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EFFECT OF EARLY INORGANIC LEAD EXPOSURE ON RAT BLOOD–BRAIN BARRIER PERMEABILITY TO TYROSINE OR CHOLINE*

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Abstract—The primary objective of this research was to test the hypothesis that low level lead (Pb) exposure during early life leads to disruption in blood–brain barrier (BBB) function in the young rat. Newborn rats received lead via milk from lactating dams that were drinking water containing 0.1% lead acetate $Pb(Ac)_2$. Pups were weaned to, and maintained on, 0.1% $Pb(Ac)_2$-containing solution up to 70 days of age. Growth was no different from that of coetaneous controls. Experimental animals displayed elevated blood lead (15 μg/dl) within 2 days from the onset of exposure, and it increased to 35–40 μg/dl between 13 and 22 days of age. Following weaning to the higher lead source, blood lead values continued to increase (55 μg/dl) but, then, appeared to decline after 55 days of age. Control animals consistently possessed blood lead values of less then 5 μg/dl. The brain capillary (BBB) transport of the neurotransmitter precursors, choline and tyrosine, was studied at 55 and 70 days of age using intracarotid injections of a bolus containing ^{14}C-labeled substrate and ^{3}HOH as a diffusable reference (Brain Uptake Index). There was no difference in the transport of either choline of tyrosine in lead-intoxicated rats compared to controls. Suspected adverse psychoneurological effects of low level inorganic lead probably relate to the parenchymal cells of the CNS and not to the brain capillary endothelial cells.

During the past decade, there has been a growing concern that low level lead exposure (>30<50 μg/dl blood lead) during early development may be detrimental to the behavioral and intellectural development of the young child. The most frequently described neuropathological finding in post-mortem brain tissue from lead-poisoned children relates to vascular changes accompanied by vasogenic edema [1–12]. Survivors of pediatric lead encephalopathy frequently possess sequelae of a neuropsychiatric nature, such as behavioral disorders and mental retardation [13]. Fortunately, due to public awareness and enlightened legislation, fulminating lead encephalopathy is now an infrequent occurrence. It was estimated, as recently as 1970, that 200,000 to 400,000 children in the United States, although asymptomatic for lead poisoning, possessed elevated blood lead levels (>40 μg/dl) and, of these, 1% were said to suffer from brain damage [14]. Indeed, many investigators have expressed concern regarding possible lead-related neurologic impairment in this asymptomatic child population [15–18]. There are some who hold the view that there is no threshold for lead and that biologically adverse effects exist in a continuum. Essentially it has been argued that neurologic damage increases with tissue lead concentration and that this is a progressive, rather than an all-in-none, phenomenon. If severe biological effects are seen in high doses, then less severe effects or subtle cellular deficits may be attendant and possibly evident at lower body burdens. The lactating dam, as an animal model for lead encephalopathy [2], has become a frequently employed means for studying cerebral and behavioral effects of lead [19] in rodents. Morphologic changes indicative of cell damage appear in cerebrovascular endothelial cells before nerve and glia cells are altered, and the findings are similar to those observed in child lead encephalopathy. Radioactive lead (^{210}Pb) has been shown to localize in the endothelial cells of the brain capillaries within 1 hr following its intraperitoneal injection [3]. It is the brain capillary endothelial cells which constitute the blood–brain barrier (BBB) that possesses at least eight known enzyme carrier systems which regulate blood-borne essential metabolite entry into brain [20]. Toxicological alterations in capillary permeability and BBB transport have been shown for mercury [21, 22], nickel [23], and lead [4] following high doses of toxicant. It is possible that such agents may also have selective and more subtle effects on transport at lower concentrations.

The present study examines the hypothesis that the uptake of essential metabolites from blood into brain, across the blood–brain barrier, is altered following low level lead exposure since this is the site of disruptive action following high level lead exposure. We therefore studied carrier-mediated transport of the neurotransmitter precursors, choline and tyrosine, following intracarotid injection of a bolus containing ^{14}C-labeled substrates in the presence of a highly diffusible reference, tritiated water [24], in lead-exposed and coetaneous control rats.

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MATERIALS AND METHODS

Animals. Sixteen 1-day-old litters and their respective dams of Wistar strain rats were obtained from Charles River (Margate, Kent, U.K.). The animals were immediately transferred to the rodent housing quarters having a 12-hr light–dark cycle, ambient temperature of 22°, and relative humidity of 50%. Half the litters and their respective dams were assigned to the control and half to the test groups.

