Arch. Histol. Cytol., Vol. 65, No. 1 (2002)
p. 45-53

Effects of Postnatally Administered Inorganic Lead on the Tyrosine Hydroxylase Immunoreactive Norepinephrinergic Neurons of the Locus Ceruleus of the Rat*

Won Taek LEE[1], Ho YOON[2], Dong Joo LEE[1], Jong Eun LEE[1], Chul Hoi KOO[1] and Kyung Ah PARK[1]

Brain Research Institute and Department of Anatomy[1], Yonsei University College of Medicine, Seoul; and Department of Anatomy[2], College of Medicine, Pochon Cha University, Gyunggi-do, Korea

Received December 26, 2001

Summary. **The neurotoxic effects of inorganic lead are known to include peripheral neuropathy in adults and encephalopathy in children. The purpose of this study was to determine the effect of inorganic lead ($PbCl_2$) administration on norepinephrinergic neurons of the locus ceruleus in neonatal rats by immunocytochemical and electron microscopic analyses.**

Lead chloride solutions, 0.05%, 0.1% and 0.2% in concentrations, were prepared in distilled water and administered orally via drinking water. After 4, 8, or 12 weeks of continuous administration, the rats were sacrificed and brains were immunostained with the tyrosine hydroxylase antibody. The number of immunoreactive cell bodies in the locus ceruleus was estimated. Densitometric analysis of immunoreactive profiles visualized by electron microscopy was performed using an image analyzer.

The numbers of immunoreactive neurons in the locus ceruleus were increased statistically by lead administration. The intensity of the immunoreaction, both under the light and electron microscopes was also increased.

Degenerative changes, including intra-axonal vacuole formation and widening of the extracellular spaces, were found by electron microscopy in and around the tyrosine hydroxylase immunoreactive axons.

Increased tyrosine hydroxylase immunoreactivity may correlate with the hyper-reactivity of lead intoxicated children. Degenerative changes may account for the reported deficits in intellectual attainment and achievement in lead intoxicated children.

Lead poisoning is one of the most common environmental hazards worldwide, especially in children (KRIGMAN et al., 1980; ROSEN, 1995; WINNEKE et al., 1996; KOIKE, 1997; NEVIN, 2000). Although the toxicity of lead was recognized centuries ago, concern was restricted to overt symptoms such as colic, encephalopathy, anemia, or renal disease (GOYER, 1990). The results of recent clinical studies indicate that "chronic" low level lead exposure during development may result in behavioral alterations in the absence of overt encephalopathy (MILLER et al., 1990; ROSEN, 1995; FINKELSTEIN et al., 1998).

The neurotoxicity of low-level long-term exposure to lead has a special relevance in children. An extensive database has provided a direct link between low levels of lead exposure and deficits in the neurobehavioral-cognitive performance evidenced in childhood and adolescence (FINKELSTEIN et al., 1998). A number of studies have demonstrated a relationship between lead poisoning and the hyperactivities of lead-exposed children (WINDER, 1982; WINDER and KITCHEN, 1984; THOMSON et al., 1989; BROCKEL and CORY-SLECHTA, 1998). Correlations between lead poisoning and both learning disability and hyperactivity have been demonstrated in animal studies (WINDERLE and KITCHEN, 1984; HOLLOWAY and THOR, 1987; SHIGETA et al., 1989; FELDMAN and WHITE, 1992; JETT et al., 1997; KUHLMANN et al., 1997).

Although these behavioral changes were attributed to the monoaminergic neurotransmitters, controversy remains concerning the role of norepinephrine, dopamine and serotonin. In the mid-seventies, GOLTER and MICHAELSON (1975) reported that in chronically lead-poisoned rats endogenous levels of brain norepinephrine increased, whereas dopamine levels remained unchanged, a result suggesting a possible relationship between lead exposure during

*This work was supported by a Research Fund for Basic Medical Science Grants (W. T. LEE) from the Korean Research Foundation.

the earliest developmental periods, increased motor activity, and brain norepinephrine. DECASTRO (1990) measured plasma catecholamine concentration and found that norepinephrine and epinephrine were significantly elevated in chronic lead poisoning. Recently COMINGS et al. (2000) analyzed dopamine, serotonin, and norepinephrine genes and found that the adrenergic genes play a greater role in the attention deficit hyperactivity disorder (ADHD) than the combined effect of the dopaminergic and serotonergic genes.

Norepinephrine producing neurons are located in the brain stem, mostly within the area of the nucleus locus ceruleus (A6 nucleus of DAHLSTRÖM and FUXE); their fibers are distributed throughout the central nervous system.

We find it surprising that few reports have focused on the morphological changes in the nucleus locus ceruleus of chronically lead poisoned young rats. The purpose of this study was to investigate the immunohistochemical and immunocytochemical changes of tyrosine hydroxylase, a rate limiting enzyme of catecholamine synthesis, on the immunoreactive norepinephrinergic neurons of lead poisoned young rats.

MATERIALS AND METHODS

Sixty Sprague-Dawley rat pups of either sex, in twelve groups of five, were used for this experiment. Rats were given $PbCl_2$ (0.05%, 0.1%, and 0.2%) containing water *ad libitum* from the first day of postpartum until weaning on day 21 postpartum. The pups continued drinking the same water until sacrifice. To enhance the taste, sucrose was added to the drinking water to a ratio of 5%. Rat pups were grouped for the duration of lead administration, postnatal 4 weeks, 8 weeks, and 12 weeks, respectively. Age matched control rats were given only 5% sucrose water until sacrifice.

