Pre- and postnatal lead exposure affects the serotonergic system in the immature rat brain

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Summary. The effect of pre- and postnatal lead exposure on the development of the serotonergic system in striatum and brain stem was investigated. Serotonin and its metabolite 5-HIAA where determined by HPLC-EC. A significant decrease of 5-HT was detected in the brain stem at postnatal day 28. At both days 6 and 28 postnatal, 5-HIAA was reduced in striatum and brain stem. The results provide support to the hypothesis that developing 5-HT neurons are sensitive to relatively low levels of lead exposure.
Key words. Lead exposure (perinatal); serotonin; HPLC-EC; rat brain; development.

Lead has been demonstrated to affect the function of the brain and the immune system[1]. Recent studies suggest that the most adverse effects are exerted during development[2]. Lead is known to pass readily to the fetus through the placenta[3]. Therefore an interaction of lead with several neurochemical parameters early in development seems possible.

Brain serotonin has been related to numerous behavioral and endocrine mechanisms[4]. Investigations of the effect of lead on indoleaminergic parameters have yielded contradictory results; however, it does appear that exposure to lead may alter certain aspects of indoleaminergic function in the brain[5–8]. In order to study the effects of lead exposure on the developing central serotonergic system, we investigated the offspring of rats that had been exposed to lead during pregnancy and lactation. At the ages of one and four weeks, the amine and its metabolite were determined in the striatum, a serotonergic projection area, and in the brain stem, which contains neurons and terminals. A preliminary report has been presented[9].

Material and methods

Animals. Long Evans rats were bred in our laboratory under controlled illumination (lights on 02.00 – 16.00 h) and at a constant room temperature (22 +/− 1 °C). They received a standard diet (NAFAG 850) and water ad libitum. The duration of mating was limited to 3 h (16.00 – 19.00 h). Females exhibiting positive vaginal smears were housed in groups of two and isolated one day before parturition. The day of birth was defined as postnatal day [PN] 1. One day after parturition, litter size was reduced to 8–10 animals. Care was taken to maintain an equal sex ratio whenever possible. Offspring were weaned at PN 28.

Treatment schedule. Dams of a similar genetic background and with comparable body weight were assigned randomly to receive either lead acetate or sodium acetate (control) in a drinking solution during pregnancy and lactation (beginning on the first day of pregnancy). This treatment schedule is similar to that described by McCarren and Eccles[10] and Costa and Fox[11], with the exception that they started with lead administration upon parturition. The following protocol was used:

Lead acetate [$(CH_3COO)_2Pb \cdot 3H_2O$; p.a. (Merck, Darmstadt)] or sodium acetate was dissolved in distilled water. In order to prevent the precipitation of lead carbonate, boiled water (up to 30 min) was used and all solutions were degassed with nitrogen. The water bottles were degassed too and firmly closed. Solutions were prepared daily. Lead was administered at 2.5 g/l (designated as moderate lead [ML] exposure), the calculated lead concentration was 1363 ppm. The controls [Co] received 1.25 g/l sodium acetate in the drinking solution.

Dams were weighed at the time of conception and at the end of pregnancy. The number of pups per dam was recorded, as was also mean food intake during pregnancy. Fluid consumption was measured on days 1, 2, 3, 9, 10, 11 and 20, 21, 22 of pregnancy and one week after parturition. On postnatal days 6 and 28 (PN 6 and PN 28), pups were weighed and the brain weight determined (see below). The data were analyzed with one-way analysis of variance followed by the Student-Newmann-Keuls t-test.

At PN 6 mean blood lead levels were, 4.5 µg/100 ml in female controls and 51.5 µg/100 ml in ML-treated females, and 4.2 µg/100 ml and 60.4 µg/100 ml in males, for controls and ML-treated animals, respectively. At PN 28, the corresponding values were 2.4 µg/100 ml and 100.2 µg/100 ml in females and 1.7 µg/100 ml and 101.0 µg/100 ml in males (Widmer et al., in preparation). Mean lead content in cerebral cortical tissue of ML-treated animals was 170 ng/g wet weight at PN 6, and 660 ng/g wet weight at PN 28 (Widmer et al., in preparation).

Tissue preparation. Animals were killed by decapitation either at PN 6 or PN 28, and the brains were rapidly removed and immediately immersed in ice-cold 0.9% sodium chloride for 30 s. Striatum and brain stem were dissected on a chilled glass plate by a modification of the method of Glowinsky and Iversen[12]. Brain tissue was immediately frozen on dry ice and stored at −70 °C until HPLC analysis was performed.

