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Effect of Lead on Na^+,K^+ ATPase Activity in the Developing Brain of Intra-uterine Growth-Retarded Rats

C. CHANEZ*[1], J. F. GIGUERE,[2] M. A. FLEXOR,[1] AND J. M. BOURRE[3]

[1]INSERM U29, Centre Claude Bernard, Hôpital Port-Royal, Paris, 75674; [2]Laboratoire de Neurochimie, Centre de Recherches Cliniques, Hôpital Saint-Luc, 1058 rue St-Denis, Montréal, H2X3J4, CANADA; and [3]INSERM U26, Hôpital F. Widal, 200 rue du Faubourg Saint-Denis, Paris, 75475

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ABSTRACT

Lead (Pb) intoxication in developing mammals, including humans, produces serious brain damage. In addition, it is known that nutritional status influences the susceptibility to Pb toxicity. We developed an *in utero* undernutrition model based on restriction of blood supply to fetuses on d 17 of pregnancy (IUGR rats). The aim of this study was to investigate in vitro the possible effect of Pb on Na^+,K^+ ATPase activity in the brain of developing IUGR and control rats from 6 to 60 d after birth. In addition, we measured the stimulation of Na^+,K^+ ATPase by the monoamines noradrenaline and serotonin.

Our results show that: (1) The neurotoxic effect of Pb is an age-related phenomenon. Both IUGR and control rats were more sensitive to Pb in the first week of life. In adults, Pb had a weak inhibitory potency; (2) the delayed matured brain in IUGR animals seemed less sensitive to Pb when compared to age-paired control rats; (3) in the IUGR group, at 15 and 22 d, low doses of Pb had a stimulatory effect on Na^+,K^+ ATPase instead of an inhibitory effect; (4) noradrenaline

*Author to whom all correspondence and reprint requests should be addressed.

phic or (IUGR) an animal with weight reduction equal to or greater than 30% of the normal average weight for the same age.

Throughout the suckling period, litters of 6–8 animals, both normal and IUGR, were left with their dames. After weaning, the animals were fed *ad libitum* with a standard diet. As preliminary experiments indicated, no sex-related differences were found in Na^+,K^+ ATPase activity; both males and females were used in the following protocol. Rats of 6,15,22, and 60 d of age were sacrificed by decapitation and their forebrain quickly removed, weighted, and homogenized in 10 vol of ice-cold bidistilled water, using a Potter Elvejhem at 1000 rpm. Aliquots of 50 µL (corresponding to 0.3–0.45 mg protein) of fresh homogenate were used to assay Na^+,K^+ ATPase activity according to the technique of Abdel-Latif et al. (1967). The final 1.2-mL assay mixture consisted of 40 m*M* Tris-HCl, pH 7.4, 150 m*M* NaCl, 20 m*M* KCl, and 5 m*M* $MgCl_2$. The reaction was started by addition of 4 m*M* Tris-ATP and stopped 15 min later with 1 mL of ice-cold 10% trichloroacetic acid. Finally, the inorganic phosphate content was determined in a 0.5-mL aliquot, according to Fiske and Subbarow (1925).

The assays were performed with and without ouabain, and the Na^+,K^+ ATPase activity was estimated by subtraction of Mg^{2+} insensitive ATPase from total ATPase activity. All determinations were done in triplicate. Enzyme activity was expressed as µmol Pi liberated/mg protein/h. When present, Pb was utilized at concentrations of 1, 0.1, 0.01, and 0.001 m*M*. Serotonin and NA were added to the assay mixture to final concentrations of 1 and 0.5 m*M*, respectively. Protein content was measured by the method of Lowry et al. (1951), with bovine serum albumin as standard.

Chemicals

Serotonin creatine sulfate (Sigma); noradrenaline (Sigma); ouabain (Sigma); adenosine triphosphate free of metal ion (Sigma); and Pb acetate (Merck).