After 4 weeks, 8 weeks, and 12 weeks, respectively, the rats were anesthetized with ether or sodium pentobarbital, perfused through the left ventricle with 2% sodium nitrite-2% heparin containing solutions followed by 3% paraformaldehyde-3% glutaraldehyde-0.1% picric acid in 0.1 M phosphate buffer (pH 7.4). Under the stereomicroscope, brain stems were carefully dissected, removed, and placed in fresh fixative for 4 to 24 h at 4°C. Brain stem regions of the nucleus locus ceruleus were sectioned with a vibratome, 50 μm-thick free-floating vibratome sections were collected in tissue well plates and processed for immunohistochemistry.

In some animals, blood samples and brain tissues were prepared without fixation and processed for lead concentration determination. Lead levels in the brain and blood of the animals were determined using atomic absorption spectrophotometry.

Sections were immunohistochemically stained against tyrosine hydroxylase using the peroxidase-antiperoxidase (PAP) method by STERNBERGER (1986). Sections were placed in 3% normal goat serum (NGS) for 30 min followed by incubation with tyrosine hydroxylase (Peninsular Lab., Belmont, CA, USA) at a dilution of 1 : 2,000 for 72 h at 4°C. After several rinses in phosphate buffered saline (PBS), the tissue was placed in 3% NGS for 30 min and incubated with goat anti-rabbit IgG at a dilution of 1 : 50 for 1 h at room temperature. It was then rinsed several times with PBS and placed in 3% NGS for 30 min and incubated with PAP at a dilution of 1 : 100 for 1 h at room temperature. Following several rinses in PBS, the sections were placed in a 0.05% diaminobenzidine (DAB) solution containing 0.01% hydrogen peroxide for 10 min. Triton X-100 (0.75%) was then added to the antibody containing the solution, to enhance the penetration of antibodies. Controls were prepared using an incubating solution devoid of primary antibodies. Sections were dehydrated with graded alcohol and xylene, and coverslipped with synthetic resin. Every other serial section between the immunohistochemically stained sections was Nissl stained with cresyl violet.

For electron microscopy, the stained tissue sections were thoroughly rinsed in a phosphate buffer and placed in 1% osmium tetroxide in a 0.1 M phosphate buffer for 30 min. They were then rinsed in a maleate buffer (pH 6.0), stained with uranyl acetate in a maleate buffer, rinsed, dehydrated, and embedded with an Epon-Araldite mixture. Regions of the nucleus locus ceruleus were selected, trimmed, and mounted on an Epon chuck with a drop of Epon. Thin sections were then cut, mounted on copper grids, and viewed with or without lead citrate staining. Observations were made using a Philips electron microscope.

Tyrosine hydroxylase immunostained cells and Nissl stained cells in the nucleus locus ceruleus were counted in serial sections under an Olympus AX 80 optical microscope. Serial sections of the locus ceruleus area were alternatively stained by Nissl and next by tyrosine hydroxylase immunohistochemistry. In each slide of serial sections, all the cells in the locus ceruleus region were counted. To avoid overcounting errors, only cells with nuclei were counted in immunostained sections; in Nissl stained sections, only cells revealing nucleoli were counted, with overcounting errors corrected using KÖNIGSMARK's

method (KÖNIGSMARK, 1970). Total counts of the locus ceruleus neurons were doubled against the sum of the cell count of every other serial section in one series of rat specimens. Two researchers separately counted the same sections to ensure more reliable results. The differences in counts by two researchers were within a 10% limit, and we averaged the data of the two.

Gray scale densities of tyrosine hydroxylase immunoreactive profiles in electron photomicrographs were measured using an image analyzer program (Optima VI). Electron photomicrographs were placed under the CCD camera connected to personal computers. In an OptimaVI program, the gray scale was divided by 256 (2^8) densities from 1 (pure white) to 256 (pure black), and background correction was made by the OptimaVI densitometry software with the lightest background as pure white. Nerve cell counts and electron microscopic densities were analyzed statistically using the ANOVA and Mann-Whitney U test.

RESULTS

Changes in body weight

The average weights of control rat pups were 92.7± 19.1 g at 4 weeks of age, and 199.6±27.7 g at 8 weeks. Rat pups treated with 0.05% lead weighed 85.3±24.0 g at 4 weeks, and 184.3±38.8 g at 8 weeks, showing no significant change. Animals treated with 0.1% lead weighed 81.6±32.3 g at 4 weeks, and 191.5±36.7 g at 8 weeks. In the 0.2% lead administered group, average weights decreased to 53.8±22.3 g (58% of the control pups) at 4 weeks, but returned to 183.4±28.8 g (approximately 90% of the control pups) at 8 weeks of age. At 12 weeks of age no significant body weight changes for rats treated with 0.2% lead (231.7±33.6 g) and the control animals (243.5±29.5 g) were apparent.

Changes of lead concentration in the blood and brain

Lead concentrations in the blood and the brain increased after lead administration. They gradually increased according to the duration and concentration of the lead administered (Table 1). The lead levels in the blood and brain increased dramatically at 8 weeks in 0.1% lead treated rats. In the 0.2% lead administered group, the levels were higher than in the 0.1% lead treated rats.