Diet. Upon arrival, the dams and their respective litters were transferred to plastic tubs containing sawdust bedding and roofed with stainless steel wire covers. All lactating rats and their eventually weaned offspring were given *ad lib* access to solid rodent diet (Diet No. 41B, Grain Harvesters, Sussex, U.K.). The lead content of the diet or tap water was not estimated. A preweighed amount of food was provided each morning following weighing of the food that remained in the hopper. The difference was the amount consumed and was recorded daily except on weekends when three times the normal amount was provided. Controls received tap water whereas experimental animals received a solution of 0.1% lead acetate [$Pb(Ac)_2$] *ad lib*. The 546 ppm lead (Pb) solution was prepared by dissolving 2.5 g $Pb(Ac)_2$ in 2.5 liters of glass-distilled water to which 1.0 ml of concentrated HCl was added. This precluded the precipitation of insoluble lead salts (lead carbonate). A similar amount of acid was added to the tap water provided to the control animals. Water bottles were weighed each morning, and the volume consumed the previous 24 hr was recorded. The dietary regimen was initiated on the day the dams and their 1-day-old litters arrived at the laboratory. All pups were weaned at 21 days of age to the same food, water or Pb solutions consumed by their dams. Consequently, experimental pups moved from a relatively low Pb exposure via lead-containing milk to 546 ppm Pb-containing drinking water. This regimen was maintained throughout the length of the experiment.

Animal redistributions. Pups were kept undisturbed with their natural dams for 72 hr. The six control dams were transferred to separate clean cages. Six male pups from each litter were distributed among the dams so that no one dam had more than one of her natural pups to nurture. Three female pups were added to each group so that each litter consisted of nine pups. A similar pattern existed for the experimental group. The females from these twelve litters and the pups from the remaining four litters were used for blood lead analysis in those animals less than 23 days of age. The protocol allowed for a 6×6 Latin Square for subject selection, minimizing potential genetic effects.

Growth. Dams and each entire litter were separately weighed daily from day 1 through day 21. After weaning only offspring were weighed individually.

Blood lead (BlPb)analysis. Cardiac puncture was not successful in the very young animals. Two, 13- and 22-day-old neonates were decapitated, and trunk blood was collected directly into M-L Brand Blood Collecting vials containing lithium heparin, frozen, and saved for future lead analysis. Blood was collected from older animals via cardiac puncture just prior to measurement of Brain Uptake Index (BUI) and was processed in the same manner as for the neonates.

Lead analysis. BlPb analysis was done by anodic stripping voltammetry employing a model 3010 A Trace Metal Analyzer (Environmental Sciences Associates, Bedford, MA, U.S.A.). The volume of blood employed was 0.1 ml in duplicate. The lower limit of sensitivity was 5 μg/dl.

Buffer (injectate). An injection solution was prepared which consisted of a Ringer's solution containing 147 mM NaCl, 4 mM KCl, and 2 mM $CaCl_2 \cdot 2H_2O$ [Na^+ 147, K^+ 4, Ca^{2+} 4, and Cl^- 155 m-equiv./liter] buffered to pH 7.56 with 10 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) buffer (CalBiochem, LaJolla, CA), to which the radiolabeled substrate was added. The temperature of the injectate was 22–25°.

Blood–brain transport of substrates. The relative entry into the brain of blood-borne substrates, which serve as precursors of the neurotransmitters, acetylcholine and the catecholamines, was determined by using the common carotid single injection technique [24]. Rats were anesthetized with sodium pentobarbitone (Sagatal, 60 mg/kg, i.p.). One milliliter of blood was removed via cardiac puncture for BlPb analysis. The right common carotid artery was exposed, and a 0.2-ml bolus of buffered Ringer's solution containing approximately 0.5 to 1.0 μCi of ^{14}C test substance and 2.0 μCi of ^3HOH was injected rapidly (<1 sec). Rats were decaptitated 15 sec later. The brain was rapidly removed and washed in cold saline, and the cerebellum, optic lobes and areas rostal to the quadri-gemini were discarded. The ipsilateral half of the brain was washed, blotted dry on filter paper, thoroughly mixed into a paté-like consistency, and approximately 100 mg was extruded through a 20-gauge hypodermic needle into a preweighed scintillation vial and weighed. This was done in duplicate.

Counting procedure. Each tissue sample, irrespective of actual weight, was digested in exactly 1 g of Soluene at room temperature for at least 24 hr. A suitable aliquot of the injection mixture was treated in a similar manner. To each was added 15 ml of toluene-based scintillation fluid (Liquifluor, New England Nuclear Corp., Boston, MA). The combined ^{14}C- and ^3H-activities were estimated in a Packard Tricarb liquid scintillation spectrometer. The radioactivities due to each isotope were determined by computer, which was programmed to correct for differential quenching and for changes in crossover of ^{14}C-activity into the ^3H-counting channel and ^3H-activity into the ^{14}C-counting channel.

Brain Uptake Index. The Brain Uptake Index (BUI) was calculated according to reported procedures [24] and was determined from the ratio of radioactivities for the ^{14}C- and ^3H-isotopes in brain tissue divided by the ratio of the isotopes in the injection solution. The BUI is the fractional uptake of ^{14}C test substance (choline or tyrosine) from blood into brain in a single circulatory pass relative to that fractional uptake into brain of the $^3[H]$water reference compound after common carotid injection.

Chemicals and drugs. [Methyl-^{14}C]Choline chloride (59.8 mCi/mmole, 424 μCi/mg), L-[U-^{14}C]-

tyrosine (514 mCi/mmole, 2.609 mCi/mg), and tritiated water (^{3}HOH) (5 mCi/ml) was obtained from the Radiochemical Centre, Amersham, U.K. These radiolabeled compounds were not checked for their relative degrees of purity. Other chemicals and reagents used in scintillation counting, formulation of buffer, and lead solution were of the highest purity commercially available. Pentobarbitone sodium (Sagatal) was obtained from May & Baker, Dagenham, U.K.