RESULTS

Body Weight, Forebrain Weight Gain, and Protein Content in Developing IUGR and Control Rats

From the first day after ligature (d 18 of pregnancy), the IUGR fetuses showed a 20% reduction of body weight relative to normal rat (Table 1). This percentage increased steadily until birth, at which time it reached an average of 40–50%. This significant reduction persisted to adulthood, whatever the rearing conditions. Results shown in Table 1 also indicated that the forebrain weight of IUGR rats at postnatal d 6 was

markedly less affected than body weight since only 27% reduction was found when compared to age-paired control rats. This difference decreased with age and leveled to about 20% at 60 d. No change was noticed in the concentration of forebrain protein in IUGR as compared to control rats. Forebrain protein concentration rose with age, but no effect on IUGR was noticed.

Development of Na^+,K^+ATPase Activity in IUGR and Normal Rats

Before 6 d of age the Na^+,K^+ ATPase activity was too low to permit a reliable dosage. At d 6, we observed a significant difference between IUGR and control rats (Table 2). The enzyme activity increased steadily during postnatal development, but the IUGR deficit persisted up to 3 wk of age. In mature animals (60-d-old) we observed activities 3.5 and 5 times higher than at 6 d in normal and IUGR groups, respectively. Such differences were not dependent on the expression of results in mg protein since the protein content of the forebrain was not significantly different in IUGR and control at any studied age (Table 1).

Effects of Pb on Basal Na^+,K^+ATPase Activity

Figure 1 shows the effects of Pb on forebrain Na^+,K^+ ATPase in developing rats.

In normal rats (Fig. 1a), Pb produces a dose-dependent inhibition. The sensitivity (i.e., the lowest Pb concentration that induced a significant inhibition) and maximal inhibition (at 1 m*M* Pb) varied with age. Older animals seemed to be less affected than younger ones. The

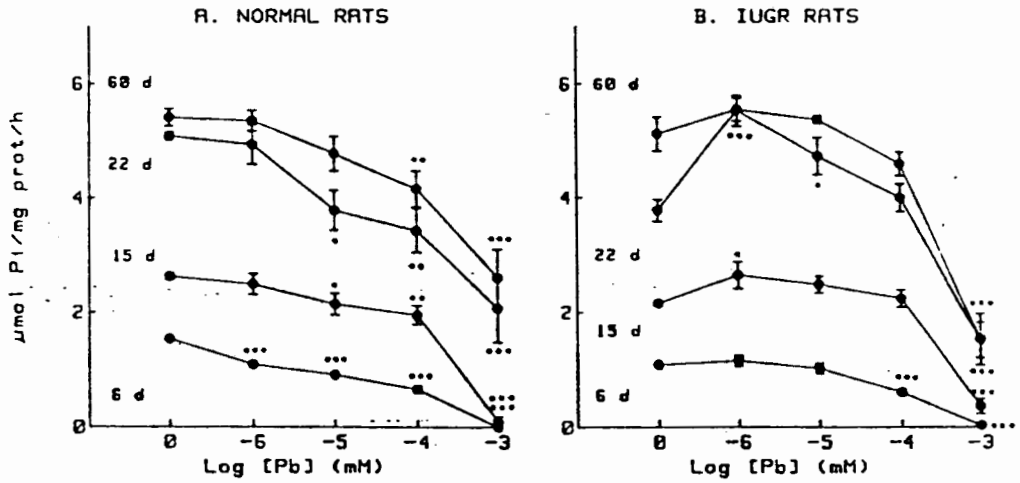

Fig. 1. Effects of Pb (0.001–1 m*M*) on cerebral Na^+,K^+ ATPase activity in developing control (A) and IUGR (B) rats. Data expressed as µmol Pi liberated/mg protein/h as mean value (without Pb) by analysis of variance: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$: squares are circles ± SEM problems with computer.

6-d-old group showed a significant (44%) inhibition at the minimal (0.001 mM) Pb concentration studied (Fig. 1b).

In the 15- and 22-d groups, the decrease was seen at concentrations higher than 0.01 mM and smaller (20 and 25%, respectively) than at 6 d. Finally, the 60-d-old adult group reacted only to Pb higher than 0.1 mM, with a significant 23% inhibition. The same age-dependent relation existed for maximal inhibition (1-mM Pb). Indeed 6-, 15-, 22- and 60-d-old rats exhibited maximal inhibition to levels respectively equivalent to 100, 97, 60, and 52%.

