Korean J. Toxicol.
Vol. 6, No. 2, pp. 131-142, 1990

SELECTIVE TOXICITY OF CHRONIC LEAD INGESTION TO CENTRAL CATECHOLAMINERGIC NERVOUS SYSTEM IN RATS

Jong Hoon Ryu, Jae Hoon Cheong, Kang Chin* and Kwang Ho Ko

Department of Pharmacology, College of Pharmacy, Seoul National University and
**National Institute of Safety Research, Seoul, Korea*

(Received July 10, 1990)
(Accepted August 30, 1990)

ABSTRACT: *The selective toxicity of lead was tested in central catecholaminergic nervous system of postnatally lead exposed rats. Three groups of animals were prepared; 1) rats exposed to low dose of lead (0.05% PbAc); 2) rats exposed to high dose of lead (0.2% PbAc); 3) age-matched normal control rats. At 2, 4, 6 and 8 weeks of age brain and body weight gain, and lead concentrations in brain tissues were measured. At the same ages tyrosine hydroxylase and Na-K ATPase activities were measured in the 4 brain areas of each animal. Body weight gain was decreased after 6 weeks of age in rats exposed to high dose of lead. Concentrations of lead in whole brain tissues were increased from 0.37 to 0.83 (ng/mg wet tissue) in these animals. In lead exposed rats, tyrosine hydroxylase activities were higher but Na-K ATPase activities were lower than those of age-matched control animals. Brain areas where tyrosine hydroxylase activities were detected without concomitant changes of Na-K ATPase activities were pons-medulla (2 weeks of age) and telencephalon (6 weeks of age) in rats exposed to low dose of lead, and those in rats exposed to high dose of lead were midbrain (4 and 6 weeks of age). These data indicate that catecholaminergic nervous system in the brain areas described above could selectively be affected by lead.*

INTRODUCTION

Lead poisoning produces toxic effects on several biological systems, such as heme-hemoprotein system (Bondy, 1986), kidney (Fowler *et al.*, 1980), central nervous system (Costa and Fox, 1983; Walsh *et al.*, 1986), etc. (Hammond, 1977; Vallee and Ulmer, 1972). Toxicity of lead to central nervous system is known to be critical and common, and has been reported to produce behavioral abnormalities including decreased learning performance, altered behavior, irritability and headache (Brown, 1975; Palmer, 1985; Sauerhoff and Michaelson, 1973). It has been reported that these behavioral effects may be related to the decreased neurite processes and synapses, hypomyelination, delayed maturation of glucose metabolism, and biochemical alterations of several nervous systems including catecholaminergic nervous

system in brain (Dorothy *et al.*, 1982; Holtzman *et al.*, 1987; Regunathan and Sundaresan, 1984; Silbergeld and Alden, 1978).

Although the existing literatures indicate that abnomalities in several nervous systems including catecholaminergic, serotonergic and cholinergic nervous systems play a pathological role in animals poisoned with lead, differentiation of the roles of several nervous systems in causing diseases induced by lead has not been clearly demonstrated. There are always possibilities that in lead intoxicated animals abnormalities in a particular nervous system such as catecholaminergic nervous system observed in brain were produced by a selective action on that nervous system or by a nonselective action of lead on all the CNS tissue. Purpose of the present investigation is to provide clarification of this issue. In this investigation, 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 CNS tissues, we measured the activity of Na/K-ATPase which is located in all tissues of brain.

MATERIALS AND METHODS

Materials

Ouabain, Trisma base, Tris-ATP, deffated Bovine Serum Albumine, Folin-Ciocalteu reagent, DL6-methyltetrahydropterine (DMPH), tyrosine, mercaptoethanol, and catalase (C-10) were obtained from Sigma Chemical Co. (St. Louis, Mo). H-[3,5]-L-tyrosine (303 mCi/mg) and Aquasol were purchased from either New England Nuclear (Boston, Ma) or from Amersham Searle (Clearwater, Il). Tritiated tyrosine used was lyophillized before tyrosine hydroxylase assay. All inorganic chemicals were reagent grade.