For the analysis, frozen brain tissue was weighed and then ultrasonicated on ice in 600 µl of 0.1 M citric acid/

0.2 M sodium dihydrogen phosphate buffer (pH 3.4, 0 °C) by an ultrasonic disintegrator ([model MSE]; peak-peak amplitude 9 µm) for 5 s. Then 5 µl of perchloric acid was added to the homogenate. The homogenate was centrifuged at 15000 g for 10 min at 4 °C. 50 µl 0.1 N NaOH was mixed with 450 µl supernatant. This solution was rapidly frozen on dry ice and stored at −25 °C until further analysis.

High performance liquid chromatography (HPLC) analysis and protein determination. Serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were quantified in the supernatant (filtered through a 0.45-µm filter [Gelman Sciences]) by reverse-phase HPLC with electrochemical detection (HPLC-EC), (for review of methods see Ribary[13]). Protein content was determined in the pellet by the method of Lowry and coworkers using bovine serum albumin as a standard[14]. A BAS LC-4B amperometric detector equipped with a glassy carbon working electrode and a Ag/AgCl reference electrode was used. The monoamines and their metabolites were oxidized at + 0.7 V relative to the reference electrode.

For each test, animals from 4 litters were investigated. Values were examined with Student's two-tailed t-test. The ratio of 5-hydroxyindoleacetic acid/5-hydroxytryptamine was taken as an estimate of serotonin turnover. Values are expressed as a percentage of the control. Control levels in brain stem were as follows: 5-HT PN 6, 3.5 to 4 pmol/mg protein; 5-HT PN 28, 35 to 40 pmol/mg protein; 5-HIAA PN 6, 7 to 10 pmol/mg protein; 5-HIAA PN 28, 40 to 45 pmol/mg protein. Control levels in the striatum were: 5-HT PN 6, 22 to 25 pmol/mg protein; 5-HT PN 28, 13 to 14 pmol/mg protein; 5-HIAA PN 6, 27 to 28 pmol/mg protein; 5-HIAA PN 28, 11 to 11.8 pmol/mg protein.

Results

The level of lead exposure used in this study did not affect the body weight of the dams, the number of pups to which they gave birth, nor the food intake. Fluid consumption was found to be significantly decreased

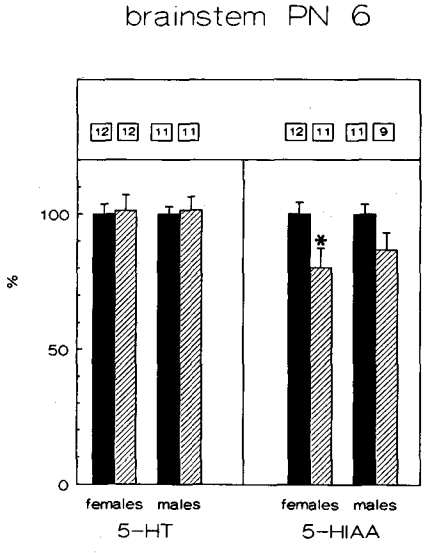

Figure 2. Effect of pre- and postnatal lead exposure on 5-HT and 5-HIAA levels at postnatal day 6 in the brain stem of male and female rats. Data are expressed as percentage of control (see fig. 1). Shaded bars = controls; hatched bars = ML-treated. * different for $p < 0.05$ vs control.

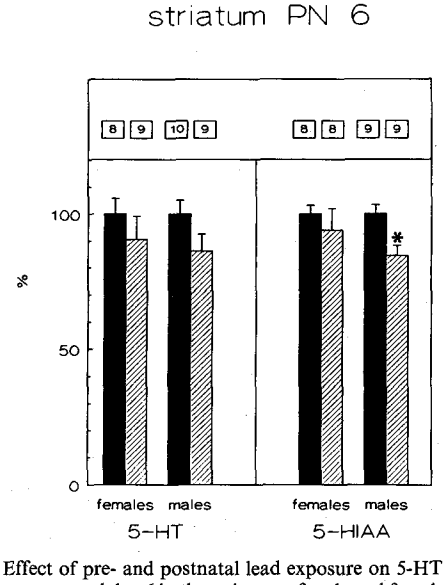

Figure 1. Effect of pre- and postnatal lead exposure on 5-HT and 5-HIAA levels at postnatal day 6 in the striatum of male and female rats. Data are expressed as percentage of control (see methods, mean + /− SEM). Number in boxes indicate number of investigated animals. Shaded bars = controls; hatched bars = ML-treated. * different for $p < 0.05$ vs control.

