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Na^+K^+ATPase ACTIVITY IN SYNAPTOSOMES AND MYELIN OF DEVELOPING CONTROL AND INTRA-UTERINE GROWTH RETARDED RATS: EFFECTS OF LEAD AND SEROTONIN

CLAUDE CHANEZ*, PASCAL BARONE*, MARIE-ANGE FLEXOR* and JEAN-MARIE BOURRE†
*INSERM Unité 29, Hôpital Port-Royal, 123 Boulevard de Port-Royal, 75014 Paris, France and †INSERM Unité 26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris, France

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Abstract—An intrauterine growth retarded (IUGR) model based on restriction of blood supply to fetuses at 17 days of pregnancy in rats was studied. We investigated *in vitro* the effects of lead on Na^+K^+ATPase activity in synaptosomes and myelin of IUGR and control rats from 6 to 60 days after birth. In both groups an age-dependent effects existed in synaptosomes for the lowest doses of lead. The experimental group tended to be more sensitive to the metal than the control group and the Na^+K^+ATPase activity was less inhibited in the younger rats as compared to mature rats. Serotonin (5-HT) added to the subcellular preparations produced different changes in Na^+K^+ATPase activity. In synaptosomes, 5-HT stimulated the enzyme activity in a dose-related manner and apparently reversed the inhibiton induced by lead up to 22 days after birth in the control group. This action was less marked in the IUGR group. In myelin fractions, the Na^+K^+ATPase activity was inhibited by lead in both groups but the "protective effect of monoamines" was never observed. The Na^+K^+ATPase activity was modulated by monoamines in synaptosomes and not in myelin, perhaps through a mechanism involving soluble factor(s).

Lead compounds are known to be environmental contaminants. A major target of these poisons is the central nervous system. Heavy metals, in general, have been shown to interact with the cell membrane and to alter its function. Lead toxicity causes growth retardation, intellectual impairment, neurophysiological and electroencephalographic abnormalities (Bushnell and Bowman, 1979; Mahaffey, 1981: Sauerhoff and Michaelson, 1973; Sobotka and Cook, 1974).

Neonatal lead toxicity induces a variation in the regional distribution of the monoamine neurotransmitters in the brain (Govoni *et al.*, 1980; Silbergeld and Goldberg, 1975). Moreover, several studies have shown that certain divalent ions of toxicological interest are potent inhibitors of brain Na^+K^+ATPase (Chanez *et al.*, 1987; Hexum, 1977; Prakash *et al.*, 1973). *In vitro* and *in vivo* effects of lead on membrane-bound ATPase have also been reported (Nechay and Saunders, 1978). Na^+K^+ATPase in various subcellular fractions is considered to be responsible for the maintenance of an inward sodium electrochemical gradient across almost all biological membranes (Abdel-Latif *et al.*, 1967; Anner, 1985; Schurmans-Stekhovens and Bonting, 1981; Schwartz *et al.*, 1972) Altered activity of this enzyme could therefore account for neuronal dysfunction in undernourished rats during the perinatal period (Kissane and Hawrylewicz, 1978).

(1) In a recent study (Chanez *et al.*, 1987), we have demonstrated in brain homogenates that the neurotoxic effect of lead is an age-related phenomenon. Lead alters the Na^+K^+ATPase activity to a lower extent in Intra Uterine Growth Retarded (IUGR) rats than in age-paired controls.

(2) We developed an "*in utero*" undernutrition model based on restriction of blood supply to fetuses on the 17th day of pregnancy (Wigglesworth, 1964). This method has many advantages compared to models of malnutrition by protein deprivation of dams during gestation and or lactation. Firstly, the fetuses are directly undernourished, thus excluding the indirect influence of nutritional or endocrine imbalances in the mother. Secondly, since only one uterine horn is operated, normal and IUGR animals are obtained in the same litter. Finally, this type of undernutrition has clinical relevance since it corresponds to the situation frequently encountered in neonatology (Chanez *et al.*, 1985; Morand *et al.*, 1982).

(3) We have previously demonstrated in our IUGR model a significant decrease of Na^+K^+ATPase activ-

ity and altered levels of serotonin or norepinephrine in different brain areas (Chanez *et al.*, 1985). Our aim is this study was to examine the effect of lead on synaptosomes and myelin both of which contain Na^+K^+ATPase an enzyme of considerable importance to the functional development of the CNS. By selecting synaptosomes and myelin, rather than whole brain homogenates, we could measure more accurately the effects of lead on Na^+K^+ATPase activity and the apparent reversal effect of 5-HT in our experimental model during postnatal development.