Experimental Design

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

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 of parturition, experimental mothers nursing their pups were given drinking water containing lead acetate 0.2% or 0.05%, ad libitum. After weanings, rat pups continued to receive

drinking water containing lead acetate 0.2% or 0.05%, throughout the expeirment. In all cases the litters were normalized to 10 animals and the pups were separated from their dams at 3 weeks after birth. Rat pups in the control group received normal tap water. The animals were sacrificed by decapitation between 9 and 10 A.M. of the day when animals become 2, 4, 6 and 8 weeks of age. Brains were rapidly removed from animals and dissected by the method of Miller *et al.* (1970) into four areas: telencephalon, diencephalon, midbrain and pons-medulla.

Determination of Na-K ATPase Activity

The activity of Na-K ATPase were assayed by the method of Silva *et al.* (1973) using microsomal fraction which were prepared by the method of Morgan *et al.* (1971). The brain regions were homogenized with tissuemizer (Tekmar Co. Oh) 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 × g (15 min, 4°C) and supernatants obtained were centrifuged at 70,000 × g (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 substrating Mg- ATPase activity (ouabain-insensitive) from total ATPase activity. The medium used for estimation of total ATPase activity consisted of final concentrations (mM) of: Tris-HCl buffer (pH 7.4), 50; MgCl, 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 containing 0.1 mM ouabain. In all experiments reaction mixture was 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 a 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 Lebel *et al.* (1978). To 0.2 ml supernatant 0.6 ml of Cupper acetate (0.25% $CuSO_4$ and 4.6% NaAc in 2N acetic acid, pH 4) and 0.1 ml of 5% ammonium molibdate 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 the 870 nm using spectrophotometer (LKB, Biochrom, Engl.). The activity of enzyme 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 methods of Nagatsue *et al.* (1964) and Reinhard *et al.* (1986), which determines tritiated water produced during hydroxylation of H-[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 × g (15 min, 4°C). The supernatant served as the enzyme source to determine the activity of tyrosine hydroxylase which was added catalase (4 units/ml). Reaction was carried out in a total volume of 110 ul in a glass test tube. Each reaction mixture contains the following components: 2-mercaptoethanol (0.29 M), sodium acetate buffer (0.5M, pH

6.0), tyrosine-HCl (2 mM), enzyme source with catalase (50 u*l*). Reaction was initiated by adding solution containing H-[3,5]-L-tyrosine (1 uCi) with MPH, cofactor (75 ug) and was incubated for 10 min at 37°C. The reaction was terminated by adding 750 u*l* 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 × g for 5 min with laboratory capped centrifuge. Aliquots of the eluted water were transferred to scintillation vials containing 10 m*l* of Aquasol, and counted for tritium in a liquid scintillation counter (LKB, Biochrom, Engl.). Tyrosine hydroxylase activity was expressed as nmoles tyrosine converted per milligram of protein per hour.

Determination of Lead Concentrations in Tissues

Lead levels were measured in the whole brain tissue by the inductively coupled plasma (Perkin-Elmer, USA). Desiccated frozen tissue sample was pulverized to a homogeneous powder. Each pulverized sample was placed in a test tube and 10 m*l* of acid mixture (nitric acid: perchloric acid = 1:1 (v/v)) was added. The test tube incubated at 40°C until no solid material was visible and applied directly.

Protein Assay

Protein content was measured by the method of Lowry *et al.* (1954). Bovine serum albumine was used as standard.

Statistical Analysis

Significant difference between groups was determined by the unpaired t-test. Differences between means were considered stastically significant when the calculated p values were less than 0.05.

RESULTS

Effects of Lead on Brain and Body Weight Gain

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

Lead Concentrations in Brain Tissues

Lead concentrations in whole brain were increased slightly from 0.25 to 0.27 (ng/mg wet tissue) in low dose- exposed rats, and highly from 0.37 to 0.83 (ng/mg wet tissue) in high dose-exposed rats (Fig. 3).

