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Effect of Organic and Inorganic Mercuric Salts on Na+K+ATPase in Different Cerebral Fractions in Control and Intrauterine Growth-Retarded Rats: Alterations Induced by Serotonin

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ABSTRACT: *An intrauterine growth-retarded (IUGR) model based on restriction of blood supply to the rat fetus at the 17th day of pregnancy was studied. We investigated in vitro the effects of thimerosal and mercuric chloride on Na+K+ATPase activity in total brain homogenate, synaptosomes and myelin at weaning. In addition, we evaluated the reversal effect of serotonin on mercury-inhibited Na+K+ATPase activity. The toxicity, in terms of inhibition of Na+K+ATPase activity was greater with mercuric chloride than with thimerosal. Synaptosomes and principally myelin were more sensitive to the metal salts than total homogenate. Serotonin stimulated the Na+K+ATPase activity in total brain homogenate and synaptosomes but inhibited the enzyme in the myelin fraction. This effect was more marked in the IUGR group than in the control group. Serotonin (1 mM) added to total homogenate pretreated with the mercury salts produced variable reversal effects. In the synaptosomal fraction reverse effect was noted with serotonin. In myelin fraction, added serotonin increased inhibition caused by thimerosal.* © 1989 Intox Press, Inc.

Key Words: Thimerosal, Mercuric Chloride, Serotonin, Na+K+ATPase, Intrauterine Growth-Retarded Rats

INTRODUCTION

Mercurial compounds have been shown to be particularly damaging to the developing brain, resulting in degenerative alterations and biochemical changes in various brain regions both in humans and experimental animals (Reuhl and Chang, 1979; Bartolome *et al.*, 1984; Cheung and Verity, 1985; Choi, 1986). Different reports have shown that heavy metals, Hg, in particular, effect directly myelin and Schwann cells and can induce hypomyelinization in developing brain (Grundt *et al.*, 1980; Ganser and Kirsher, 1985). Modifications in neurotransmitter levels and of uptake and turnover of dopamine and noradrenaline have also been observed, (Komulainen and Tuomisto, 1981; Rajana and Hobson, 1985). Moreover, the mercurials have been associated with changes in the activity of certain enzymes containing active sulfhydryl groups such as Na+K+ATPase (Grundt *et al.*, 1982; Kaplan and Mone, 1985; Magour, 1986; Unnikumar *et al.*, 1987). This enzyme plays a major role in neuronal functions and in the

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maintenance of an inward directed sodium electrochemical gradient across the membranes and also in the control of the uptake and release of neurotransmitters at the presynaptic level (Schwartz, 1972; Schurmans-Stekovens, 1981; Anner, 1985).

We have previously demonstrated that lead induces significant inhibition of Na^+K^+ATPase in developing rats (Chanez *et al.*, 1987; Chanez *et al.*, 1988). Since nutritional status influences susceptibility to metal toxicity, we have developed an undernourished model based on the restriction of the blood supply on the 17th day of gestation (Wigglesworth, 1964). Using this intrauterine growth-retarded model (IUGR), we have shown, *in vitro*, different susceptibilities to the metal, in terms of enzyme activity or effects of neurotransmitters on enzyme activity (Chanez, 1985).

In this study, we evaluate the different toxic effects on the central nervous system of thimerosal (an organic compound that may cross the blood brain barrier) and mercuric chloride (inorganic salt) by measuring Na^+K^+ATPase activity in total brain homogenate, synaptosomes and myelin.

MATERIALS AND METHODS

In female rats of Shermann strain the uterine blood supply was restricted according to the method of Wigglesworth (1964). After birth 4 control and 4 IUGR pups were kept with lactating mothers until 22 days post-natal. Rats were decapitated at 10 a.m. and their brain quickly removed and dissected at 4°C. Preliminary experiments indicated that there was no sex-related difference, in Na^+K^+ATPase activity and both males and females were used at random.

Synaptosomes were prepared according to the method of Hajos (1975).