EXPERIMENTAL PROCEDURES

Female rats of Sherman strain were fed to Extralabo standard diet *ad libitum*. Gestational age was ascertained by allowing access to the male on one single occasion. Uterine blood supply was restricted according to the method of Wigglesworth (1964). Briefly, on the 17th day of gestation a laparotomy was performed under ether anesthesia, the uterine horns were exposed and both the uterine artery and vein were ligated at the lowest part of one horn. The closer a fetus was to the ligation, the smaller its weight; near the ligation some fetuses were even resorbed completely. At birth, an animal was considered as being IUGR when its body weight was reduced by at least 40% as compared to controls from the same litter. Using this criterion, approximately 35% of the mothers undergoing laparotomy had offspring showing IUGR. Eighty-seven per cent of these animals were viable and showed no malformation. After birth, 4 control and 4 IUGR pups were kept with lactating mothers until weaning. For the whole period, all animals had free access to standard chow and water. At various time intervals rats were decapited between 10 a.m. and 12 a.m. and their brains 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.

Preparation of synpatosomes fractions

Fractions were prepared according to the method of Hajos (1975) with slight modifications. Forebrains were homogenized in 0.32 M sucrose and centrifuged at 1500 *g* for 10 min and the supernatant centrifuged at 8500 *g* for 20 min. The pellet was then resuspended in 0.32 M sucrose layered onto 0.80 M sucrose and centrifuged in a SW28 Beckmann at 8000 *g* for 20 min. Myelin floating on the top of the gradient was discarded and synaptosomes were taken from the 0.80 M sucrose fraction without the pellet. Finally, synaptosomes were concentrated by centrifugation at 11,000 *g* for 30 min. The synaptosomal purity was checked at all rat ages by electron microscopy and assay of enzymes associated with membranes (ATPase) or cytoplasm (lactic acid deshydrogenase). The relative advantages of this method are the speed of preparation (90 min), the enrichement in synaptosomes (60 to 80%) and the low contamination by various particles principally free mitochondria (about 20%).

Preparation of myelin fractions

Myelin was prepared from fresh rat brain according to the procedure of Norton (1973). Tissue was homogenized in 0.32 M sucrose and an initial density gradient centrifugation performed over a layer of 0.85 M sucrose. The myelin fraction was washed once with deionized water and resulting pellets were resuspended in 0.32 M sucrose, layered on 0.85 M sucrose and centrifuged from 35 min at 75,000 *g*. The myelin was removed from the interface, washed twice with deionized water and centrifuged at 12,000 *g* for 15 min. In the experiments with 13- and 16-day-old rats, the six crude myelin layers were pooled in two or three centrifuge tubes. All steps were performed at 0–4°C. The samples of myelin were frozen and stored at −80°C. 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).

Determination of Na^+K^+ATPase activity

Purified synaptosomes and myelin were homogenized in ice-cold bidistilled water. Na^+K^+ATPase activity was measured (Abdel-Latif *et al.*, 1967) in a mixture of 40 mM Tris-HCl, 150 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, in the presence or absence of 0.75 mM ouabain; pH 7.4, total volume 1.1 ml. After preincubation for 10 min at 37°C the reaction was started by addition of 4 mM Tris-ATP and stopped 15 min later by addition of 1 ml ice-cold 10% trichloroacetic acid. Additives were introduced at the beginning of the preincubation period. After centrifugation at 3000 *g* for 15 min, inorganic phosphate was determined in an aliquot (0.5 ml) of the supernatant according to the method of Fiske and Subbarow (1925). Na^+K^+ATPase activity was estimated by subtracting Mg^{2+} ouabain-insensitive ATPase from total ATPase activity. Under such conditions, Na^+K^+ATPase activity was linear for at least 30 minutes at 37°C. All determinations were made in triplicate. Enzyme activity was expressed as μ mol Pi liberated per mg of protein per h. 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 to be non-significant.