Effect of Lead on Tyrosine Hydroxylase Activity

The effect of lead ingestion to rat pups on tyrosine hydroxylase from birth in various brain areas was summarized in Table 1. In lead exposed rats, tyrosine hydroxylase ac-

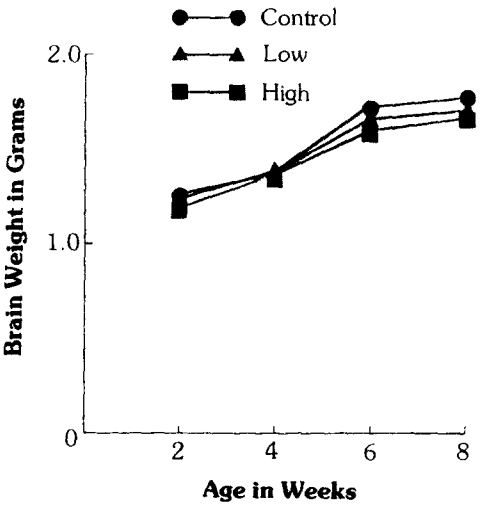

Fig. 1. Whole brain weight of offspring exposed to lead throughout experimental period. Each point represents of the mean value from at least 4 animals.

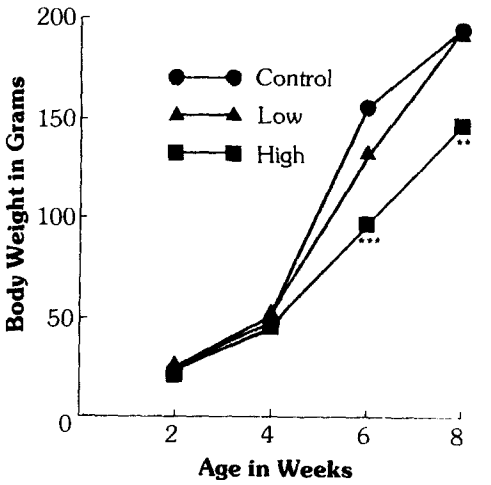

Fig. 2. Body weight of offsprings chronically exposed to lead throughout experimental period. Each point represents the mean value from at least 4 animals. *indicates a significant difference from control group (**: $P<0.01$, ***: $P<0.001$).

tivities were consistently higher than those of control group. Brain areas which exhibited a significant increase in tyrosine hydroxylase activity in rats exposed to low dose of lead were diencephalon, pons-medulla (2 weeks of age) and telencephalon (6 weeks of age), and those in rats exposed to high dose of lead were diencephalon and pons-medulla (2 weeks of age), midbrain and pons-medulla (4 weeks of age), telencephalon, midbrain and pons-medulla (6 weeks of age).

Effect of Lead on Na-K ATPase Activity

The effect of lead ingestion to rat pups on Na-K ATPase activity in various brain

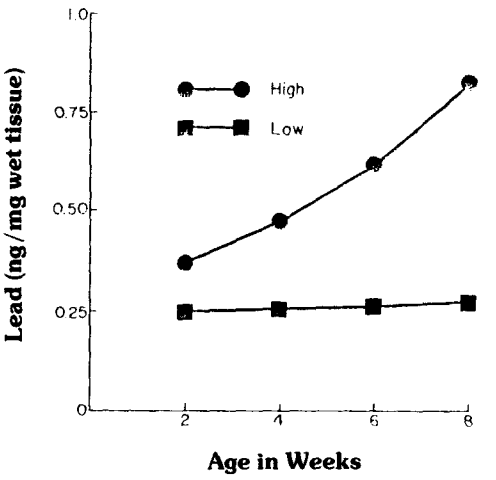

Fig. 3. Concentrations of lead in brain tissues from rat pups postnatally exposed to either low or high dose of lead acetate dissolved in drinking water. Brain tissues per each assay were pooled from at least 4 animals. Each point represents the mean value from 3 replicates.

