SELECTIVE EFFECT OF CHRONIC LEAD INGESTION ON TYROSINE HYDROXYLASE ACTIVITY IN BRAIN REGIONS OF RATS

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Abstract—Alterations of tyrosine hydroxylase activity in various regions of brain from rats postnatally exposed to lead were tested. Three groups of animals were prepared; (1) Rats exposed to lead at a low dose (0.05 % lead acetate, PbAc); (2) Rats exposed to lead at a high dose (0.2 % PbAc); (3) Age-matched normal control rats.

At 2, 4, 6, and 8 weeks of age, weight of brain and body, and concentrations of lead in whole brain of animals in each group were measured. Activities of tyrosine hydroxylase and Na+K+ ATPase were also measured at the same ages in 4 brain regions of each animal. Body weight gain was decreased after 6 weeks of age in rats exposed to lead at a high dose. Concentrations of lead in whole brain were increased from 0.37 to 0.83 (ng/mg wet tissue) in these animals. Exposure of rats to lead generally increased tyrosine hydroxylase activity and decreased Na+K+ ATPase activity. However, changes of tyrosine hydroxylase activity were detected without concomitant changes of Na+K+ ATPase activity in pons-medulla at 2 weeks of age and telencephalon at 6 weeks of age in rats exposed to lead at a low dose, and in midbrain at 4 and 6 weeks of age in rats exposed to lead at a high dose. These data imply that catecholaminergic nervous system in the brain regions described above could be selectively affected by lead.

Key words: Lead toxicity, tyrosine hydroxylase, Na+K+ ATPase, selectivity, brain, rat.

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INTRODUCTION

One of the critical and common toxicity of lead poisoning is toxicity to central nervous system (Costa and Fox, 1983; Walsh et al., 1986) which includes encephalopathy and behavioral disorders (Shin and Hanin, 1978). Although the biochemical basis of neurotoxic effects remains to be completely elucidated, it has been known that concentrations of neurotransmitters and activities of enzymes were altered from several nervous systems in brain of animals exposed to lead (Caspers, 1982; McGinnis and Michaelson, 1987; McIntosh et al., 1988; Meredith et al., 1988). Concentrations of norepinephrine and dopamine in brain (Golter and Michaelson, 1975) as well as those of homovanillic acid and vanilmandelic acid in urine were changed after lead treatment (McIntosh et al., 1988; Ong et al., 1989). Also, the activities of tyrosine hydroxylase, monoamine oxidase and phenylethanolamine N-methyltransferase in brains of animals were affected by lead intoxication (Caspers, 1982; Meredith et al., 1988; Unni and Caspers, 1985). Although alterations of neurochemical factors in these findings may represent the effect of lead on catecholaminergic nervous system, it is not clear whether it was caused as a consequence of selective effect of lead on this particular nervous system or not. Since factors such as ATPase (Markovac and Goldstein, 1988) and amino acid which are not solely neuronal were also affected by lead (Unni and Caspers, 1985), there are always possibilities that abnormalities in lead intoxicated animals in a particular nervous system such as catecholaminergic nervous system were caused by a selective effect on that nervous system or as a part of nonselective effect of lead on all brain tissues. The objective of the present investigation is to clarify this issue. In this study, as an index of lead toxicity to central catecholaminergic nervous system, we measured the activity of tyrosine hydroxylase which is uniquely located in the catecholaminergic nervous system, and as an index of lead toxicity to all the nonspecific tissues in brain, we measured the activity of Na\(^+\)-K\(^+\) ATPase which is located in all tissues of brain. By comparing both indices in brain regions of rats exposed to the same dose of lead, selectivity to catecholaminergic nervous system of lead toxicity was evaluated.