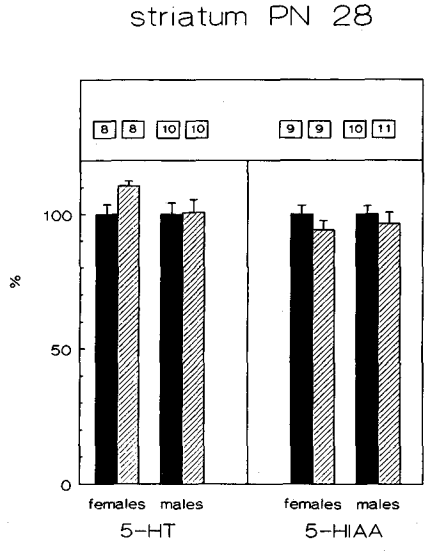

Figure 3. Effect of pre- and postnatal lead exposure on 5-HT and 5-HIAA levels at postnatal day 28 in the striatum of male and female rats. Data are expressed as percentage of control (see fig. 1). Shaded bars = control; hatched bars = ML-treated. * different for $p < 0.05$ vs control.

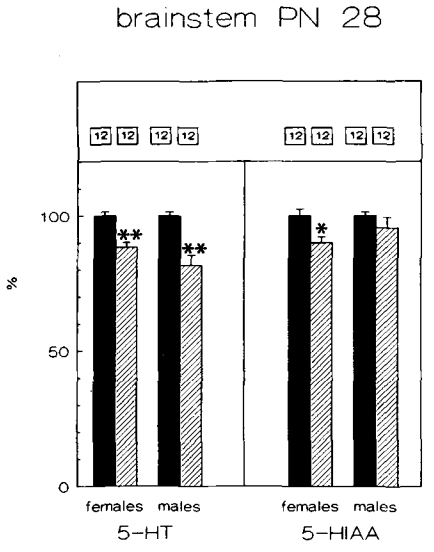

Figure 4. Effect of pre- and postnatal lead exposure on 5-HT and 5-HIAA levels at postnatal day 28 in the brain stem of male and female rats. Data are expressed as percentage of control (see fig. 1). Shaded bars = control; hatched bars = ML-treated. * different for $p < 0.05$ vs control; ** different for $p < 0.005$ vs control.

($p < 0.01$) at days 9, 10 and 11 of pregnancy, but no significant differences were detected at any other stage. Lead-treated offspring did not show any significant differences from controls in body weight, brain weight or age of eye opening.

At PN 6, 5-HT levels in striatum and in brain stem were not significantly changed, but tended to be reduced in the striatum of both sexes. The metabolite 5-HIAA was reduced in both brain regions examined, but the reduction was significant only in the brain stems of females (fig. 1 and fig. 2).

At PN 28, levels of 5-HT and, in females, 5-HIAA, were significantly decreased in brain stem, while no significant effects were observed in striatum (fig. 3 and fig. 4). The changes in the brain stem resulted in an increase in the 5-HIAA/5-HT ratio of male animals.

Discussion

Pre- and postnatal lead exposure affects the serotonergic system in a complex way. In the brain stem, where both cell bodies and terminals are present, lead-exposed females show normal 5-HT in the presence of reduced 5-HIAA at PN 6, and reduced 5-HT with reduced 5-HIAA at PN 28. As a result, the 5-HIAA/5-HT ratio is diminished at PN 6 but normalized by PN 28. In lead-exposed males, 5-HT is likewise normal at PN 6 and reduced at PN 28, but 5-HIAA is not significantly changed. In the 5-HT terminal area of the striatum, only minor effects were noted; in particular a reduction of 5-HIAA in males at PN 6. In connection with behavioral alterations, only small changes in neurochemical parameters are to be expected, since more important neurochemical changes would result in severe functional deficits.

The changes we observed were not accompanied by significant general toxic effects. The general toxicity for dams and pups appears to have been very low, since none of the investigated growth parameters revealed any significant difference between control animals and lead-treated ones, with the exception of a reduction in water intake by the dams at three days of pregnancy (GD 9, 10, 11). The effects on growth parameters observed in this study are in accordance with previous findings of this and similar lead dosing models[10,11,15–17]. However, changes in placental function cannot be completely ruled out.