Myelin was prepared according to the procedure of Norton (1973). Freshly thawed material was used in each experiment. The Na^+K^+ATPase activity was greater in frozen myelin than in fresh myelin. Purity of myelin was verified by electron microscopy, lipid analysis, protein electrophoresis and radioimmunoassays as previously reported (Bourre *et al.*, 1984).

Total brain, purified synaptosomes and myelin were homogenized in ice-cold bidistilled water and the Na^+K^+ATPase activity was measured by the method of Abdel-Latif *et al.* (1967).

Ethylmercurithiosalicylic acid (thimerosal) or mercuric chloride ($HgCl_2$) were introduced at the beginning of the preincubation period. All determinations were made in triplicate. Enzyme activity was expressed as µmol Pi liberated per mg of protein and per hr (Fiske and Subbarow, 1925). Protein concentration was determined according to the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. Statistical calculations were performed according to Snedecor (1967), when $P > 0.05$ (Student's t-test) the difference was considered not to be significant.

RESULTS

The protein content in total homogenate, synaptosome and myelin fractions was similar in both groups. The synaptosomes/wet brain weight and myelin/wet brain weight ratios were also similar (Chanez *et al.*, 1988).

Basal and Serotonin-Stimulated Na^+K^+ATPase Activity in Total Brain Homogenate, Synaptosomes and Myelin in IUGR and Control Rats

Na^+K^+ATPase in total homogenate was significantly lower in the IUGR group than in the control group. This difference existed until 60 days (Chanez, 1985). 1 mM of serotonin added to the medium produced a maximal stimulatory effect on basal enzyme activity (22% for the control and 38% for IUGR rats). (Fig. 1A and 2A).

In the synaptosomal fraction, the Na^+K^+ATPase activity was higher than in total homogenate but no significant difference was observed between the IUGR and the control groups. As in total homogenate, serotonin (1mM) produced a marked stimulatory effect