MATERIALS AND METHODS

Experimental design: Animals were divided into three groups using Wistar rat pups. Each group consisted of almost the same numbers of both male and female rats. The first group postnatally received lead acetate at a low concentration (0.05%) for up to 8 weeks. The second group postnatally received lead acetate at a high concentration (0.2%) for up to 8 weeks. The third group received the same treatment as the first and the second groups without lead acetate. Each group was divided into 4 sub-groups, and concentrations of lead, activities of tyrosine hydroxylase and Na\(^+\)-K\(^+\) ATPase in regions of brain and body weights were determined in each sub-group; the first sub-group at 2 weeks of age, the second at 4
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weeks of age, the third at 6 weeks of age and the last at 8 weeks of age, respectively.

**Materials**: Ouabain, Trisma base, Tris-ATP, defatted bovine serum albumin, Folin-Ciocalteu reagent, 6-methyltetrahydropterine (MPH), tyrosine, mercaptoethanol, and catalase (C-10) were obtained from Sigma Chemical Co. (St. Louis, Mo., U. S. A.). [\(^{3}H\)]-[3, 5]-L-tyrosine (303 mCi/mg) and Aquasol were purchased from both New England Nuclear (Boston, Ma., U. S. A.) and from Amersham Searle (Clearwater, Ill., U. S. A.). Tritiated tyrosine used was lyophilized before tyrosine hydroxylase assay. Nitric acid and sulfuric acid were purchased from WAKO Pure Chemical Co. (Osaka, Japan). The maximal limit of lead concentration in nitric acid was 0.00002% and that in sulfuric acid 0.00001%. All inorganic chemicals were reagent grade.

**Animals**: Wistar rat pups of both sexes were used. Male and female rats supplied from the Laboratory Animal Center of Seoul National University were mated at 10 weeks of age. Pregnant rats were selected and caged individually.

Within 1 day from parturition, dams nursing their pups were given drinking water containing 0.05% or 0.2% of lead acetate, ad libitum.

After weanlings, rat pups continued to receive drinking water containing 0.2% or 0.05% of lead acetate throughout the experiment. In all cases number of litters nursed by each dam was equalized to ten in order to minimize differences of nutritional effect during the period of nursing. For example, if one pregnant rat delivered twelve litters and the other eight, two litters among twelve delivered by the first rat were removed from their dam and transferred to the second rat for nursing which delivered only eight litters. All pups were separated from their dams at 3 weeks after birth. Rat pups in the control group received normal tap water. Animals were sacrificed by decapitation between 9 and 10 A. M. of the day when animals became 2, 4, 6, and 8 weeks of age. Brains were rapidly removed from animals and dissected by the method of Glowinski and Iverson (1966) into four anatomical regions: telencephalon, diencephalon, midbrain and pons-medulla.

**Determination of Na\(^{+}\)-K\(^{+}\) ATPase activity**: The activity of Na\(^{+}\)-K\(^{+}\) ATPase was assayed by the method of Silva et al. (1973) using microsomal fraction prepared by the method of Morgan and Matthews (1971). The brain regions were homogenized with tissueemizer (Tekmar Co., Oh, U. S. A.) set at 60 in 10 volumes of solutions (w/v) containing 0.32 M sucrose, 2.4 mM sodium deoxycholate, 2 mM EDTA and 50 mM Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 14,000 xg (15 min, 4°C) and supernatants obtained were centrifuged at 70,000 xg (10 min, 4°C). The pellet was suspended by adding medium containing 0.32 M sucrose, 20 mM EDTA and 50 mM Tris-HCl buffer (pH 7.4) enough to bring the final protein concentrations to 0.4–0.5 mg/ml. All samples were stored at −70°C until used for assays.