Blood lead levels of PN 6 (50 to 60 µg/100 ml) were about twice the level reported for young urban children, which can exceed 35 µg/100 ml[18]. The effective threshold for symptomatic intoxication in children is about 80 to 100 µg/100 ml[19]. The maximum safe blood lead concentration for a child has been considered to be 25 µg/100 ml[2], though behavioral disturbances have more recently been reported for lower concentrations[20].

The alterations induced by perinatal lead exposure clearly depend upon the developmental stage and also are influenced by sex. The difference between PN 6 and PN 28 might be linked with the difference in plasma and brain lead levels (see methods). However, it should be noted that chemicals can also initiate developmental changes that outlast the initial effect[21]. The contrasting observations in striatum and brain stem are not easy to interpret. There is no indication for a significant change in 5-HT turnover in striatum, i.e. in a 5-HT terminal area. In brain stem, the alterations could be due either to changes specific for neuronal perikarya, or to effects on other 5-HT terminal fields. Reduced 5-HT levels might reflect a reduced synthetic capacity. In those cases where reduced concentrations of 5-HIAA occur in the presence of normal 5-HT levels, they could be brought about either by a change in 5-HT turnover or, alternatively, by a direct effect of lead on monoamine oxidase. The cofactor of this enzyme has been shown to be bound covalently to the enzyme by way of a thioether linkage from the isoalloazine ring to a cysteinyl residue of the protein[22]. Since, at physiological pH values, the SH groups of some amino acids, such as cysteine, in proteins, play a primary role in the binding of lead in vivo[23], it is possible that lead could interact with this enzyme.

Serotonin neurons become detectable in rat midbrain from gestational day (GD) 13, and 5-HT projections in the forebrain from GD 14[24–26]. Serotonergic binding sites of the S_2-type are found in the forebrain of the rat at gestational day 15 3/4[27]. Schlumpf and coworkers[28] reported the presence of S_1 binding sites in brain stem at GD 18 and at GD 20 in neocortex. Therefore, the possibility of interactions of prenatal lead exposure with the early development of serotonergic systems exists. This idea is in keeping with a recent study which reported increased hippocampal S_1 receptor densities in rats chronically treated with lead during neonatal life. The

treatment schedule used was comparable to that of this study, since lead was administered to the animals as a 0.2% lead acetate drinking solution. In contrast, no difference was found when the exposure occurred after weaning[8].

The observations of this study lend further support to the idea that developing serotonergic neurons are also affected by chronic lead exposure.

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Melatonin modulates apomorphine-induced rotational behaviour

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Summary. Prior melatonin administration (1 and 10 mg/kg b.wt) causes a significant reduction in apomorphine (1 mg/kg b.wt) induced rotational behaviour in both 6-hydroxydopamine and quinolinic acid lesioned rats.
Key words. Melatonin; apomorphine; 6-hydroxydopamine; quinolinic acid; rotational behaviour.

The regulatory function of the pineal gland, ideally situated anatomically to integrate and compare information from both extra- and intra-cranial sources, appears to be mediated through release of the principal pineal hormone, melatonin (MEL)[1]. Apart from the neuroendocrine role of MEL[2], animal and human studies have implicated MEL as an important modulator of behaviour.

Conflicting reports on the effect of MEL in Parkinson's disease – a movement disorder characterized by striatal DA deficiency exist. Anton Tay et al. report improvement of symptoms on administration of 1.2 g/day of MEL to Parkinsonian patients[6] while Shaw et al. report that doses of MEL up to 1 g/day did not alter the Parkinsonian syndrome[7]. In schizophrenia – a disorder possibly associated with DA hyperactivity, MEL metabolism appears to be altered[8] and reduced midnight levels of MEL coupled with raised cortisol levels have been reported[9]. The role of the pineal gland and MEL's antidyskinetic activity has begun to be recognised in neuroleptic-induced tardive dyskinesia, a disorder associated with a supersensitivity of DA receptors[10]. MEL has also been demonstrated to cause 'psychomotor retardation' when administered to patients with Huntington's chorea, a movement disorder characterized by hyperkinetic choreiform movements, which is primarily treated with DA antagonists[11].

High affinity binding sites for ^{125}Iodo-MEL have been identified in the striatum, as well as the hippocampus, hypothalamus, cortex and amygdala of both the male