Table 1. TH activities in brain areas of CNS of postnatally lead-exposed rats

Brain Area		Na-K ATPase Activity (umole/mg protein/hr.)			
		2 Weeks of Age	4 Weeks of Age	6 Weeks of Ages	8 Weeks of Age
TELENCEPHALON	Control	3.90±0.23	6.65±0.35	5.90±0.26	5.93±0.20
	0.05%	4.50±0.33	6.90±0.14	7.49±0.19**	6.21±0.17
	0.2%	4.60±0.26	7.31±0.34	7.93±0.27**	6.36±0.23
DIENCEPHALON	Control	6.45±0.30	4.90±0.21	5.02±0.56	3.70±0.37
	0.05%	8.64±0.23**	4.88±0.41	4.49±0.05	4.78±0.45
	0.2%	7.78±0.25**	5.78±0.41	4.75±0.73	4.09±0.21
MIDBRAIN	Control		9.24±0.55	7.58±0.34	6.21±0.32
	0.05%		10.68±0.28	7.15±0.30	6.19±0.14
	0.2%		11.81±0.28*	9.77±0.23**	6.23±0.21
PONS-MEDULLA	Control	1.39±0.05	1.87±0.02	1.82±0.14	1.85±0.07
	0.05%	2.17±0.08**	1.99±0.99	1.69±0.08	1.96±0.08
	0.2%	1.65±0.07*	2.18±0.02**	2.30±0.04**	1.78±0.14

Each value represents the mean ± S.E.M. of data from at least 4 animals.
*indicates a significant difference from control group.
(*; $P<0.05$, **; $P<0.01$, ***; $P<0.001$)
Data of DIENCEPHALON at 2 weeks of age are shown as enzyme activities from areas containing DIENCEPHALON and MIDBRAIN.

areas was 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 always lower than those of control group. Brain areas whch exhibited a significant decrease in Na-K APTase activity in rats exposed to low dose of lead were diencephalon and pons-medulla (2 weeks of age), telencephalon and midbrain (8 weeks of age), and those in rats exposed to high dose of lead were telencephalon, diencephalon and pons-medulla (2 weeks of age), diencephalon and pons-medulla (4) weeks of age), telencephalon, diencephalon and midbrain (8 weeks of age).

Table 2. Na-K ATPase activities in brain areas of CNS of postnatally lead-exposed rats

Brain Area		Na-K ATPase activity (umole/mg protein/hr.)			
		2 Weeks of Age	4 Weeks of Age	6 Weeks of Ages	8 Weeks of Age
TELENCEPHALON	Control	9.36±0.50	12.23±0.29	16.02±0.89	18.50±0.56
	0.50%	7.60±1.16	10.24±1.90	11.55±1.38	15.56±0.43*
	0.2%	5.98±1.15*	9.39±1.37	11.40±0.95*	13.98±1.09*
DIENCEPHALON	Control	11.00±0.74	12.23±0.88	18.23±2.38	19.44±0.08
	0.05%	7.53±0.35**	11.91±0.61	15.39±0.86	14.46±1.71
	0.2%	8.26±0.77*	9.70±1.10*	10.38±0.86*	17.79±0.25*
MIDBRAIN	Control		14.04±1.04	19.35±2.10	23.17±1.12
	0.05%		11.77±4.09	18.90±0.32	16.94±1.15**
	0.2%		12.04±1.67	13.03±1.53	14.88±0.87
PONS-MEDULLA	Control	10.38±0.79	12.33±0.96	18.85±0.83	20.60±2.15
	0.05%	7.58±0.57	12.55±0.52	15.39±1.34	15.95±0.93
	0.2%	6.84±1.39*	10.16±1.80*	11.88±0.61**	15.10±1.73

Each value represents the mean ± S.E.M. of data from at least 4 animals.
*indicates a significant difference from control group.
(*; $P<0.05$, **; $P<0.01$, ***; $P<0.001$)
Data of DIENCEPHALON at 2 weeks of age are shown as enzyme activities from areas containing DIENCEPHALON and MIDBRAIN.