Na\(^{+}\)-K\(^{+}\) ATPase activity was determined by subtracting Mg\(^{2+}\)-ATPase activity (ouabain-insensitive) from total ATPase activity. The medium used for estimating total ATPase activity consisted of final concentrations (mM) of: Tris-HCl buffer

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(pH 7.4), 50 ; MgCl₂, 5 ; KCl, 20 ; NaCl, 100 ; and 0.2 ml of enzyme suspension which was stored. Mg-ATPase activity was measured in the above medium without NaCl and KCl and 0.1 mM ouabain. In all experiment reaction mixtures were preincubated in shaken-water bath for 10 min at 37°C. The reaction was started by addition of 5 mM Tris-ATP and incubated in water bath at 37°C. After 20 min, reaction was stopped by adding 1 ml of 10% trichloroacetic acid in an ice bath, and centrifuged for 10 min at 4°C. The inorganic phosphate liberated was measured by the method of Level et al. (1978). To 0.2 ml of supernatant, 0.6 ml of cupric acetate (0.25 % CuSO₄ and 4.6 % NaAc in 2N acetic acid, pH 4) and 0.1 ml of 5 % ammonium molybdate were added and mixed rapidly. Then, 0.1 ml of 2 % Elon in 5 % sodium sulfite was added and mixed. Seven minutes later, the absorbance was read at 870 nm using spectrophotometer (LKB, Biochrom, UK). The enzyme activity was expressed as micromoles of inorganic phosphate liberated per milligram of protein per hour.

**Determination of tyrosine hydroxylase activity**: Tyrosine hydroxylase activity was assayed by radioassay method modified from the method of Reinhard et al. (1986), which determined tritiated water produced during hydroxylation of [³H]-[3, 5]-L-tyrosine. Each tissue prepared was homogenized in 5 volumes (w/v) of ice-cold 5 mM Tris-acetate buffer (pH 6.0) with glass and teflon homogenizer, and centrifuged at 40,000 xg (15 min, 4°C). The supernatant served as the enzyme source to determine the activity of tyrosine hydroxylase to which catalase (4 units/ml) was added. Reaction was carried out in a total volume of 110 μl in a glass test tube. Each reaction mixture contained the following components: 2-mercaptoethanol (0.29 mM), sodium acetate buffer (0.5 M, pH 6.0), tyrosine-HCl (2 mM), enzyme source with catalase (50 μl). The reaction was initiated by adding solution containing [³H]-[3, 5]-L-tyrosine (1 uCi) with MPH, cofactor (75 μg) and incubated for 10 min at 37°C. The reaction was terminated by adding 750 μl of 10 % trichloroacetic acid. The reaction mixture was then rapidly vortexed for 3–5 seconds and stored in ice box. After 5 min, the reaction mixture was loaded on the selectapette pipette tip (Clayadams, NJ.) packed with activated charcoal, and centrifuged at 1,000 xg for 5 min with laboratory capped centrifuge. Aliquots of the eluted water were transferred to scintillation vials containing 10 ml of Aquasol, and counted for tritium in a liquid scintillation counter (LKB, Biochem, UK). Tyrosine hydroxylase activity was expressed as nmoles tyrosine converted per milligram of protein per hour.

**Determination of concentrations of lead in tissues**: Process to digest brain tissue was basically adopted from that of Barry (1975). Whole brain tissues were lyophilized and then digested overnight in acid mixture of nitric acid and sulfuric acid. After standing overnight, the sample which was yellowish solution with foam was sonicated for 2 hours, and then the sample which was yellowish clear solution was diluted by distilled and deionized water (DDW) to make the volume of 80–100 ml. Concentration of lead in tissue was measured by the ICP (Perkin-Elmer,
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USA) method. The sample was analyzed with ICP for lead at the emission wavelength: 220.353. Selected wavelength was recommended by the instrument manufacturer, and represented the wavelength giving the strongest signal to noise ratio. The instrument was calibrated daily with external standard (Junsei Chemical Co., Japan). Nominal recovery for lead was 97 ± 1.2 % and recovery efficiency for lead was determined at 1 ppm. The lower limit of sensitivity was 0.1 μg/ml.

Protein assay: Protein content was measured by the method of Lowry et al. (1951). Bovine serum albumin was used as standard.

Data analysis: Activities of Na⁺–K⁺ ATPase and tyrosine hydroxylase were examined by analyses of variance (ANOVA) in each brain region for a given condition with concentration of lead and time after exposure to lead (at each age of animals) as the main factors. One-way ANOVA compared means from exposed (to lead either at low or high concentrations) and control groups in animals. Weights of brain and body as well as concentrations of lead in brain tissues from exposed and control groups in animals were compared by the same procedure. When ANOVA indicated the presence of a significant exposure effect, standard t test was used to compare means from exposed and control animals to determine the basis of the difference.