RESULTS

1. Body weight, forebrain weight, synaptosomal and myelin protein content in developing IUGR and control rats

On the first day after the ligation (day 17 of pregnancy) IUGR fetuses showed a 20% reduction of body weight relative to control fetuses. This percentage increased until birth when it reached an average of 40%. This significant reduction persisted to adulthood whatever the rearing conditions. Data (Table 1) indicate that the forebrain weight of IUGR rats was markedly less affected than the body weight since only an 18% reduction was found compared to age-paired control animals. This significant difference persisted in IUGR rats six weeks after birth. The protein content of synaptosomes and myelin determined as mg/100 mg of fresh tissue increased during

Table 1. Developmental changes in the weights of body and forebrain, and protein contents of synaptosomes and myelin in IUGR and control rats

Postnatal age (days)		Body weight (g)	Forebrain weight (g)	Synaptosomes Prot. (mg/100 mg wet tissue)	Myelin Prot. (mg/100 mg wet tissue)
6	C	15.5 ± 0.8	0.48 ± 0.03	2.62 ± 0.24	ND
	E	10.1 ± 0.9*	0.41 ± 0.01*	2.45 ± 0.24	
15	C	38.1 ± 2.1	0.98 ± 0.05	3.20 ± 0.15	0.57 ± 0.05
	E	25.2 ± 3.1*	0.84 ± 0.07*	2.79 ± 0.22	0.47 ± 0.04
22	C	53.2 ± 6.7	1.07 ± 0.06	4.17 ± 0.14	1.49 ± 0.14
	E	31.8 ± 5.1*	0.92 ± 0.09	3.61 ± 0.20	1.52 ± 0.16
30	C	111.7 ± 2.5	1.12 ± 0.02	ND	1.98 ± 0.12
	E	84 ± 3.6*	1.02 ± 0.01*		1.85 ± 0.07
60	C	242.0 ± 29.8	1.31 ± 0.09	3.33 ± 0.26	2.39 ± 0.15
	E	141.5 ± 14.1*	1.16 ± 0.05*	2.97 ± 0.17	2.01 ± 0.20

Each value expressed (in mg of g) is the mean ± SEM of 10–20 individual determinations. $P < 0.05$ compared to values from age-paired control rats. *C = control rats and E = experimental rats (IUGR); ND = not determined.

development from 2.62 mg six days after birth to 3.3 mg sixty days after birth for synaptosomes and from 0.57 mg to 2.33 mg for myelin (Table 1). No significant difference in the protein contents was found between IUGR and control rats at any age.

2. Basal and 5-HT-stimulated $Na^+ K^+$ ATPase activity in total homogenates, synaptosomes and myelin from developing IUGR and control rats

Immediately after birth, $Na^+ K^+$ ATPase activity was too low to permit significant measurements. In both groups, $Na^+ K^+$ ATPase activity in forebrain homogenates, synaptosomes and myelin increased markedly during postnatal development.

In homogenates, $Na^+ K^+$ ATPase was low in the pups but in mature animals (60 days old) we observed activities 3.5 to 5 times higher than at 6 days. Significant differences noted on the 6th postnatal day between IUGR and control rats persisted for at least one month after birth (Fig. 1, 2).

In synaptosomes (Fig. 1), the activity was 2 times higher than in homogenates but no significant difference was observed between IUGR and control rats. Serotonin (1 mM) added to synaptosomes produced a stimulatory effect on $Na^+ K^+$ ATPase activity in experimental and control animals. The potency of this monoamine varied with age of rats.

In myelin fractions (Fig. 2), $Na^+ K^+$ ATPase activity was considerably higher than in total homogenates. The enzyme activity on day 13 (not shown) and 16 in IUGR and control rats was 5 times higher than in the older group. The values of enzyme activity decreased during development and after the weaning, the $Na^+ K^+$ ATPase activity in the older group was only 1.5 to 2 times higher than in total brain homogenate.

The increased values found in myelin fraction in the younger rats were estimated after numerous dosages. In fact, since the quantities of myelin collected per animal were too low for correct manipulation, it was necessary to stock the myelin pellets over a period of two to three weeks. However after this period, we observed a substantial decrease of $Na^+ K^+$ ATPase activity in relation to freezing time. Therefore, we measured enzyme in myelin from fresh-frozen pellets.

In contrast to findings in synaptosomes:

(i) Significant differences were observed in myelin preparations between IUGR and control rats. At the 13th (not shown) and 16th postnatal days, the $Na^+ K^+$ ATPase activity was lower in IUGR than in control rats.

Opposite results were noted in 60 day-old rats.

(ii) $Na^+ K^+$ ATPase activity was inhibited by 5-HT (1 mM) in myelin preparations. This inhibitory action was particularly marked in the 16-day-old animals: $14.38 \pm 1.02\ \mu M$ Pi basal activity versus 5.87 ± 0.70 with 5-HT (1 mM) in the control group and $10.91 \pm 0.9\ \mu M$ Pi basal activity versus 4.90 ± 1.02 with 5-HT (1 mM) in the IUGR group.