Light microscopy

The nucleus locus ceruleus in the A6 group of DAHLSTRÖM and FUXE was clearly demarcated on the lateral side of the fourth ventricle. It appeared from the upper end of the ventricle (around Bregma -9.1 mm) to the level of the abducens nucleus (around Bregma -10.6 mm). Nissl stained preparations showed closely aggregated cell bodies and a clear boundary around the nucleus locus ceruleus.

Tyrosine hydroxylase immunoreactive neurons were concentrated in the nucleus locus ceruleus region. Small numbers of the immunoreactive neurons were observed outside the nucleus (Fig. 1). Axons were also stained immunohistochemically within and outside the nucleus. In the lead treated groups, cells and processed were more darkly stained than those of the controls (Figs. 1, 2). However, no differences were found in animals treated with different lead concentrations.

Tyrosin hydroxylase immunoreactive neurons

Table 1. Average lead concentration in the blood and brain.

	Blood lead concentration (μg/dl) N=8 (each group)	Brain lead concentration (μg/dl) N=3 (each group)
Control group	14.72± 2.84	2.44± 0.65
Lead treated group		
0.1% 4 weeks	30.20± 4.86	18.18± 2.21
0.1% 8 weeks	94.75±16.33	25.32± 6.59
0.1% 12 weeks	97.30±14.65	32.68± 6.45
0.2% 4 weeks	74.32±11.21	38.68± 4.68
0.2% 8 weeks	106.49±19.66	40.98± 9.44
0.2% 12 weeks	121.25±24.72	46.18±14.21

Mean±S.D.

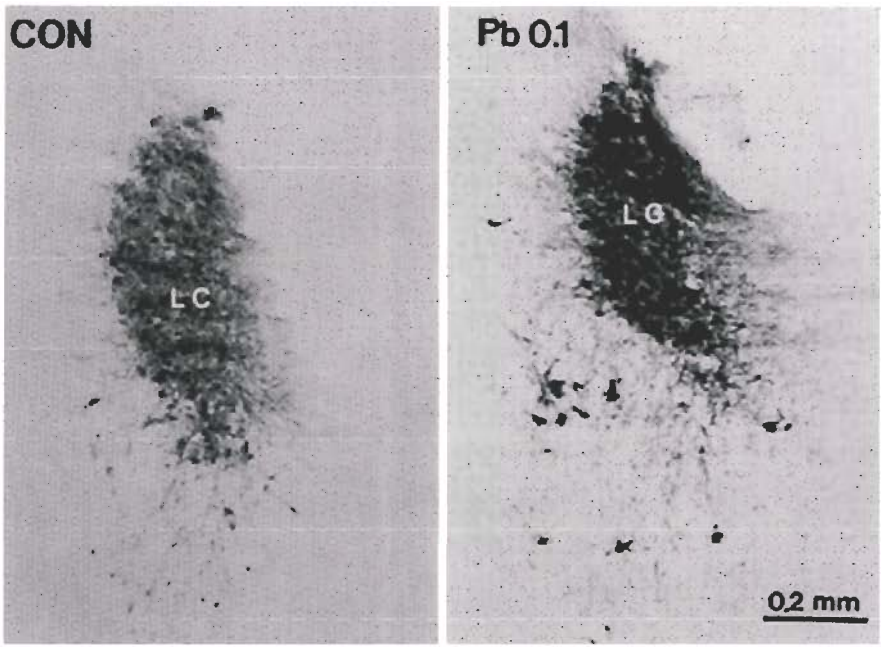

Fig. 1. Tyrosine hydroxylase immunoreactivity in the nucleus locus ceruleus (*LC*) in a control rat (**CON**) and in a 0.1%-8 week lead treated rat (**Pb 0.1**). Nerve cells and processes are more intensely immunoreactive in the lead treated animal. ×75