RESULTS

Whole brain weight gain in rats postnatally exposed to lead acetate either at low or high doses was not significantly different from that in normal control animals as illustrated in Fig. 1, but body weight gain (Fig. 2) was decreased in rats exposed to lead at high dose after 6 weeks of age.

Lead concentrations in whole brain as shown in Fig. 3 were significantly increased (from 0.37 to 0.83 ng/mg wet tissue) in rats exposed to lead at a high dose, but were not significantly changed in rats exposed to lead at low dose.

The effect of exposure of pups to lead from birth on tyrosine hydroxylase in various brain regions is summarized in Table 1. Brain regions which exhibited a significant increase in tyrosine hydroxylase activity in rats exposed to lead at low dose were diencephalon, pons-medulla at 2 weeks of age and telencephalon at 6 weeks of age, and those in rats exposed to lead at a high dose were diencephalon and pons-medulla at 2 weeks of age, midbrain and pons-medulla at 4 weeks of age, telencephalon, midbrain and pons-medulla at 6 weeks of age.

The effect of exposure of pups to lead on Na⁺–K⁺ ATPase activity in various brain regions is summarized in Table 2. The activities of Na⁺–K⁺ ATPase were consistently increased in all groups with age. In lead-exposed rats, the activities of Na⁺–K⁺ ATPase were in most cases lower than those of control group. Brain regions which exhibited a significant decrease in Na⁺–K⁺ ATPase activity in rats exposed to lead at a low dose were diencephalon and pons-medulla at 2 weeks of age, telencephalon and midbrain at 8 weeks of age, and those in rats exposed to lead at a
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Fig. 1. Whole brain weight of rats postnatally exposed to lead at low (0.05 %) and high (0.2 %) doses as lead acetate through drinking water. Each point represents the mean ± S. D. of the data from 4-5 animals.

high dose were telencephalon, diencephalon and pons-medulla at 2 weeks of age, diencephalon and pons-medulla at 4 weeks of age, telencephalon, diencephalon and midbrain at 8 weeks of age.

The effect of postnatal exposure of pups to lead on the activities of tyrosine hydroxylase and Na\(^+\)-K\(^+\) ATPase are summarized in Tables 3 and 4 for comparison.

The change of tyrosine hydroxylase activity was detected, but not Na\(^+\)-K\(^+\) ATPase activity, in pons-medulla at 2 weeks of age and telencephalon at 6 weeks of age in animals exposed to lead at a low dose, and detected in midbrain at 4 and 6 weeks of age in rats exposed to lead at a high dose.

**DISCUSSION**

The effect of lead on body weight gain was observed after 6 weeks of age in rats exposed to lead at a high dose (Fig. 2). Decreases in body weight gain were
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Fig. 2. Body weight of rats postnatally exposed to lead at low (0.05 %) and high (0.2 %) doses as lead acetate through drinking water. Each point represents the mean ± S. D. of the data from 4-5 animals. * indicates a significant difference from control group (*: P<0.05).

considered to be the sign of lead toxicity in animals since there were no changes in mortality as a result of exposure to lead in this study.

Patterns of brain weight gain were not affected in both groups of rats exposed to lead at either low or high doses throughout the entire stages of experiment. Similar results have been reported by other investigators (Costa and Fox, 1983).

Na⁺–K⁺ ATPase is the enzyme which is known to be located in all neural systems (Godfraind et al., 1975). Na⁺–K⁺ ATPase activity was increased with age in all brain regions of any groups of animals tested in this experiment. In groups of rats exposed to lead at low or high doses, Na⁺–K⁺ ATPase activity in most brain regions was lower than that in age matched control animals. The difference in enzyme activity due to exposure to lead tended to be dose-dependent in most cases (Table 2). The inhibitory effect of lead on Na⁺–K⁺ ATPase from the present data is in agreement with the finding of others (Markovac and Goldstein, 1988).