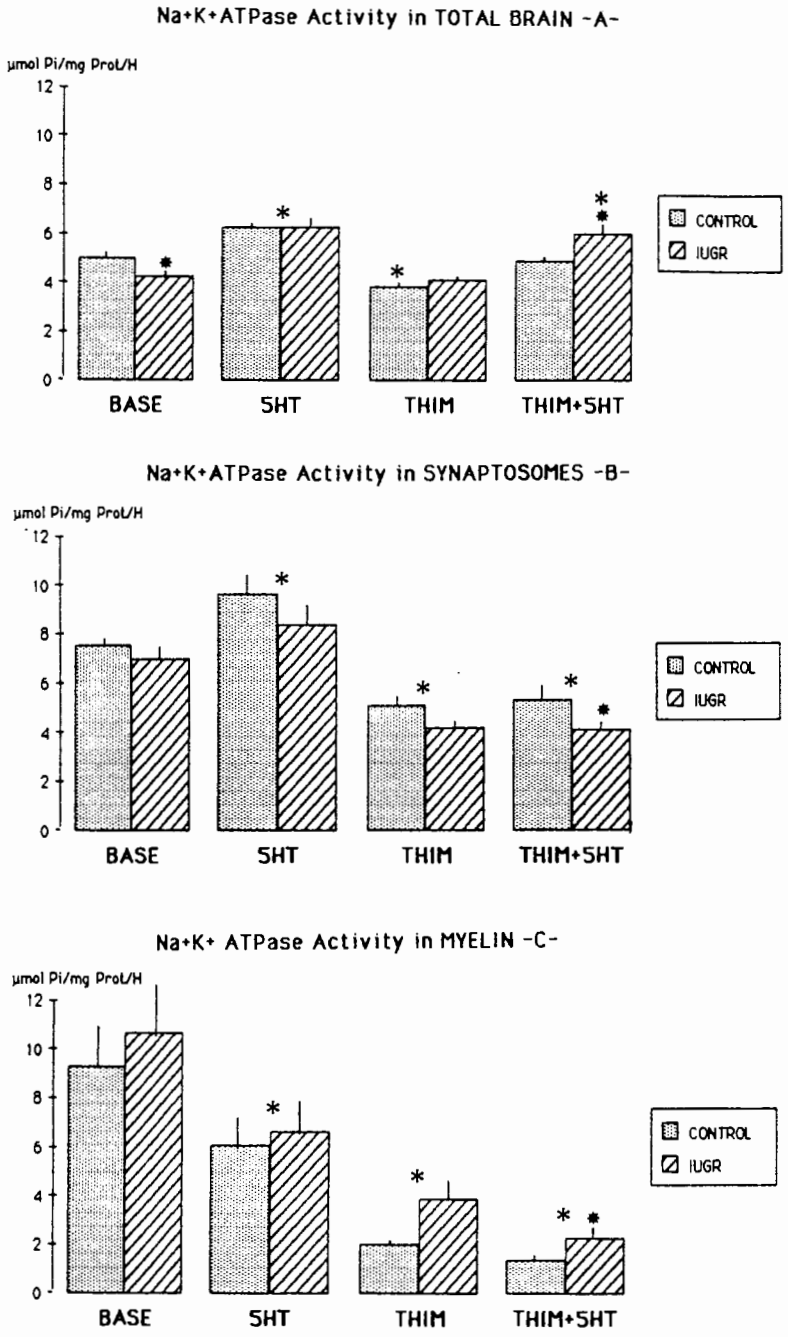

FIG. 1. A.B.C. Effect of thimerosal (5 x 10^{-5}M) onNa^+K^+ATPase activity in total brain homogenate (A), synaptosomes (B) and myelin (C), in the presence or the absence of 5-HT (1mM). Each bar represents the mean (SEM) of 6 to 8 individual measurements ofNa^+K^+ATPase activity (in µmol Pi per mg protein per hr).

*$p < 0.05$ compared to values of pair-aged control rats.