Table 3. The selective effect of lead on TH activity in CNS of rats (low dose)

	2 Weeks of Age	4 Weeks of Age	6 Weeks of Age	8 Weeks of Age
TH (+)/			TELENCEPHALON	
Na-K ATPase (-)	PONS-MEDULLA			
TH (+)/	DIENCEPHALON			
Na-K ATPase (+)				
TH (-)/	TELENCEPHALON	TELENCEPALON	DIENCEPHALON	TELENCEPHALON
		DIENCEPHALON	MIDBRAIN	DIENCEPHALON
		MIDBRAIN	PONS-MEDULLA	MIDBRAIN
		PONS-MEDULLA		PONS-MEDULLA

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

Table 4. The selective effect of lead on TH activity in CNS of rats (high dose)

	2 Weeks of Age	4 Weeks of Age	6 Weeks of Age	8 Weeks of Age
TH (+)/		MIDBRAIN	MIDBRAIN	
Na-K ATPase (-)				
TH (+)/	DIENCEPHALON		TELENCEPHALON	
Na-K ATPase (+)	PONS-MEDULLA	PONS-MEDULLA	PONS-MEDULLA	
TH (-)/	TELENCEPHALON	TELENCEPHALON	DIENCEPHALON	TELENCEPHALON
		DIENCEPHALON		DIENCEPHALON
				MIDBRAIN
				PONS-MEDULLA

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

Selective Effect of Lead on Tyrosine Hydroxylase Activity

The effect of postnatal lead ingestion to rat pups on activities of tyrosine hydroxylase and Na-K ATPase were summarized in Table 3 and 4. Brain areas where changes of activities were detected not for Na-K ATPase but for tyrosine hydroxlase were pons-medulla at 2 weeks of age and telencephalon at 6 weeks of age in animals exposed to low dose of lead, and those in rats exposed to high dose of lead were midbrain at 4 and 6 weeks of age.

DISCUSSIONS

Decreases in body weight gain was taken as the sign of lead toxicity in animals since there were no changes of mortality as a result of lead exposure in the current study. The effect of lead on body weight gain was observed after 6 weeks of age in rats exposed to high dose of lead. Patterns of brain weight gain were not affected in both groups of rats exposed to either low or high dose of lead throughout the entire stages of experiment. Similar results have been reported by investigators (Costa and Fox,1983).

Na-K ATPase is the enzyme which is known to be located in all neural systems. It is involved in the active transport of ions across the cell membrane and that enzyme existing in the nerve terminal is associated with the release and reuptake of neurotransmitters (God frained *et al.*, 1975). Na-K ATPase activity was increased with age in all brain areas of any groups of animals tested in this experiment. In groups of rats exposed to low or high dose of lead, Na-K ATPase activities in all brain areas were lower than those in age matched control animals and these differences of enzyme activities due to lead exposure were dose-dependent. These data indicate that the decreased Na-K ATPase activates in brain areas are caused by inhibitory effect of lead on that enzyme. The inhibitory effect of lead on Na-K ATPase could primarily be ascribed to suppression of either development or function of neural tissue. In agreement with this interpretation we and Phillis (1981) reported that Na-K ATPase activity increases during the period for the formation of dendrites and with an increase in electrical activity of neural tissue. The mechanism of lead for inhibition of Na-K ATPase activity was not further experimented in this study, but several possibilities accepted from other reports might be offered. Decrease in the Na-K ATPase activity could be results derived from reduction in the energy production by inhibition of enzymes involved in the Krebs cycle and mitochondrial respiration (Gmerek *et al.*, 1981; Mailman, 1980; Sterling *et al.*, 1982).