Tyrosine hydroxylase is the initial and rate-limiting enzyme in catecholamine
biosynthesis and it is uniquely located in the catecholaminergic nervous systems (Shiman et al., 1971). Its activity could be activated by several factors such as nerve stimulation (Morganroth et al., 1974), drug treatment (Zigmond et al., 1974), and anaerobic conditions (Pastuszko et al., 1985). Tyrosine hydroxylase activity was found to be increased in some regions of brains from animals exposed to lead as shown in Table 1. In rats exposed to lead at a low concentration, the greater activity of tyrosine hydroxylase was observed in telencephalon at 6 weeks of age, diencephalon at 2 weeks of age and pons-medulla at 2 weeks of age, respectively, than that in age-matched normal control animals. Similar patterns of the effect of lead on tyrosine hydroxylase were observed in regions of brain from rats exposed to lead at a high dose. Overall effects of lead on the activity of tyrosine hydroxylase seemed to be dependent on regions of brain. Since the concentrations of lead in brain tissues

Fig. 3. Concentrations of lead in brain tissues of rats postnataally exposed to lead at low (0.05 %) and high (0.2 %) doses as lead acetate through drinking water. Brain tissues pooled from 4-5 animals were used for each assay. Each point represents the mean value from 3 replicates. Concentrations of lead from control rats were not detectable.
Table 1. TH activity in brain areas of CNS of postnatally lead-exposed rats.

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Group</th>
<th>2 Weeks of Age</th>
<th>4 Weeks of Age</th>
<th>6 Weeks of Age</th>
<th>8 Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TELENCEPHALON</td>
<td>Control</td>
<td>3.90±0.51</td>
<td>6.65±0.78</td>
<td>5.90±0.58</td>
<td>5.93±0.45</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>4.50±0.74</td>
<td>6.90±0.31</td>
<td>7.49±0.42*</td>
<td>6.21±0.38</td>
</tr>
<tr>
<td></td>
<td>0.2 %</td>
<td>4.60±0.58</td>
<td>7.31±0.76</td>
<td>7.93±0.60*</td>
<td>6.36±0.51</td>
</tr>
<tr>
<td>DIENCEPHALON</td>
<td>Control</td>
<td>6.45±0.67</td>
<td>4.90±0.47</td>
<td>5.02±1.25</td>
<td>3.70±0.83</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>8.64±0.51*</td>
<td>4.88±0.92</td>
<td>4.49±0.11</td>
<td>4.78±1.01</td>
</tr>
<tr>
<td></td>
<td>0.2 %</td>
<td>7.78±0.56*</td>
<td>5.78±1.10</td>
<td>4.75±1.63</td>
<td>4.09±0.47</td>
</tr>
<tr>
<td>MIDBRAIN</td>
<td>Control</td>
<td>9.24±1.23</td>
<td>7.58±0.76</td>
<td>6.21±0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td></td>
<td>10.68±0.63</td>
<td>7.15±0.67</td>
<td>6.19±0.31</td>
</tr>
<tr>
<td></td>
<td>0.2 %</td>
<td></td>
<td>11.81±0.63*</td>
<td>9.77±0.51*</td>
<td>6.23±0.47</td>
</tr>
<tr>
<td>PONS/MEDULLA</td>
<td>Control</td>
<td>1.39±0.11</td>
<td>1.87±0.05</td>
<td>1.82±0.31</td>
<td>1.85±0.16</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>2.17±0.18*</td>
<td>1.99±0.20</td>
<td>1.69±0.18</td>
<td>1.96±0.18</td>
</tr>
<tr>
<td></td>
<td>0.2 %</td>
<td>1.65±0.16*</td>
<td>2.18±0.05*</td>
<td>2.30±0.09*</td>
<td>1.78±0.31</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S. D. of data from 5-6 animals.
* indicates a significant difference from control group (P<0.05).
# # : Data for midbrain at 2 weeks of age are represented by those for diencephalon since assays were carried out using both tissues unseparated.
Table 2. Na/K-ATPase activity in brain areas of postnatally lead-exposed rats.