**$p < 0.05$ to 0.001 for 5-HT, Thim + 5-HT compared to basal values.

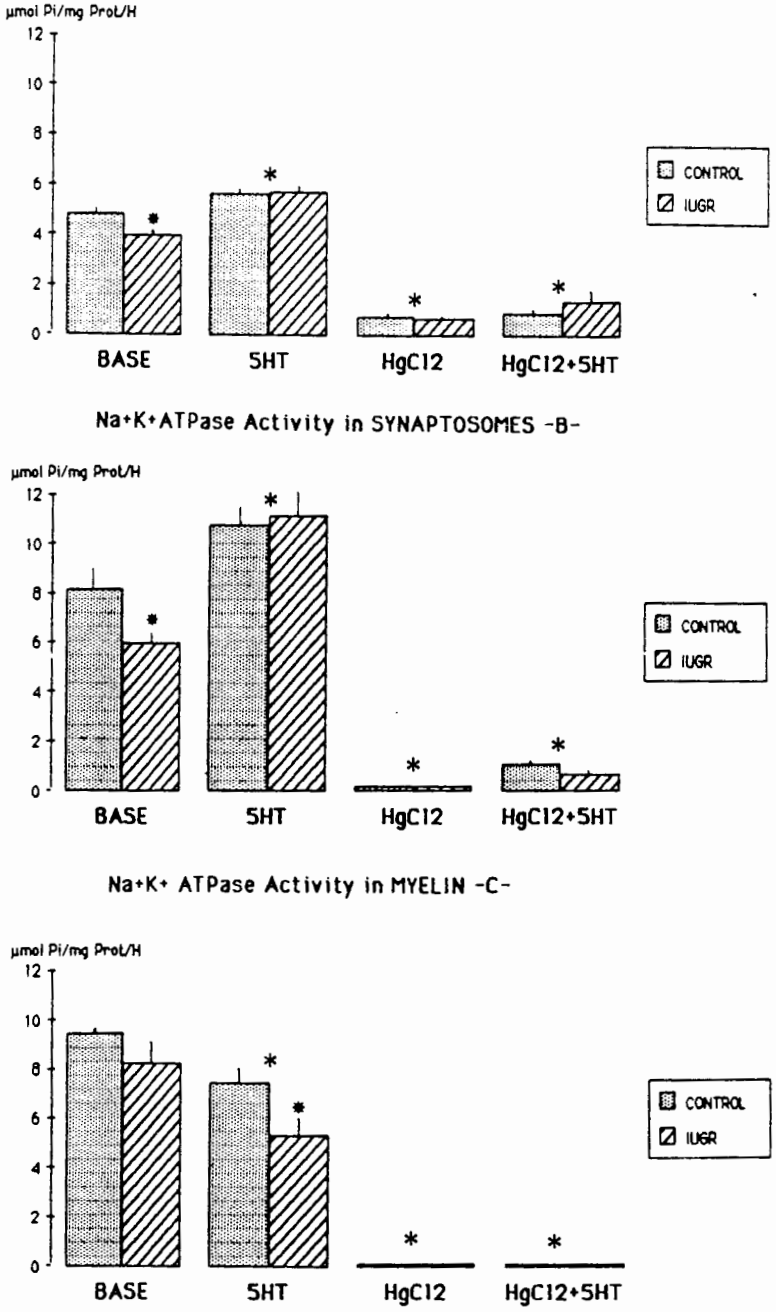

FIG. 2. A.B.C. Effect of mercuric chloride (1 x 10^{-5}M) on Na+K+ATPase activity in total brain homogenate (A) synaptosomes (B) and myelin (C), in the presence or the absence of 5.HT (1mM). Each bar represents the mean (± SEM) of 6 to 8 individual measurements of Na+K+ATPase activity (in µmol Pi per mg protein per hr).
*$p < 0.05$ compared to pair-aged control rats.
**$p < 0.05$ to 0.001 for 5-HT, Thim and Thim 5-HT compared to basal values.

on Na^+K^+ATPase activity especially in the IUGR rats. (Fig. lB and 2B).

In the myelin fraction, the basal Na^+K^+ATPase activity in both groups was 2-fold higher than in total homogenate. These levels correspond to adult levels. In contrast to findings in total homogenate and synaptosome, 5-HT in myelin preparations inhibits Na^+K^+ATPase activity. 9.39 ± 0.9 micromoles Pi basal activity versus 6.7 ± 0.9 with 5-HT in control rats and 9.44 ± 1.22 micromoles Pi basal activity versus 5.98 ± 1 with 5-HT in IUGR rats (Fig. lC and 2C).

Effects of Thimerosal and Mercuric Chloride in Total Homogenate, Synaptosomes and Myelin in IUGR and Control Rats

The effects of thimerosal (5 x 10^{-5}M) and $HgCl_2$ (1 x 10^{-5}M) at a concentration approximately equal to IC50 on Na^+K^+ATPase activity were compared in three different brain fractions. Pretreatment of total homogenate (Fig. 1A) with thimerosal moderately affected the Na^+K^+ATPase activity of control rats (26% reduction). The IUGR rats were less sensitive since similar Na^+K^+ATPase values were found with and without thimerosal. Pretreatment with $HgCl_2$ produced a strong inhibition of the enzyme activity in both groups (about 84%). The synaptosomal preparation was more sensitive to mercuric salts than total brain homogenate. Thimerosal inhibited enzyme activity by about 36% in control and IUGR rats but 100% of inhibition occurred with $HgCl_2$ (Fig. 1B and 2B). In myelin preparation, this inhibition with thimerosal was 78% in control rat and 65% in IUGR rat and 100% for $HgCl_2$ (Fig. lC and 2C).

5-HT was added to the different media to a final concentration of 1 mM. This concentration yielded maximal stimulation in total homogenate and synaptosomes without the metal salts. The reversal effect of this neuro-transmitter was expressed as the capacity to restore mercury inhibited activity to basal values.