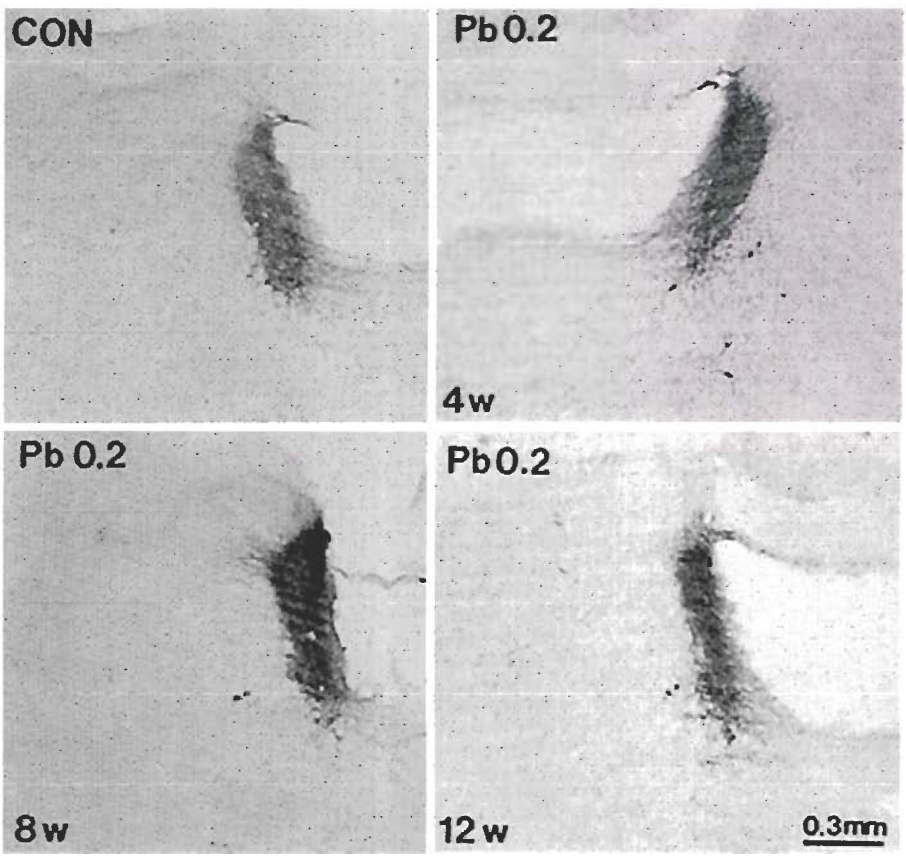

Fig. 2. Tyrosine hydroxylase immunoreactivity in the locus ceruleus of a control rat (**CON**) and in 0.2% (**Pb 0.2**)-4 week, 8 week and 12 week lead treated animals. ×38

were also found in other locations, including the dopaminergic neurons of the substantia nigra pars compacta (A9 group of DAHLSTRÖM and FUXE). It was however hard to find differences in the intensity of the immunoreactivity except in the nucleus locus ceruleus (not shown).

Changes in cell counts

In the control group, the average number of tyrosine hydroxylase immunoreactive cells in the nucleus locus ceruleus was 418.6±65.1 at 4 weeks, 453.2±68.4 at 8 weeks, and 464.1±55.4 at 12 weeks. In the lead treated groups, statistically significant ($p<0.05$) changes in cell counts were apparent compared with equivalent aged controls, but no statistically significant changes were observed between the lead treated groups (Table 2).

The average number of Nissl stained cells in the nucleus locus ceruleus in the control group was 550.7±93.4 at 4 weeks, 565.8±76.5 at 8 weeks, and 558.3±89.0 at 12 weeks. In the lead treated groups, no statistically significant changes in cell counts were apparent compared with equivalent aged controls (Table 3).

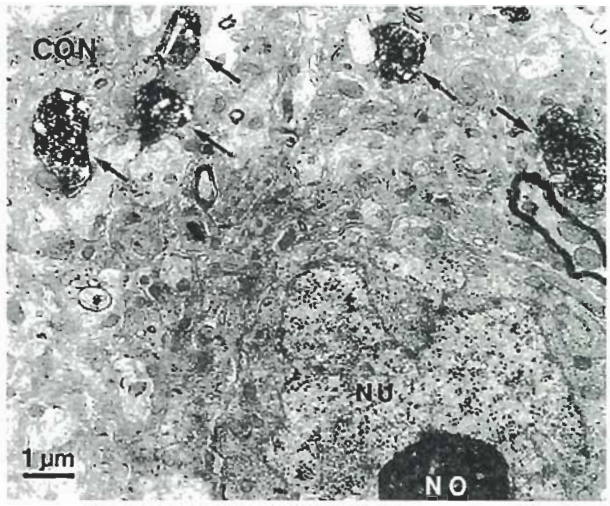

Fig. 3. Election micrograph showing tyrosine hydroxylase immunoreactive profiles in the locus ceruleus of a control rat. Numerous immunoreactive axons (*arrows*) are shown in the vicinity of a locus ceruleus neuron. *Nu* nucleus, *No* nucleolus, × 7,000

Table 2. Changes in tyrosine hydroxylase immunoreactive neurons in the nucleus locus ceruleus of the rat.

	4 weeks	8 weeks	12 weeks
Control group	418.6± 65.1	453.2± 68.4	464.1± 55.4
Lead treated group			
0.05%	493.3± 79.4	501.2±101.5	562.1± 91.0
0.1%	481.6± 94.3	483.7± 77.5	573.2±103.4
0.2%	509.9±112.7	538.1± 81.2	570.1±126.8

N=5 (each group), Mean±S.D.

Table 3. Changes in Nissl stained neurons in the nucleus locus ceruleus of the rat.

	4 weeks	8 weeks	12 weeks
Control group	550.7± 93.4	565.8± 76.5	558.3± 89.0
Lead treated group			
0.05%	569.1±107.1	546.4±101.7	583.2±101.7
0.1%	554.8± 84.7	873.2±117.1	575.9± 86.3
0.2%	570.0±103.5	560.9±105.6	581.6±111.2

N=5 (each group), Mean±S.D.