The IUGR rats reacted differently to in vitro Pb inhibition (Fig. 1b). In the 6-d-old group the Pb inhibition appeared significant at 0.1 mM (56% inhibition) instead of 0.001 mM, as seen in normal animals of the same age. This age group thus seems to be less sensitive to Pb than age-paired controls. Indeed, the inhibited (0.001 and 0.01 mM Pb) Na^+, K^+ ATPase activity in the normal group reached the basal levels encountered in the IUGR group. It is interesting to note that the difference in basal activity between IUGR and normal rats of 6 d disappears in the presence of low Pb concentrations. On the other hand, the maximal inhibition was the same (100%) in both groups.

In the 15- and 22-d-old groups, Pb produces a totally different effect on Na^+,K^+ ATPase activity in the IUGR group when compared to the normal group. Instead of an inhibition, we found, at low dose, a stimulation of the enzyme or a "restoration" of the normal basal activity. At 0.001 mM, Pb produced significant increases of 23 and 46% in the 15 and 22-d-old groups, respectively. Furthermore, the 0.01-mM dose still had this stimulatory effect (24%) in the latter group. In the presence of this Pb-induced stimulation, the IUGR Na^+,K^+ ATPase activity significantly exceeded the normal values. We thus observed a biphasic reaction with a stimulatory effect at a lower dose and an inhibitory effect at the highest doses of Pb. The latter was in the same order of magnitude than that seen in normal rats. The adult group did not exhibit this stimulation at low Pb dose, and there were no differences between the normal and IUGR groups.

Effects of NA and 5-HT on Basal Activity of Na^+,K^+ ATPase in Developing IUGR and Normal Rats

In Table 2 we observed that the two monoamines produced a stimulatory effect on basal Na^+,K^+ ATPase activity in both groups. The potency of these drugs varied with age and group. Noradrenalin and 5-HT were more potent in IUGR than in normal rats until 3 wk of age. As shown in Fig. 2, IUGR and normal rats reached the same stimulated activity. In adulthood these drugs had the same effect in both groups, since the basal activity was similar.