The inhibition caused by thimerosal in total homogenate was almost reversed by 5-HT in the control group. In IUGR rats, the enzyme was not inhibited by thimerosal. The 5-HT added to the medium with thimerosal stimulated the Na^+K^+ATPase activity to the same extent as that in a medium with 5-HT alone (Fig. 1A and 2A). Slight differences were observed in synaptosomal fraction (Fig. 1B and 2B).

Interestingly, in the myelin fraction, 5-HT not only failed to reverse mercuric salt inhibition but itself had an inhibitory effect on Na^+K^+ATPase activity. The addition of 5-HT to myelin pretreated with thimerosal increased enzyme inhibition by 41% and 31% in IUGR and control rats respectively. This increase corresponds to the inhibitory effect of 5-HT on the myelin preparation without thimerosal (Fig. 1C and 2C).

DISCUSSION

The neurotoxicity of two mercuric salts during perinatal development results in marked and variable modifications in the specific Na^+K^+ATPase activity in total and subcellular fractions in IUGR and control rats. In this study, the difference in Na^+K^+ATPase activity found in synaptosomes and myelin was not a consequence of other cellular contaminants (see Methods).

In synaptosomes as in total brain homogenate, the stimulatory effect of 5-HT on Na^+K^+ATPase activity was significantly increased in IUGR and control rats compared to basal values. The Na^+K^+ATPase activity was not modulated by 5-HT in myelin fractions. Discrepancies between sensitivity of synaptosomes and myelin to 5-HT may be due to different washing protocols used to obtain this subcellular fraction. Some authors have proposed that monoamines do not directly stimulate Na^+K^+ATPase but rather reverse the inhibitory effect on the enzyme of a water-soluble and dializable factor(s) obtained in cytoplasm by high-speed centrifugation (Schaefer *et al.*, 1972; Gilbert *et al.*, 1980; Chanez *et al.*, 1987; Chanez *et al.*, 1988). In the absence of such factor(s), the monoamines have an inhibitory effect on Na^+K^+ATPase activity which is not mediated by adrenergic receptors.

The different inhibitory actions of metal salts on the activity of Na^+K^+ATPase have been examined, and it may be assumed that $HgCl_2$ (which binds strongly to SH-groups) may alter the stereospecificity of the enzyme, thus reducing access to the catalytic site of ATPase (Vallee and Ulmer, 1972). The difference in inhibitory potency between bivalent mercuric ions and nonvalent organomercurials can be explained by the fact that the inorganic mercuric ions can react with two sulfhydryl equivalents whereas organic mercuric salt can react with only one thiol group of the enzyme (Patzelt-Wenczler and Schoner, 1981). Henderson *et al.*, 1979, suggested also that mercuric salts can have selective or nonspecific effects on Na^+K^+ATPase activity by inhibition of the enzyme alone, or of both the enzyme and partial enzyme reactions.

Because of previous reports on the metal chelating potency of monoamines for reversing *in situ* inhibition caused by divalent metals or for preventing lipid peroxidation (Hexum, 1977; Sawas and Gilbert, 1982; Chanez, *et al.*, 1988; Chapman and Greenwood, 1988), we tested the chelating effects of serotonin. The significant reversal effect obtained with 5-HT observed especially in total homogenate compared to myelin fraction could suggest the hypothesis of an action of a soluble factor or digitalis-like factor in the inhibitory mechanism (Morise *et al.*, 1988; Rodriquez de Lores Arnaiz *et al.*, 1988; Rauch and Buckalew, 1988). A nonspecific competitivity or affinity between $HgCl_2$, 5-HT and the factor(s) and Na^+K^+ATPase could partly explain our different results with homogenate and myelin, the principle of which would be a reduction of inhibition. This mechanism being more marked in IUGR argues for a higher level of these factors in total brain homogenate in this experimental group.

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