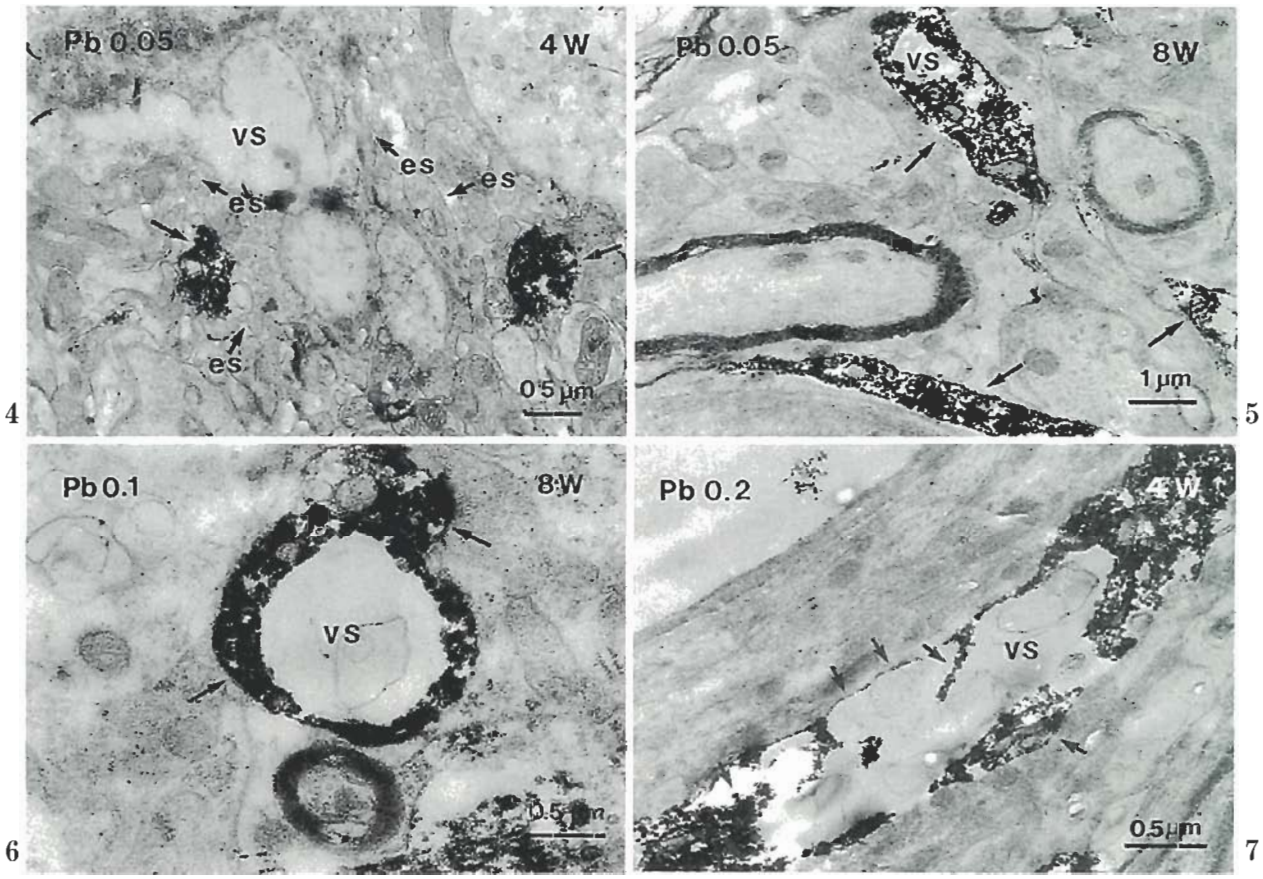

Fig. 4. Tyrosine hydroxylase immunoreactive profiles (*arrows*) in the nucleus locus ceruleus of a lead treated rat (0.05%, 4 weeks). Densely positive granular structures are shown within axons. Widened extracellular spaces (*es*) and large vacuolar spaces (*VS*) are noted. Intracellular organelles are disorganized in some fibers. × 16,000

Fig. 5. Tyrosine hydroxylase immunoreactive profiles (*arrows*) in a lead treated rat (0.05%, 8 weeks). Darkly immunoreactive granules and a large vacuolar region (*VS*) is seen in the axons. Vacuolar regions are not demarcated by membranes. × 9,000

Fig. 6. Tyrosine hydroxylase immunoreactive profiles (*arrows*) in a lead treated rat (0.1%, 8 weeks). The centrally located large vacuolar region (*VS*) in the axon, is surrounded by densely immunoreactive granules. Note that the vacuolar region is not demarcated by membranes. × 22,000

Fig. 7. Longitudinally sectioned tyrosine hydroxylase immunoreactive axon in a lead treated rat (0.2%, 4 weeks). Vacuolar spaces (*VS*) are located in the central region of the axon, while minute immunoreactive granules (*arrows*) are found in the periphery. Almost intact portions of tyrosine hydroxylase immunoreactive granules are found in the proximal and distal part of the axon. × 22,000

Electron microscopic observation

In the control animals, the immunoreactivity for tyrosine hydroxylase was visualized under the electron microscope as electron-dense granules in axons and neuronal cell bodies. The tyrosine hydroxylase immunoreactive axons were unmyelinated. Their axoplasm was rather evenly filled with the electron-dense granules. The granules were clearly demarcated from membrane structures (Fig. 3).