3. Effects of lead on $Na^+ K^+$ ATPase activity in synaptosomes and myelin fractions in developing IUGR and control rats

The action of various doses of lead in the two subcellular fractions was measured:

1. In synaptosome preparations (Fig. 1) lead produced dose-dependent inhibition. The mean lead

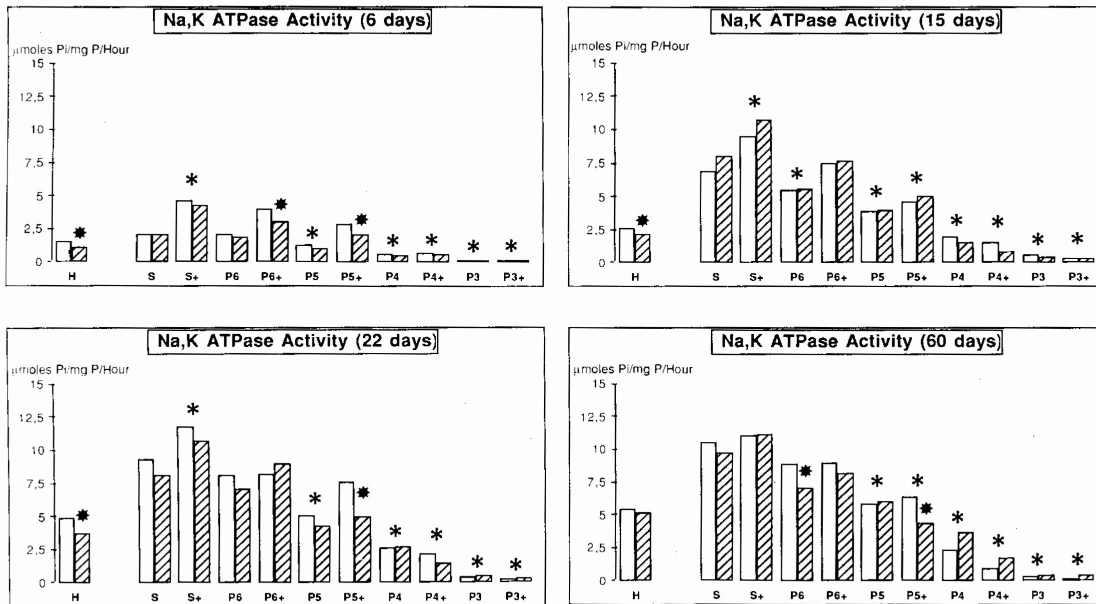

Fig. 1. Effects of P6 (Pb 10^{-6} M) P5 (Pb 10^{-5} M) P4 (Pb 10^{-4} M) and P3 (Pb 10^{-3} M) on Na^+K^+ATPase activity in synaptosomes in the presence (+) or the absence of 1 mM 5HT. The homogenate values (H) are given for comparison with synaptosomal values (S). Each bar represents the mean ± SEM of 6–8 individual measurements of Na^+K^+ATPase activity (in μmol Pi per mg protein and per hour). ✱: $P < 0.05$ compared to values of age-paired control rats; *: $P < 0.05$ to 0.001 compared to synaptosome basal values. Empty bars: control rats; hatched bars: IUGR rats.