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 nerve stimulation (Morganroth III. *et al.*, 1974), drug treatment (Zigmund *et al.*, 1974), and anaerobic conditions (Pastuszko *et al.*, 1985). In groups of animals exposed to lead, tyrosine hydroxylase activity was higher than that in age-matched control group. The extent of increase of activity was dose-dependent. Because Na-K ATPase is located in all neural and non-neural tissues of brain and tyrosine hydroxylase is uniquely located in the catecholaminergic nervous system. The selective toxicity of lead to central catecholaminergic nervous system can

be concluded when experimental findings are defined as following: tyrosine hydroylase activity is changed without concomitant change of 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 (2 weeks of age) and telencephalon (6 weeks of age) of rats exposed to low dose of lead. Of rats exposed to high dose of lead such data were collected only in the midbrain (4 and 6 weeks of age). These data indicate that catecholaminergic nervous system in those brain areas are selectively affected by lead. It is different to reach a concrete interpretation for the mechanism of selective toxicity of lead 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. In all the remaining areas of brain where Na-K ATPase activity was changed with or without change of tyrosine hydroxylase activity following the exposure of animals to lead it may be interpreted that the effect of lead was nonsensitive on both non-neural and neural tissues including catecholaminergic nervous system. An alternative interpretation for the effect of lead is also possible in the brain areas where changes of both enzyme activities were found; although catecholaminergic nervous system could still be selectively affected by lead exposure in those brain areas the dosage of lead employed in the present experiment is not low enough to induce differential changes of activities of two different enzymes. 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). The findings of the present study showing the selective toxicity of lead to catecholaminergic nervous system suggest a possibility that another particular nervous system in central nervous system can also be selectively affected following exposure of animals to lead. Based on this finding it may be suggested that another particular nervous system in central nervous system can also be selectively affected following exposure of animals to lead.

ACKNOWLEDGEMENT

This work was partially supported by grants from Korea Science and Engineering Foundation (KOSEF 86-0610) and Research Institute for Pharmaceutical Sciences of Seoul National University.

REFERENCES

Andrews, D.W., Langan, T.A. and Weiner, N. (1983): Evidence for the involvement of a cAMP-independent protein kinase in the activation of soluble tyrosine hydroxylase from rat striatum, *Proc. Natl. Acad. Sci. (USA)*, **80,** 2097-2101.

Bondy, S.C. (1986): Effect of triethyl lead chloride on d-aminolevulnic acid dehydratase, *J. Toxicol. Environ. Health*, **18,** 639-649.

Browder, A.A., Joselow, M.M. and Louria, D.B. (1973): The problem of lead poisoning, *Medicine*, **52,** 121-139.

Brown, D.R. (1975): Neonatal lead exposure in the rat: Decreased learning as a func-

tion of age an blood lead concentrations, *Toxicol. Appl. Pharmacol.*, **32,** 628-637.

Costa, L.G. and Fox, D.A. (1983): A selective decrease of cholinergic muscarinic receptors in the visual cortex of adult rats following developmental lead exposure, *Brain Res.*, **276,** 259-266.

Dorothy, J.B., Wooley, D.E., Vijayan, V.J. and Overman, S.R. (1982): Morphometric effects of postnatal lead exposure on hippocampal development of the 15 day old rat, *Develop. Brain Res.*, **3,** 595-612.

El Mestikaway, S., Glowinski, J. and Hamon, M. (1966): A kinetic study of bovine adrenal tyrosine hydroxylase. *J. Biol. Chem.*, **241,** 4452-4456.

Forbes, G.B. and Reina, J.C. (1972): Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rats, *J. Nutr.*, **102,** 647-652.

Fowler, B.A., Kimmel, C.A., Woods, J.S., McConnell, E.E. and Grant, L.D. (1980): Chronic low level lead toxicity in the rat III. An integrated assessment of long-term lead toxicity with special reference to the kidney, *Toxicol. Appl. Pharmacol.*, **56,** 59-77.