In the lead treated animals, extracellular spaces were widened and large vacuolar spaces were present within the axons. Axonal organelles were disorganized in some fibers (Fig. 4). Frequently, large vacuolar

regions were found in tyrosine hydroxylase immunoreactive axons, and these were surrounded by immunoreactive granules and vacuoles not demarcated by membranes (Figs. 5, 6). Large vacuolar spaces were often located in the central region, while extremely small tyrosine hydroxylase immunoreactive granules were found in the periphery. Almost intact portions of the immunoreactive granules were found in the proximal and distal parts of the axons (Fig. 7). Organelles such as mitochondria and vesicles were absent from the large vacuolar central region, but observed between the immunoreactive granules.

The severity of these changes was unrelated to the lead concentration administered. The vacuolar changes were frequently encountered even in the rats treated with the smallest doses (0.05%) of lead. On the other hand, the severity of the changes seemed somewhat related to the duration of the lead exposure. Widened intercellular spaces and intracellular vacuoles were more frequently found in rats treated with lead for 12 weeks than in the animals treated for 4 weeks.

Tyrosine hydroxylase immunoreactive granules were more darkly stained in the lead treated rats than in the control animals. Repeated careful electron microscopy revealed differences in the immunostaining intensity between both groups. Gray scale (256 grade) densities of each electron photomicrograph were measured using an image analyzer with the Optomax VI software program. The average gray scale values of the granules in the control rats were 223.6 ± 15.6, while those in the lead treated rats were 248.7 ± 7.1, for a significantly difference (<0.05).

DISCUSSION

Ever since DAVID et al. (1972) found that chronic exposure to low level lead could be associated with hyperactivity in children, studies have concentrated on the relationships between lead levels, hyperactivity and catecholamines. GOLTER and MICHAELSON (1975) found a 13% increase in the level of brain norepinephrine, whereas dopamine levels remained unchanged during the early stages of chronic lead exposure in rats with signs of hyperactivity. SILBERGELD and GOLDBERG (1975) also reported that there was a 27% increase in norepinephrine but no change in the level of dopamine in the brain of mice chronically exposed to lead. DECASTRO (1990) measured plasma catecholamine concentrations and found that norepinephrine and epinphrine were significantly elevated in children with chronic lead poisoning. The exposure of rats to low doses of lead selectively increased tyrosine hydroxylase activity in the pons-medulla region (CHIN et al., 1992). In multivariate linear regression step-forward models, 24-h exertions of epinephrine and norepinephrine were significantly and positively associated with blood lead, whereas, both 24-h dopamine and 2-h serotonin excretions were negatively associated with blood lead in 645 male participants (PAYTON et al., 1993). Recently COMINGS et al. (2000), who analyzed dopamine, serotonin, and norepinephrine genes by multivariate regression analysis, found that the adrenergic genes play a greater role in attention deficit hyperactivity disorder (ADHD) than the dopaminergic and serotonergic genes combined.

In this study, tyrosine hydroxylase immunoreactive profiles in the locus ceruleus, both by light and electron microscopy, showed darker, denser staining after chronical exposure to lead. This finding supports the view that the norepinephrinergic system might be activated by that treatment, and this in turn may be responsible for hyperactivity. These results were further substantiated by an increased number of tyrosine hydroxylase immunoreactive neuronal cell bodies with no changes in Nissl stained cells, which represent functional hyperactivity, in the nucleus locus ceruleus region of rats chronically exposed to lead.

SAUERHOFF and MICHAELSON (1973), on the other hand, reported increased levels of dopamine in the brain, and no changes in the levels of norepinephrine in rats during early lead exposure. LASLEY et al. (1984) found that there were no changes in either norepinephrine or dopamine levels. DUBAS and HRDINA (1978) reported significant decreases in cortical norepinephrine, dopamine and serotonin, midbrain dopamine, striatal norepinephrine and hypothalamic dopamine and serotonin, whereas the norepinephrine level in the midbrain was elevated in 8-week-old hyperactive rats during early lead exposure. On the other hand, SINGH and ASHRAF (1989) recorded decreased levels of norepinephrine in the whole brain of early lead-exposed rats.

Changes in the activity of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis, have previously been explored, though with controversial results. Some investigators have reported decreased enzyme activity, especially in relation to dopaminergic systems; the decrease was seen region specifically in the nucleus accumbens, hypothalamus, in combination with no alterations in other regions (MEREDITH et al., 1988; MCINTOSH et al., 1988, 1989; JADHAV and RAMESH, 1997; KOHLER et al., 1997; RAMESH and JADHAV, 1998). CHIN et al. (1992) reported increased tyrosine hydroxylase activity in the

pons-medulla region. The findings of these authors on the regional differences in tyrosine hydroxylase activity and increased enzyme activity in the pons-medulla region are in accordance with our histochemical data on the enzyme activity in the nucleus locus ceruleus.

The mechanism of the increased activity of tyrosine hydroxylase by lead is largely unknown. Evidence is available suggesting that lead stimulates brain protein kinase C (MARKOVAC and GOLDSTEIN, 1988; CHEN et al., 1998) and consequently induces *c-fos* mRNA in PC 12 cells (KIM et al., 1997, 2000), and it is known that *c-fos* mRNA may induce enzyme activation. It is also possible that *c-fos* mRNA induction could be responsible for the activation of tyrosine hydroxylase in neurons of the nucleus locus ceruleus, but further investigation is needed to clarify this hypothesis.