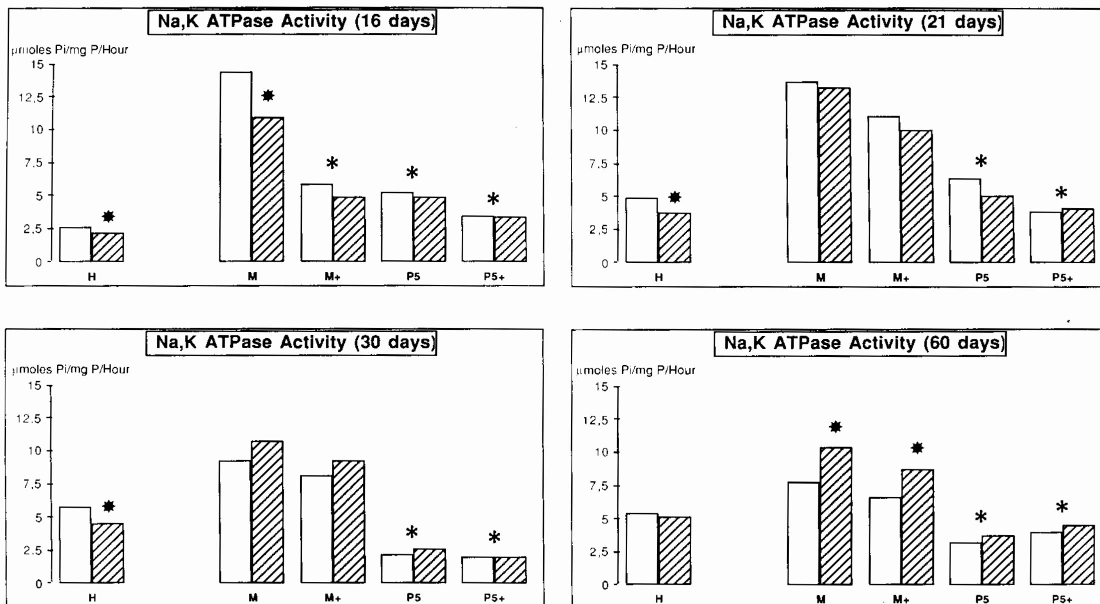

Fig. 2. Effects of P5 (Pb 10^{-5} M) on Na^+K^+ATPase activity in the myelin in the presence (+) or absence of 1 mM 5HT. The homogenate values (H) are given for comparison with myelin values (M). Each bar represents the mean ± SEM of 6–8 individual measurements of Na^+K^+ATPase activity (in μmol Pi formed per mg protein and per hour). ✱: $P < 0.05$ compared to values of age-paired control rats; *: $P < 0.05$ to 0.001 compared to myelin basal values. Empty bars: control rats; hatched bars: IUGR rats.

inhibition expressed as % of control was 96 ± 7; 73 ± 7; 45 ± 5 and 15 ± 5 for 1; 0.1; 0.01 and 0.001 mM Pb respectively. The younger rats seemed less sensitive to the toxic effects of lead as compared to older rats: for example, with 0.001 mM Pb, the $Na^+K^+ATPase$ values for young rats were similar to the basal values whereas at 60 days the enzyme activity was inhibited by about 20%. A trend to increasing lead sensitivity was found in IUGR rats such that at 22 and at 60 days the values from IUGR animals were significantly lower than those from age-paired controls.

2. In myelin fractions, the sensitivity of $Na^+K^+ATPase$ to the inhibitory action of 0.01 mM Pb was marked at 16, 21, 30 and 60 days. The mean of enzyme inhibition reached about 60% in all animals studied except in 30-day-old rats (75%).

IUGR and control rats reacted similarly on lead administration; inhibition of $Na^+K^+ATPase$ by 0.01 mM Pb in the experimental group was the same as in control group. In IUGR and controls rats an age-dependent inhibition of $Na^+K^+ATPase$ activity was seen in synaptosomal fractions at the lowest Pb doses but this was not observed in the myelin preparations.

4. Reversal of lead inhibition by 5-HT in synaptosomes and myelin fractions in developing IUGR and control rats

5-HT was able to increase $Na^+K^+ATPase$ activity in the synaptosomal fractions but not in the myelin fractions. Lead produced a dose-dependent inhibition in both preparations. The activity of $Na^+K^+ATPase$ with the two effectors (lead and 5HT) was plotted in $P_6 + P_5 + P_4 + P_3$. Figures 1 and 2 show the inhibitory effects of various doses of lead on $Na^+K^+ATPase$ activity when 5-HT was added to the medium at a final concentration of 1 mM. The reversal potency of 5-HT was seem as its capacity to restore lead inhibited activity to basal levels (**S or M**) or stimulated levels (**S+ M+**) (i.e. to values without lead).

—**In synaptosomes,** the inhibition caused by 0.001 mM Pb was entirely reversed by 5-HT (1 mM) at 6, 15 and 22 days. The action was especially marked in 6-day-old rats since the reversed values were similar to stimulated control values. In these animals similar inhibitory effects were also induced by 0.01 mM Pb. In the oldest rats at all Pb concentrations added to the medium, basal $Na^+K^+ATPase$ values were not restored; 0.01 mM Pb and 0.1 mM Pb doses induced decreases that were only partially reversed. The maximal inhibition (at 1 mM) was not sensitive to 5-HT stimulation even when the monoamine was used in quantities equimolar to Pb.

In IUGR rats, the protective effects of 5-HT was significantly less marked than in the control group: at 0.001 mM Pb, reversal of lead inhibition was significantly lower in 6- and 60-day-old IUGR rats and at 0.01 mM Pb, in 16-, 22- and 60-day-old rats compared with age-paired controls.