Gmerek, D.E., McCaffety, M.R., O. Neill, K.J., Melamed, B.R. and O. Neill, J.J. (1981): Effect of inorganic lead on rat brain mitochondrial respiration and energy production. *J. Neurochem.*, **36,** 1109-1115.

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

Hammond, P.B. (1977): Exposure of humans to lead, *Ann. Rev. Pharmacol. Toxicol.*, **17,** 197-214.

Holtzman, D., Hsu, J.S. and Mortell, P. (1978): The pathogenesis of lead encephalophathy in the rat pup: Effect of maternal $PbCO_3$ feedings from birth, *Pediat. Res.*, **12,** 1073-1082.

Holtzman, D., Olson, J.E., DeVries, C. and Bensch, K. (1987): Lead toxicity in primary cultured cerebral astrocytes and cerebellar granular neurons, *Toxicol. Appl. Pharmacol.*, **89,** 211-225.

Hussain, T., Ali, M.M. and Chandra, S.V. (1987): The combined effect of lead and manganese on monoamine uptake and sodium-potassium ATPase in striatal synaptosomes, *J. Appl. Toxicol.*, **7,** 277-280.

Joh, T.H., Park, D.H. and Reis, D.J. (1978): Direct phosphorylation of brain tyrosine hydroxylase by cAMP-dependent protein kinase: Mechanism of enzyme activation, *Proc. Natl. Acad. Sci., USA.* **75,** 4744-4748.

Kuhn, D.M. and Lovenberg, W. (1984): Calcium and Cell Function. Vol. V. (Cheng, W.Y. ed.) pp. 311-323, Academic Press. New York.

Lebel, D., Poirier, G.G. and Beaudoin, A.R. (1978): A convenient method for the ATPase assay, *Anal. Biochem.*, **85,** 86-89.

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,** 265-275.

Mailman, R.B. (1980): Introduction to biochemical toxicology (Hodgson, E. and Guthrie, F.E. eds.) pp. 227-232, Elsevier, New York.

McGinnis, P.M. and Michaelson, I.A. (1987): The effect of chronic lead exposure on tyrosine hydroxylase activity in the striatum of rats, Diss. Abstr. In. B. **47,** 2823.

Miller, F.P., Cox, R.H. Jr., Snodgrass, W.R. and Maickel, R.P. (1970): Comparative effects of *p*-chlorophenyl-alanine, *p*-chloroamphetamine and *p*-chloro-N-methyl-amphetamine on rat brain norepinephrine, 5-hydroxytryptamine, and 5-hydroxy-indolacetic acid, *Biochem. Pharmacol.*, **19,** 435-442.

Minnema, D.J., Greenland, R.D. and Michaelson, I.A. (1986): Effect of *in vitro* inorganic lead on dopamine release from superfused rat striatal synaptosomes, *Toxicol. Appl. Pharmacol.*, **84,** 400-411.

Modak, A., Weinstein, S., Stabinoha, W.B. (1975): Effect of chronic lead ingestion on the central cholinergic system in rat brain regions, *Toxicol. Appl. Pharmacol.*, **34,** 340-347.

Morgan, I.G. and Mattews, D.A. (1971): Synaptic plasma membrane from rat brain synaptosomes, Biochem. Biophys. Acta., **248,** 380-

Morgenroth, V.H., III., Boadle-Biber, M. and Roth, R.H. (1974): Tyrosine hydroxylase: Activation by nerve stimulation. *Proc. Natl. Acad. Sci. (USA),* **71,** 4283-4287.

Nagatsue, T., Levitt, M. and Udenfriend, S. (1964): A rapid and simple radioassay for tyrosine hydroxylase activity, *Anal. Biochem.*, **9,** 122-126.

Nathanson, J.A. (1977): Lead-inhibited adenylate cyclase. A model for the evaluation of chelating agents in the treatment of CNS lead toxicity, *J. Pharm. Pharmacol.*, **29,** 511-513.

