophan concentration in rats. Values are means ±SD (n=6). Significant differences by student's t-test **P<0.01 from their respective saline treated controls.

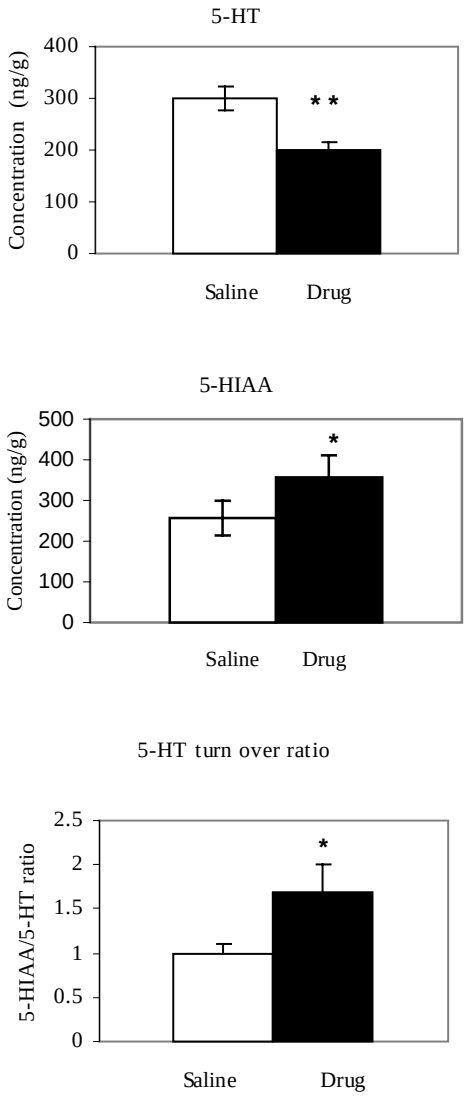

Fig. 5: Effects of mercuric chloride on 5-HT 5-HIAA and 5H1AA/5HT in rats. Values are means ±SD (n=6). Significant differences by student's t-test *P<0.05 and**P<0.01 from their respective saline treated controls.

DISCUSSION

Previous studies have shown that mercuric chloride used in the treatment of rheumatoid arthritis could produce autoimmune side effects in humans (Enestrom and Hultman, 1991; Goldman *et al*., 1991). Therefore dental amalgams are thought to be a risk factor in some patients with autoimmune disease (Bartova *et al*, 2003). Our data also supports the notion of the previous researchers (Goldman *et al*., 1991; Enestrom and Hultman, 1995). We report that a fairly good percentage (33%) of rats were susceptible to mercuric chloride induced autoimmune disorder at a dose of 1 mg/ml/kg and exhibited ANA positive reactions (Fig. 3)

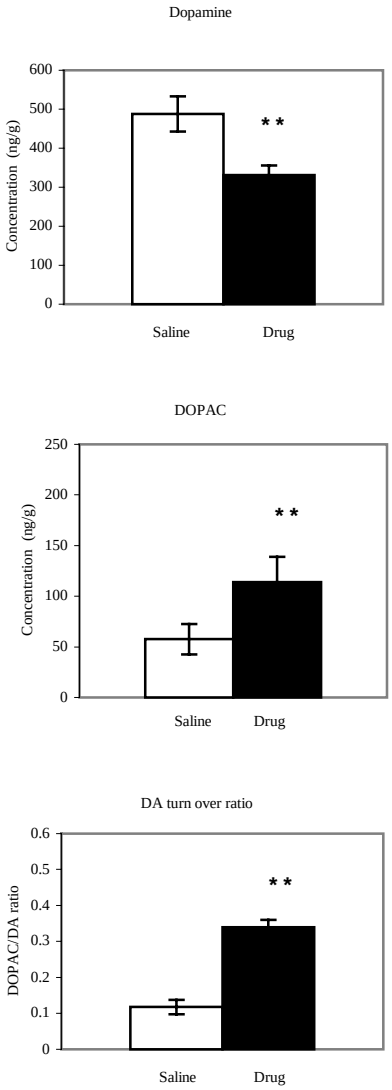

Fig. 6: Effects of mercuric chloride on DA, DOPAC and DOPAC/DA in rats. Values are means ±SD (n=6). Significant difference by student's t-test **P<0.01 from their saline injected counterparts.

The serotonergic system is implicated in the regulation of food intake. Enhancing 5-HT functions decreases food intake in experimental animals (Christensen and Brooks, 2006). Increased availability of tryptophan (5-HT precursor) to the brain has been documented as a common pathogenic mechanism for anorexia associated with different diseases (Cangiono *et al*., 1991 and Coskun *et al*., 2006).

In the present study brain tryptophan levels and 5-HIAA levels increased (fig. 4 and 5), suggesting an increase in 5-HT release. Indeed 5-HT turnover also increased in rats treated with mercuric chloride (fig. 5). A decrease of food intake in mercuric chloride treated animals, as reported by other authors (Lakshmana *et al*., 1993 and Abu *et al*., 2004), is therefore explainable in terms of an increase in serotonin induced satiety signal (Sugrue, 1987, Haider and Haleem, 2000 and Hsiao *et al*, 2006).

Administration of mercuric chloride induced increases in locomotor activity is also reported previously (Rocha *et al*., 2001) (fig. 2). It's however difficult to explain mercuric chloride induced hyperactivity in terms of increased 5-HT turnover because an increase in 5-HT functions decreases motor activity (Villegiar *et al*, 2006). Conversely an increase in dopamine metabolism increases motor activity (McNamra *et al*., 2006). The present results are therefore consistent with the previous studies showing that an increase in dopamine metabolism is involved in mercuric chloride hyperactivity. Serotonergic system interacts with Dopamine neurons at the level of caudate to control motor activity (Vihavainen, 2006). 5-HT acts via 5-HT2C receptors on the dopamine neurons to decrease motor activity (Pessia *et al*., 1994; Kapur and Ramington; 1996). Stimulation of somatodendritic 5-HT receptors by enhanced 5-HT turnover in the raphe region could decrease the availability of 5-HT in the caudate, releasing dopamine neurons from the inhibitory effects of 5-HT and elicit hyperactivity. It is therefore possible that administration of mercuric chloride largely increases brain 5-HT metabolism in the somatodendritic region to decrease the availability of 5-HT at DA neurons and elicit hyperactivity. Brain regional studies may be of use to further elucidate the role of 5-HT in mercuric chloride induced hyperactivity.

To conclude, we can suggest that along with tissue necrosis and other adverse effects; administration of mercuric chloride-induced increases of brain 5-HT and DA metabolism may tend to contribute to anorexia and hyperactivity seen in mercuric chloride exposed rats.

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