—**In myelin fractions,** in contrast to synaptosomes, the inhibition induced by Pb 0.01 mM was not reversed by 1 mM 5-HT.

DISCUSSION

This study confirms an earlier report that lead causes *in vitro* a dose-dependent inhibition of $Na^+K^+ATPase$ activity in brain homogenates of developing IUGR and control rats (Chanez *et al.*, 1987). In this report we have also measured the effect of lead on $Na^+K^+ATPase$ activity in synaptosomes and myelin and the reversal action of 5-HT. A comparison has been made with results obtained using brain homogenate. The effects on $Na^+K^+ATPase$ activity in the subcellular fractions *in vitro* may be related to the overall neurotoxicity of metals observed *in vivo* during development.

In synaptosomes and myelin, the basal activity of $Na^+K^+ATPase$ at all ages studied was significantly higher compared with basal homogenate values in both IUGR and control rats. Previous reports have consistently shown $Na^+K^+ATPase$ activity to be present in isolated myelin fractions (Bourre *et al.*, 1982; Reiss *et al.*, 1981; Zimmerman *et al.*, 1982), although this has been interpreted as possible contamination of myelin by other membranes (Danks *et al.*, 1979). However, biochemical and immunocytochemical studies (Mrsulja *et al.*, 1985; Schwartz *et al.*, 1980), have shown $Na^+K^+ATPase$ to be present in the myelin. The use of various protocols for myelin isolation probably explains controversial results in the values reported for enzyme activity. In the present study, the purity of synaptosomes and myelin preparations was assayed at 6, 16, 22 and 60 days by electron microscopy and marker enzyme. The enrichment in $Na^+K^+ATPase$ found in synaptosomes and myelin was not a consequence of other subcellular contaminants.

The relative increase of $Na^+K^+ATPase$ activity found in synaptosomes and myelin compared to homogenate was more marked in young than in mature animals. In myelin fraction, the enzyme activity was inversely modified by lead in the youngest and oldest IUGR rats as compared with age-paired controls. In this fraction, the decrease of $Na^+K^+ATPase$

response to 5-HT (1 mM) was particularly significant in 16-day-old IUGR and control rats. Similar results were observed with norepinephrine at concentrations of 5×10^{-4} M to 5×10^{-6} M added to the medium (not shown). In synaptosomes as in homogenate, the stimulatory potency of 5-HT was significantly increased in both IUGR and control rats during the developmental stage (6–22 days) which corresponds to the most rapid period of synaptogenesis (Wu and Phillis, 1981b). The reduction in the responsiveness of the enzyme in the oldest animals may be due to the increasing contribution of glial Na^+K^+ATPase to the total enzyme pool. This suggestion is in agreement with the findings of Sweadner (1979).

Discrepancies between sensitivities of synaptosomes and myelin Na^+K^+ATPase to monoamines may be due to different protocols used to obtained the subcellular fractions. In homogenates, synaptosomes and particular fractions, several authors (Rodriguez de Lores Arnaiz and Mistrorigo de Pacheco, 1978; Gilbert *et al.*, 1980; Godfraind *et al.*, 1974; Schaefer *et al.*, 1972) have demonstrated that monoamines do not directly stimulate Na^+K^+ATPase activity but rather reverse the inhibitory effect on the enzyme of a heat stable and dialyzable factor contained in cytoplasm. This soluble factor is obtained by high-speed centrifugation and requires complete disruption by freezing or osmotic shock. These authors reported that noradrenaline stimulated Na^+K^+ATPase in the presence of the fraction containing this factor, but in its absence noradrenaline (concentration $> 10^{-4}$ M) had a weak inhibitory effect on Na^+K^+ATPase which has not mediated by adrenergic receptors. In myelin preparations several wash centrifugations eliminated this soluble factor and the serotonin and noepinephine added in the medium can inhibit Na^+K^+ATPase activity. In this study with similar concentration of 5-HT: 10^{-3} M or NA: 10^{-4} M (not shown) we noted a significant inhibitory effect in the younger IUGR and control animal. At the other ages the monoamines added in the medium failed to enhance the enzyme activity.

The inhibitory action of metals on the activity of the enzyme has been extensively examined *in vitro* in brain homogenates, synaptosomal and microsomal preparations (Chandra *et al.*, 1984; Chanez *et al.*, 1987; Hexum, 1977; Kai *et al.*, 1980; Prakash *et al.*, 1973; Rajana *et al.*, 1983). The two subcellular fractions synaptosomes and myelin were affected in the same way by the divalent metal and the ability of lead to inhibit Na^+K^+ATPase activity varied in parallel with the results found in total brain homogenates.