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Group</th>
<th>2 Weeks of Age</th>
<th>4 Weeks of Age</th>
<th>6 Weeks of Age</th>
<th>8 Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TELENCEPHALON</td>
<td>Control</td>
<td>9.36 ± 1.33</td>
<td>12.13 ± 0.71</td>
<td>16.02 ± 1.26</td>
<td>18.50 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>7.60 ± 2.02</td>
<td>10.24 ± 3.81</td>
<td>11.55 ± 2.39</td>
<td>15.56 ± 0.86</td>
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<tr>
<td></td>
<td>0.2%</td>
<td>5.98 ± 1.99*</td>
<td>9.39 ± 2.38</td>
<td>11.40 ± 1.90*</td>
<td>13.98 ± 1.88*</td>
</tr>
<tr>
<td>DIENCEPHALON</td>
<td>Control</td>
<td>1.00 ± 1.95</td>
<td>12.23 ± 1.53</td>
<td>18.23 ± 4.76</td>
<td>19.44 ± 0.13</td>
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<tr>
<td></td>
<td>0.05%</td>
<td>7.54 ± 0.60*</td>
<td>16.55 ± 4.41</td>
<td>15.39 ± 1.49</td>
<td>14.46 ± 3.42</td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>8.26 ± 1.54*</td>
<td>12.53 ± 1.66*</td>
<td>10.38 ± 1.50*</td>
<td>17.79 ± 0.50*</td>
</tr>
<tr>
<td>MIDBRAIN</td>
<td>Control</td>
<td></td>
<td>14.04 ± 1.80</td>
<td>19.34 ± 5.93</td>
<td>23.18 ± 2.24</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td># #</td>
<td>11.77 ± 7.08</td>
<td>18.90 ± 0.55</td>
<td>16.94 ± 2.30*</td>
</tr>
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<td></td>
<td>0.2%</td>
<td></td>
<td>12.04 ± 2.89</td>
<td>13.03 ± 3.06</td>
<td>14.88 ± 1.50*</td>
</tr>
<tr>
<td>PONS/MEDULLA</td>
<td>Control</td>
<td>10.38 ± 2.09</td>
<td>12.34 ± 1.93</td>
<td>18.85 ± 1.66</td>
<td>20.60 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>7.58 ± 0.80</td>
<td>12.55 ± 0.73</td>
<td>15.39 ± 1.90</td>
<td>15.95 ± 1.86</td>
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<tr>
<td></td>
<td>0.2%</td>
<td>6.84 ± 2.41*</td>
<td>10.16 ± 3.12*</td>
<td>11.89 ± 1.22*</td>
<td>15.10 ± 3.00</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S. D. of data from 5-6 animals.
* indicates a significant difference from control group (P<0.05).
# # : Data for midbrain at 2 weeks of age are represented by those for diencephalon since assays were carried out using both tissues unseparated.
Table 3. The selective effect of lead at low dosage on TH activity in CNS of rats.

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>2 Weeks of Age</th>
<th>4 Weeks of Age</th>
<th>6 Weeks of Age</th>
<th>8 Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH (+)</td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
</tr>
<tr>
<td>Na/K-ATPase (-)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TH (+)</td>
<td>DIENCEPHALON/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na/K-ATPase (+)</td>
<td>MIDBRAIN</td>
<td>MIDEA</td>
<td>MIDEA</td>
<td>MIDEA</td>
</tr>
<tr>
<td>TH (-)</td>
<td></td>
<td></td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
</tr>
<tr>
<td>Na/K-ATPase (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+) indicates change of enzyme activity following lead intoxication compared with control group.
(-) indicates no changes of enzyme activity following lead intoxication compared with control group.

Table 4. The selective effect of lead at high dosage on TH activity in CNS of rats.