Under the electron microscope, generalized degenerative profiles, such as widened extracellular spaces, large vacuolar spaces within processes, and disorganized intra-axonal organelles were observed in this study. DE GENNARO (1978) found that lead effects the ultrastructure of the developing spinal cord and results in prominent morphological changes, namely, extensive vacuolation and disorganization of the endoplasmic reticulum of neuroglial astrocytes. Postnatal lead administration reportedly altered the development of cerebellar Purkinje cell morphology (MCCONNELL and BERRY, 1979; PATRICK and ANDERSON, 2000). In our present study, degenerative changes were region-specifically evident in the tyrosin hydroxylase immunoreactive axons of the nucleus locus ceruleus region. Large vacuolar regions surrounded by darker tyrosine hydroxylase immunoreactive granular profiles were frequently encountered under the electron microscope.

The average blood lead concentration in Americans was reported to be 8.2 g/d*l* (4.0–26.0 g/d*l*) as measured in 645 male subjects (PAYTON et al., 1993), which is slightly lower than that measured in our control rats (14.72 ± 2.84 g/d*l*). Blood lead levels have steadily decreased in Americans, but this is not a worldwide trend (KOIKE, 1997). In our present study, changes in neurons in the nucleus locus ceruleus were evident at a blood lead level of 0.1% in 4 week lead-exposed rats, just twice the level of control rats. Our result further suggests that the severity of the effects of lead was not related to the lead level but to the duration of exposure. The results of our study would hopefully reinforce the importance of avoiding chronic lead exposure, especially in childhood.

REFERENCES

BROCKEL, B. J. and **D. A. CORY-SLECHTA:** Lead, attention, and impulsive behavior: changes in a fixed-ratio waiting-for-reward paradigm. Pharmacol. Biochem. Behav. 60: 545–552 (1998).

CHEN, H. H., T. MA, A. S. HUME and **I. K. HO:** Developmental lead exposure alters the distribution of protein kinase C activity in the rat hippocampus. Biomed. Environ. Sci. 11: 61–69 (1998).

CHIN, K., J. H. RYU, J. H. CHEONG, K. H. KO and **Y. KUROIWA:** Selective effect of chronic lead ingestion on tyrosine hydroxylase activity in brain regions of rats. J. Toxicol. Sci. 17: 197–210 (1992).

COMINGS, D. E., R. GADE-ANDAVOLU, N. GONZALEZ, S. WU, D. MUHLEMAN, H. BLAKE, G. DIETZ, G. SAUCIER and **J. P. MACMURRAY:** Comparison of the role of dopamine, serotonin, and noradrenaline genes in ADHD, ODD and conduct disorder: multivariate regression analysis of 20 genes. Clin. Genet. 57: 178–196 (2000).

DAVID, O., J. CLARK and **K. VOELLER:** Lead and hyperactivity. Lancet 1: 900–903 (1972).

DE GENNARO, L. D.: The effects of lead nitrate on the central nervous system of the chick embryo I. Observations of light and electron microscopy. Growth 42: 141–155 (1978).

DECASTRO, F. J.: Plasma catecholamine activity in chronic lead poisoning. J. Vet. Hum. Toxicol. 32: 152–153 (1990).

DUBAS, T. C. and **P. D HRDINA:** Behavioural and neurochemical consequences of neonatal exposure to lead in rats. J. Environ. Pathol. Toxicol. 2: 471–84 (1978).

FELDMAN, R. G. and **R. F. WHITE:** Lead neurotoxicity and disorders of learning. J. Child Neurol. 7: 354–359 (1992).

FINKELSTEIN, Y., M. E. MARKOWITZ and **J. F. ROSEN:** Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. Brain Res. Rev. 27: 168–176 (1998).

GOLTER, M. and **I. A. MICHAELSON:** Growth, behavior and brain catecholamines in lead-exposed neonatal rats: A reappraisal. Science 187: 359–361 (1975).

GOYER, R. A.: Lead toxicity: from overt to subclinical to subtle health effects. Environ. Health Perspect. 86: 177–181 (1990).

HOLLOWAY, W. R. JR. and **D. H. THOR:** Low level lead exposure during lactation increases rough and tumble play fighting of juvenile rats. Neurotoxicol. Teratol. 9: 51–57 (1987).

JADHAV, A. L. and **G. T. RAMESH:** Pb-induced alterations in tyrosine hydroxylase activity in rat brain. Mol. Cell Biochem. 175: 137–141 (1997).

JETT, D. A., A. C. KUHLMANN, S. J. FARMER and **T. R. GUILARTE:** Age-dependent effects of developmental lead exposure on performance in the Morris water maze. Pharmacol. Biochem. Behav. 57: 271–279 (1997).

KIM, K. A., M. ANNADATA, G. W. GOLDSTEIN and J. P. BRESSLER: Induction of *c-fos* mRNA by lead in PC 12 cells. Int. J. Devel. Neurosci. 15: 175-182 (1997).

KIM, K. A., T. CHAKRABORTI, G. W. GOLDSTEIN and J. P. BRESSLER: Immediate early gene expression in PC 12 cells exposed to lead: requirement for protein kinase C. J. Neurochem. 74: 1140-1146. (2000).

KOHLER, K., H. LILIENTHAL, E. GUENTHER, G. WINNEKE and E. ZRENNER: Persistent decrease of the dopamine-synthesizing enzyme tyrosine hydroxylase in the rhesus monkey retina after chronic lead exposure. Neurotoxicology 18: 623-632 (1997).