A higher affinity for lead or a more efficient binding might perhaps explain the increased metal inhibition particularly noted in myelin preparations. In this subcellular fraction, an alteration in the surrounding enzymes can modify Na^+K^+ATPase activity.

Our findings demonstrate in synaptosomal preparation a complete reversal effect of the neurotransmitters at the lowest concentrations of lead. However, this effect was significantly decreased in the IUGR group. Furthermore, synaptosomes and myelin were modulated differently by the monoamines. These results obtained with a heavy metal such as lead are comparable with other studies on the mercuric salts. A more extensive study will be required to determine the nature of the soluble factor(s) and its role in the mediation of Na^+K^+ATPase activity in different subcellular brain fractions of control and IUGR rats.

REFERENCES

Abdel-Latif A. A., Brody J. and Ramahi H. (1967) Studies of Na^+K^+ATPase in nerve endings and appearance of electrical activity in developing rat brain. *J. Neurochem* **14,** 1133–1141.

Anner B. M. (1985) The receptor function of the Na^+K^+ATPase activated adenosine triphosphate system. *Biochem. J.* **227,** 1–11.

Bourre J. M., Chanez C., Dumont O. and Flexor M. A. (1982) Alteration of 5′ Nucleotidase and Na^+K^+ATPase in Central and Peripheral nervous tissue from dysmyelinating mutants (jimpy, quaking, trembler, shiverer and mld). comparison with CNPase in the developing sciatic nerve from trembler. *J. Neurochem.* **38,** 643–649.

Bourre J. M., Pascal G., Durand G., Masson M., Dumont O. and Piciotti M. (1984) Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes induced by a diet devoid of n-3 fatty acids. *J. Neurochem.* **43,** 342–348.

Bushnell P. J. and Bowman R. E. (1979) Reversing learning deficits in young monkeys exposed to lead. *Pharmacol. Biochem. Behav.* **10,** 733–742.

Chandra S. V., Murthy R. C., Husain T. and Bansal S. K. (1984) Effect of interactions of heavy metals on Na^+K^+ATPase and the uptake of ^{3}H NA in rat brain synaptosomes. *Acta Pharmac. Toxic.* **54,** 210–213.

Chanez C., Flexor M. A., and Hamon M. (1985) Long lasting effects of intrauterine growth retardation on basal and 5HT stimulated Na^+K^+ATPase in the brain of developing rats. *Neurochem. Int.* **7,** 319–329.

Chanez C., Giguere J. F., Flexor M. A. and Bourre J. M. (1987) Effects of lead on Na^+K^+ATPase activity in the developing brain intrauterine growth retarded rats. *Neurochem. Pathol.* **5,** 37–49.

Chanez C, Privat A, Flexor M. A. and Drian J. M. (1985) Intrauterine growth retardation on developmental changes in DNA and 14C Thymidine metabolism in different regions of rat brain: histological and biochemical correlations *Dev. Brain Res.* **21,** 283–292.

Danks D. and Matthieu J. M. (1979) Hypotheses regarding myelination derived from comparison of myelin subfraction. *Life Sci.* **24,** 1425–1440.

Fiske C. H. and Subbarow V. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66,** 375–400.

Gilbert J. C., Sawas A. H. and Wyllie M. G. (1980) Stimulation and inhibition of synaptosomes ATPase by noradrenaline. The involvement of cytoplasmic factor. *Arch. Int. Pharmacodyn.* **245,** 42–47.

Godfraind T., Koch M. C. and Verbeke N. (1974) The action of EGTA on the catecholamines stimulation of rat brain Na^+K^+ATPase *Biochem. Pharmac.* **23,** 3505–3511.

Govoni S., Memo M., Lucchi L., Spano P. F. and Trabucchi M. (1980) Brain neurotransmitters systems and chronic lead intoxication. *Pharmac. Res. Commun.* **12,** 447–460.

Hajos A. (1975) An improved method for the preparation of synaptosomal fractions in high purity. *Brain Res.* **93,** 485–489.

Hexum T. D. (1977) The effects of catecholamines on transport (NaK) Adenosine triphosphatase. *Biochem. Pharmac.* **26,** 1221–1227.

Kai J. C. K., Guest F. G., Leung T. K. C., Lim L. and Davison A. N. (1980) The effects of cadmium, manganese and aluminium on sodium-potasium activated and magnesium actived adenosine triphosphatase activity and choline uptake in brain rat synaptosomes. *Biochem. Pharmac.* **29,** 141–146.