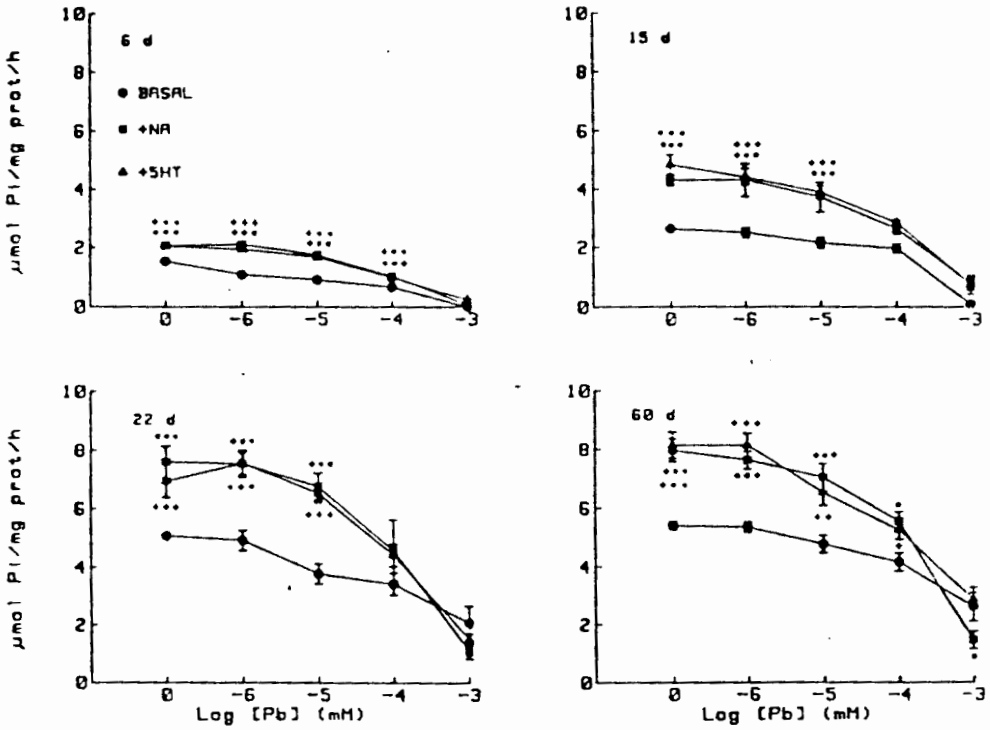

Fig. 3. Reversal of Pb-induced Na^+,K^+ATPase inhibition by NA and 5-HT in control animals. Values expressed as µmol Pi liberated/mg protein/h as mean ± SEM: ●, basal inhibited values; ■, NA-stimulated values; and ▲, 5-HT-stimulated values. Significant difference from basal inhibited value, evaluated by analysis of variance, are indicated for NA-stimulated values by: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$; and for 5-HT-stimulated values by: $^+$ $p < 0.05$; $^{++}$ $p < 0.01$; and $^{+++}$ $p < 0.001$.

only partially restored. The stimulatory effect of Pb (seen with 0.001 and 0.01 m*M* in 15- and 22-d-old rats, respectively) was not observed for the monoamine-stimulated activity. This suggests that the NA and 5-HT prevent the Pb stimulation (Fig 4) or that monoamine stimulated activity is the maximal activity.

DISCUSSION

The Na^+,K^+ATPase activity plays a key role in the maintenance of neuronal transmembrane potential (Schwartz et al., 1972) as well as neurotransmitter uptake and release (Logan and O'Donovan, 1980; Wu and Phillis, 1981). In a previous report (Chanez et al., 1985) we observed a decrease in the Na^+,K^+ATPase activity of different brain structures in IUGR rats as well as a greater sensitivity to the stimulatory effect of 5-HT when compared to age-paired control rats. The present study reveals an equivalent in vitro stimulation of Na^+,K^+ATPase activity with NA.

sitive to Pb action than older animals. Clinically, Pb intoxication in children is far more dramatic than in the adult. From this point of view, it is interesting to note in vitro greater Pb-induced Na^+,K^+ ATPase inhibition in young. Further studies are needed to implicate a Pb-induced Na^+,K^+ ATPase defect in the pathophysiology of Pb encephalopathy in young.

The IUGR Na^+,K^+ ATPase seemed less sensitive to Pb than control Na^+,K^+ ATPase. An obvious change was observed in 15- and, particularly, 22-d-old IUGR rats. The lowest Pb doses restored the IUGR deficient basal activity to normal in vitro values of basal activity. Shukla et al. (1983) described a similar biphasic effect on Na^+,K^+ ATPase with Mn^{2+}. In the same manner, calcium, at concentrations below 3 μM, apparently stimulated Na^+,K^+ ATPase, whereas, at higher concentrations, it induced an inhibition (Rohani et al., 1981; Powis et al., 1983).

Previous reports have observed that Pb inhibition can be modified by an increasing ATP concentrations (Tice, 1969). Kissane and Hawrylewicz (1975) have reported that undernourished rats present a significant increase at ATP values and an important reduction in ADP utilization.

Preliminary studies (Chanez, unpublished results) did not reveal any significant difference in ATP brain concentration between both groups during the first 3 wk after birth. Further studies will be necessary to explain these differences.

Controversal reports have implicated different mechanisms concerning biogenic amine stimulation of Na^+,K^+ ATPase in various brain preparations (Hexum, 1977; Schaefer et al., 1974; Svoboda and Mosinger, 1981). According to these authors, the catecholamines enhanced the activity of Na^+,K^+ ATPase by chelating inhibitory divalent cations or by preventing lipid peroxidation. Our findings demonstrated that 5-HT and NA, two physiologically important monoamines, might also regulate Na^+,K^+ ATPase activity by reducing in vitro inhibition by Pb. These two monoamines reversed enzyme inhibition in relation to the concentration of metal in the reaction mixture. However, for all animals, we observed a complete reversal effect of the monoamines at the lowest Pb concentrations. The results obtained with heavy metals, such as Pb, were consistent with additional studies (Chanez, unpublished observations) on mercuric salts. We have noted that the inhibitory effects of mercurial compounds, more potent inhibitors of the Na^+,K^+ ATPase, could be reversed by NA and 5-HT in a dose-dependent manner. This reversal action was more important in the IUGR than in the control group.

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