KOIKE, S.: Low-level lead exposure and children's intelligence from recent epidemiological studies in the U.S.A. and other countries to progress in reducing lead exposure and screening in the U.S.A. Nippon Eiseigaku Zasshi 52: 552-561 (1997).

KÖNIGSMARK, B. W.: Methods for the counting of neurons. In: (ed. by) W. J. H. NAUTA and S. O. E. EBBESSON: Contemporary research methods in neuroanatomy. Springer-Verlag, New York-Heidelberg-Berlin, 1970 (p.315-340).

KRIGMAN, M. R., T. W. BOULDIN and P. MUSHAK: Lead. In: (ed. by) P. S. SPENCER and H. H. SCHAUMBURG: Experimental and clinical neurotoxicology. Williams and Wilkins, Baltimore-London, 1980 (p.490-507).

KUHLMANN, A. C., J. L. MCGLOTHAN and T. R. GUILARTE: Developmental lead exposure causes spatial learning deficits in adult rats. Neurosci. Lett. 233: 101-104 (1997).

LASLEY, S. M., R. D. GREENLAND, D. J. MINNEMA and I. A. MICHAELSON: Influence of chronic inorganic lead exposure on regional dopamine and 5-hydroxytryptamine turnover in rat brain. Neurochem. Res. 9: 1675-1688 (1984).

MARKOVAC, J. and G. W. GOLDSTEIN: Picomolar concentrations of lead stimulate brain protein kinase C. Nature 334 (6177): 71-73 (1988).

MCCONNELL, P and M. BERRY: The effects of postnatal lead exposure on Purkinje cell dendritic development in the rat. Neuropathol. Appl. Neurobiol. 5: 115-132 (1979).

MCINTOSH, M. J., P. A. MEREDITH, M. R. MOORE and A. GOLDBERG: Action of lead on neurotransmission in rats. Xenobiotica 19: 101-113 (1989).

MCINTOSH, M. J., P. A. MEREDITH, M. A. PETTY and J. L. REID: Influence of lead exposure on catecholamine metabolism in discrete rat brain nuclei. Comp. Biochem. Physiol. 89C: 211-213 (1988).

MEREDITH, P. A., M. J. MCINTOSH, M. A. PETTY and J. L. REID: Effects of lead exposure on rat brain catecholaminergic neurochemistry. Comp. Biochem. Physiol. 89C: 215-219 (1988).

MILLER, G. D., T. F. MASSARO and E. J. MASSARO: Interactions between lead and essential elements: a review. Neurotoxicology 11: 99-119 (1990).

NEVIN, R.: How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy. Environ. Res. 83: 1-22 (2000).

PATRICK, G. W. and W. J. ANDERSON: Dendritic alterations of cerebellar Purkinje neurons in postnatally lead-exposed kittens. Devel. Neurosci. 22: 320-328 (2000).

PAYTON, M. N., H. HU, D. SPARROW, J. B. YOUNG, L. LANDSBERG and S. T. WEISS: Relation between blood lead and urinary biogenic amines in community-exposed men. Amer. J. Epidemiol. 138: 815-825 (1993).

RAMESH, G. T. and A. L. JADHAV: Region-specific alterations in tyrosine hydroxylase activity in rats exposed to lead. Mol. Cell Biochem. 189: 19-24 (1998).

SAUERHOFF, E. K. and I. A. MICHAELSON: Hyperactivity and brain catecholamines in lead-exposed developing rats. Science 182: 1022-1024 (1973).

SHIGETA, S., K. MIYAKE and T. MISAWA: Critical period of brain development in learning caused by lead exposure in rats. Tokai J. Exp. Clin. Med. 14: 147-152 (1989).

SILBERGELD, E. K. and A. M. GOLDBERG: Pharmacological and neurochemical investigations of lead-induced hyperactivity. Neuropharmacology 14: 431-444 (1975).

SINGH, A. K. and M. ASHRAF: Neurotoxicity in rats sub-chronically exposed to low levels of lead. Vet. Hum. Toxicol. 31: 21-25 (1989).

STERNBERGER, L. A.: Immunocytochemistry, 3rd ed. John Wiley, New York, 1986.

THOMSON, G. O., G. M. RAAB, W. S. HEPBURN, R. HUNTER, M. FULTON and D. P. LAXEN: Blood-lead levels and children's behaviour-results from the Edinburgh Lead Study. J. Child Psychol. Psychiat. 30: 515-528 (1989).

WINDER, C.: The interaction between lead and catecholaminergic function. Biochem. Pharmacol. 31: 3717-3721 (1982).

WINDER, C. and I. KITCHEN: Lead neurotoxicity: A review of the biochemical, neurochemical and drug induced behavioral evidence. Prog. Neurobiol. 22: 59-87 (1984).

WINNEKE, G., H. LILIENTHAL and U. KRAMER: The neurobehavioural toxicology and teratology of lead. Arch. Toxicol. Suppl. 18: 57-70 (1996).

Prof. Kyung Ah PARK
Department of Anatomy
Yonsei University College of Medicine
Shinchon-dong 134, Seodaemun-gu
120-752 Seoul, Korea

Tel: +822-361-5167
Fax: +822-365-0700
E-mail: kapark@yumc.yonsei.ac.kr