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>2 Weeks of Age</th>
<th>4 Weeks of Age</th>
<th>6 Weeks of Age</th>
<th>8 Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH (+)</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
</tr>
<tr>
<td>Na/K-ATPase (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH (+)</td>
<td>DIENCEPHALON/</td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
<td>PONS-MEDULLA</td>
</tr>
<tr>
<td>Na/K-ATPase (+)</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
<td>MIDBRAIN</td>
</tr>
<tr>
<td>TH (-)</td>
<td>TELENCEPHALON</td>
<td>DIENCEPHALON</td>
<td>DIENCEPHALON</td>
<td>DIENCEPHALON</td>
</tr>
<tr>
<td>Na/K-ATPase (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+) indicates change of enzyme activity following lead intoxication compared with control group.
(-) indicates no changes of enzyme activity following lead intoxication compared with control group.
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were available only from whole brain in the present study, it is difficult to draw any conclusion whether such findings on tyrosine hydroxylase activity in particular regions of brain would be related to concentration of lead in the same region of brain from the same group of animals. Another issue on alterations in tyrosine hydroxylase activity due to exposure of rats to lead may be focused on whether such changes in certain regions of brain are transient or not. In telencephalon at 6 weeks of age and in diencephalon at 2 weeks of age, such alterations due to lead seem to be transient regardless of dosage of lead employed in this experiment, whereas in midbrain and pons-medulla, these alterations became long-lasting from transient as the dosage of lead increases. One speculation on this issue may be possible that such changes could be related to blood brain barrier which regulates the permeation of amino acid such as tyrosine. Michaelson and Bradbury (1982) have noted that [14C]-tyrosine transport across blood barrier to brain of rats exposed to lead was not different from that of normal control rats. Although concentration of lead administered through drinking water in that study was 0.1 % which was different from that in the present study, the period of exposure of animals to lead was up to 70 days of age which was far longer than that in our study. Therefore it does not seem to be plausible to think that there was a great difference between amount of lead administered to rat pups in our experiment and Michaelson's although such speculation is not fit for precise comparison of the amount of lead. Based on such comparison, it may be difficult to interpret that the changed pattern of tyrosine hydroxylase activity in regions of brain detected in the present study is related to the change of blood brain barrier permeability due to lead during development. Tyrosine hydroxylase activity is changed without concomitant change in Na⁺-K⁺ ATPase activity following the exposure of animals to lead. In the present experiment, the data where tyrosine hydroxylase activity was increased without change of Na⁺-K⁺ ATPase activity were collected in pons-medulla at 2 weeks of age and telencephalon at 6 weeks of age of rats exposed to low dose of lead (Table 3). Of rats exposed to lead at a high dose, such data were collected only in the midbrain at 4 and 6 weeks of age (Table 4). It is well known that any tissue can be affected when the dosage of lead is large enough (Bondy 1986; Fowler et al., 1980; Costa and Fox, 1983; Walsh et al., 1986). Therefore, one possible explanation for these data could be that tyrosine hydroxylase in those brain regions might selectively be affected by lead. It is difficult to reach a concrete interpretation for the mechanism whether selective toxicity was caused by different affinities of lead to these enzymes or by different degree of accumulation of lead in the regions of brain. One explanation for all the remaining regions of brain where Na⁺-K⁺ ATPase activity was changed with or without change of tyrosine hydroxylase activity following the exposure of animals to lead may be that the effect of lead was not selective on both non-neural and neural tissues including catecholaminergic nervous system. Another explanation may be that, perhaps, the dosage of lead employed in the present experiment is not low enough to induce differential
Chronic effect of lead on brain tyrosine hydroxylase

changes of the two different enzymes activity.

Since Na\(^+\)-K\(^+\) ATPase is located in all neural and non-neural tissues of brain and tyrosine hydroxylase is uniquely located in catecholaminergic nervous system, selective effect of lead on tyrosine hydroxylase in particular brain regions detected in the present study may be employed as an index representing selective toxicity of lead to catecholaminergic nervous system.

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