Kissane J. Q. and Hawrylawicz E. J. (1975) Development of Na^+K^+ATPase in neonatal brain synaptosomes after protein malnutrition. *Ped. Res.* **9,** 146–150.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 2105–2112.

Mahaffey K. R. (1981) Nutritional factors in lead poisoning. *Nutr. Rev.* **39,** 353–362.

Morand O., Chanez C., Masson M., Dumont O., Flexor M. A., Baumann N. and Bourre J. M. (1982) Alteration in fatty acid composition of neurons, astrocytes, oligodendrocytes, myelin and synaptosome in intra-uterine malnutrition. *Ann. Nutr. Metab.* **26,** 111–120.

Mrsulja B. J., Zalewski A. A. and Coping G. (1985) Ultracytochemical localization of ouabain sensitive K^+ dependent p-nitrophenylphosphatase in myelin. *Brain Res.* **343,** 154–158.

Nechay B. R. and Saunders J. P. (1978) Inhibitory characteristics of lead chloride in sodium-potassium-dependant adenosine triphosphatase preparations derived from kidney, brain and heart of several species. *J. Toxic. Environ. Health* **4,** 147–159.

Norton W. T. and Poduslo S. E. (1973) Myelin in rat brain. Method of myelin isolation. *J. Neurochem.* **21,** 748–757.

Prakash N. J., Fontana J. and Henkin R. I. (1973) Effect of transitional metal ion on Na^+K^+ATPase activity and uptake of norepinephrine and choline by synaptosomes. *Life Sci.* **12,** 246–269.

Rajana B., Hobson M., Bansal S. K. and Desaiah D. (1983) Effect of Cadmium chloride on rat brain synaptosomal ATPases. *Toxic. Lett.* **18,** 331–336.

Reiss D. S., Lees M. B. and Sapirstein V. S. (1981) Is Na^+K^+ATPase a myelin-associated enzyme? *J. Neurochem.* **36,** 1418–1426.

Rodriguez de Lores Arnaiz G. and Mistrorigo de Pacheco M. (1978) Regulation of Na^+K^+ adenosine triphosphatase on nerve endings membranes. Action of norepinephrine and a soluble factor. *Neurochem. Res.* **3,** 733–744.

Sauerhoff E. K. and Michaelson I. A. (1973) Hyperactivity and brain catecholamines in lead-exposed developing rats. *Science.* **182,** 1022–1024.

Schaefer A., Unyi G. and Pfeifer A. K. (1972) The effect of a soluble factor and of catecholamines on the activity of adenosine triphosphatase in subcellular fractions of rat brain. *Biochem. Pharmac.* **21,** 2289–2294.

Schurmans-Stekhovens F. and Bonting S. L. (1981) Transport adenosine triphosphatases: properties and functions. *Physiol. Rev.* **61,** 1–76.

Schwartz A., Lindenmayer G. E. and Allen J. C. (1972) The Na^+K^+ATPase membrane transport system importance in cellular function. In: *Current Topics in Membranes and Transport.*, Vol. 3. pp. 1–82. Academic Press, N.Y.

Schwartz M., Ernst S. A., Siegel G. J. and Agranoff B. W. (1980) Immunocytochemical localisation of Na^+K^+ATPase in the goldfish optic nerve. *J. Neurochem.* **36,** 107–115.

Silbergeld E. K. and Goldgerg A. M. (1975) Pharmacological and neurochemical investigations of lead-induced hyperactivity. *Neuropharmacology* **14,** 431–444.

Sobotka T. J. and Cook M. P. (1974) Post-natal lead acetate exposure in rats possible relationship to minimal brain dysfunction. *Am. J. Mental. Defic.* **79,** 5–9.

Sweadner K. J. (1979) Two molecular form of (Na^+K^+) stimulated ATPase in brain separation and difference in affinity for strophanthidin. *J. Biol. Chem.* **254,** 6060–6067

Wigglesworth J. S. (1964) Experimental growth retardation in the foetal rat. *J. Pathol. Bact.* **88,** 1–13.

Wu P. H. and Phillis J. W. (1981b) Noradrenaline stimulation of Na^+K^+ATPase in homogenates of the developing rat brain. *Biochem. Pharmac.* **30,** 2368–2370.

Zimmerman R. T. and Cammer W. (1982) ATPase activities in myelin and oligodendrocytes isolated from the brain developing rats and from bovine brain white matter. *J. Neurosci. Res.* **8,** 73–81.