Nathanson, J.A. and Bloom, F.E. (1975): Lead-induced inhibition of brain adenylate cyclase, *Nature,* **255,** 419-420.

Olson, L., Bjorklund, H., Henschen, A., Palmer, M. and Hoffer, B.J. (1984): Some toxic effect of lead, other metals and antibacterial agents on the nervous system. -Animal experimental model, *Acta Neurol. Scand.*, **70,** (Suppl. 100), 77-87.

Overmann, S.R., Fox, D.A. and Woolley, D.E. (1979): Neurobehavioral ontogeny of neonatally lead exposed rats. I. Relax development and somatic indices, *Neurotoxicol.*, **1,** 125-147.

Palmer, M.R., Bjorklund, H., Freedman, R., Taylor, D.A., Marwaha, J., Olson, L., Serger, A. and Hoffer, B.J. (1981): Permanent impairment of spontaneous purkinje cell discharge in cerebellar grafts caused by chronic lead exposure, *Toxicol. Appl. Pharmacol.*, **60,** 431-440.

Pastuszko, A., Wilson, D.F. and Erecinska, M. (1985): Activation of tyrosine hydroxylase in the central nervous system by anaerobiosis, *Biochem. Pharmacol.*, **34,** 2975-2985.

Regunathan, S. and Sundaresan, R. (1984): Pyruvate metabolism in the young rats intoxicated with organic lead, *J. Neurochem.*, **43,** 1346-1356.

Reinhard, J.F., Jr., Smith, G.K. and Nichol, C.A. (1986): A rapid and sensitive assay for tyrosine-3-monoxygenase based upon the release of H_2O and adsorption of [H]-tyrosine by charcoal, *Life Sci.*, **39,** 2185-2189.

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

Shiman, R., Akino, M. and Kaufman, S. (1971): Solubilization and partial purification of tyrosine hydroxylase from bovine adrenal medulla, *J. Biol. Chem.*, **246,** 1330-1340.

Silbergeld, E.K. and Alden, H.S. (1978): Subcellular mechanisms of lead neurotoxicity, *Brain Res.*, **148,** 451-467.

Silva, P., Hayslett, J.P., Epstein, F.H. (1973): The role of Na-K-activated adenosine triphosphatase in potassium adaptation, *J. Clin. Invest.*, **52,** 2665-2671.

Sterling, G.H., O Neill, K.J., McCaffety, M.R. and O Neill, J.J. (1982): Effect of chronic lead ingestion by rats on glucose metabolism and acetylcholine synthesis in cerebral cortex slices, *J. Neurochem.*, **39,** 592-596.

Unni, L.K. and Caspers, M.L. (1985): Inhibition of bovine brain monoamine oxidase by lead, *Biochem. Pharmacol.*, **34,** 2563-2566.

Vallee, B.L. and Ulmer, D.D. (1972): Biochemical effects of mercury, cadmium and lead, *Ann. Rev. Biochem.*, **41,** 91-128.

Vizi, E.S. (1977): Termination of transmitter release by stimulation of sodium-potassium activated ATPase, *J. Physiol.*, **267,** 261-280.

Walsh, T.J., Schalz, D.W. Tilson, H.A. and Dehaven, D.L. (1986): Acute exposure to triethyl lead enhances the behavioral effects of dopaminergic agonists.: Involvement of brain dopamine in organolead neurotoxicity, *Brain Res.*, **363,** 222-229.

We, P.H. and Phillis, J.W. (1981): Noradrenaline stimulation of Na-K ATPase in homogenates of the developing rat brain, *Biochem. Pharmacol.*, **30,** 2368-2370.

Zigmund, R.E., Schon, F. and Iversen, L.L. (1974): Increased tyrosine hydroxylase activity in the locus coeruleus of rat brain stem after reserpine treatment and cold stress. *Brain Res.*. **70.** 547-552.