RESULTS

Growth. Both control and Pb-exposed dams consumed comparable weights and volumes of food and fluid, respectively, resulting in the ability of the dam to maintain or increase her body weight throughout the period of lactation and suckling of her young. Suckling pups in both control and lead-exposed groups gained weight at essentially comparable rates.

Blood lead concentrations. Changes in blood lead concentrations with length of exposure (age) are illustrated in Fig. 1. Control animals consistently possessed blood lead of less than 5 μg/dl (lower limits of detectability). Blood samples from younger control animals were therefore not contaminated by blood collected from the severed trunk, a less desirable method. This may not have been the case with the trunk blood from experimental animals. Experimental animals displayed elevated BlPb values (15 μg/dl) within 2 days from the onset of exposure to the lactating dam, reflecting the rapid transfer of ingested lead to milk. By 13 days of age blood lead values of the suckling pups doubled (35 μg/dl) as a result of the increased consumption of lead-contaminated milk which, in turn, continues to be enriched with lead as the dam's body burden for lead increases [25]. Following weaning to the higher lead source, namely 546 ppm lead in drinking water, blood lead values continued to increase (45–65 μg/dl) at 55 days of age and then appeared to decline in spite of the continued ingestion of lead. These blood lead values, ranging on the average from 15 to 55 μg/dl or three to eleven times the value in the non-exposed controls, encompassed the range of blood lead values (35–60 μg/dl) found in some of the human pediatric population asymptomatic for clinical lead poisoning.

Table 1. Uptake of ^{14}C-labeled choline and [^{3}H]tyrosine in control and lead-exposed rats*

	Controls	Lead-exposed
Choline	0.064 ± 0.009 (10)	0.063 ± 0.015 (10)
Tyrosine	0.489 ± 0.075 (13)	0.454 ± 0.066 (13)

* Brain Uptake Index (BUI) values are means ± S.D. For each mean N = 10 or 13. BUI = (tissue ^{14}C/tissue ^{3}H)/(injectate ^{14}C/injectate ^{3}H), uncorrected for trapped blood.

Brain Uptake Index for tyrosine and choline. Fifty-five- and seventy-day-old rats were used for the study of brain uptake of tyrosine and choline. L-[U^{14}]Tyrosine (0.25 μCi, sp. act. 514 mCi/mmole) and ^{3}H-tritiated water (2.0 μCi, 5 mCi/ml) in 0.2 ml of Ringer's buffer, pH 7.54, were injected into the common carotid artery. This corresponded to a 2.41 μM solution of tyrosine.

In the case of choline, 1.0 μCi of [methyl-^{14}C]choline chloride (sp. act. 59.8 mCi/mmole) and 2 μCi of ^{3}H-tritiated water (5 mCi/ml) in 0.20 ml of Ringer's buffer, pH 7.54, were injected into the common carotid artery. This corresponded to an 83.6 μM solution of choline.

In both instances, the rats were decaptitated 15 sec after injection, and brain tissue was prepared as described for counting the relative contents of ^{14}C- and ^{3}H-radioactivities. The relative extractability of tyrosine and choline from blood into brain in leaded and control rats is shown in Table 1. There was no

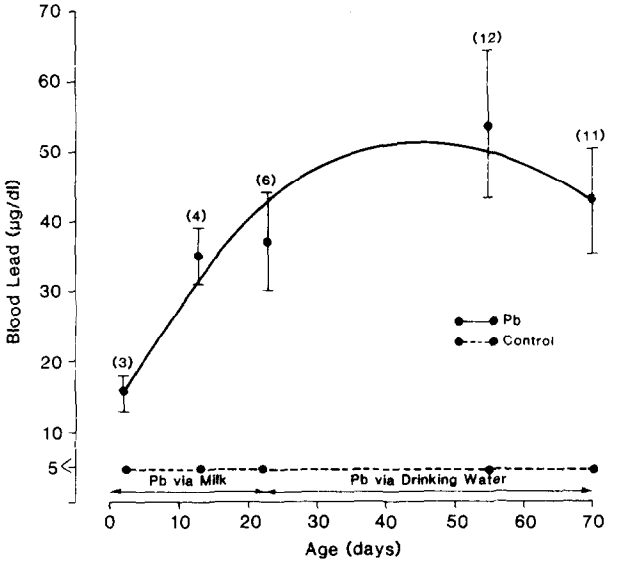

Fig. 1. Blood lead concentrations (μg/dl) in rats exposed to Pb–milk and weaned to 546 ppm lead. The curve was drawn by eye. The numbers in parentheses equal the number of subjects. Error bars indicate ± S.D.

difference in the transport of either of these metabolic substrates in leaded as compared to control animals.

DISCUSSION

A frequent feature in many fatal cases of human inorganic-lead encephalopathy is the occurrence of disruption in the morphologic integrity of the brain vasculature. Vascular lesions have been repeatedly observed [5–8] since the early studies of Tanquerel des Planches [5]. In experimental models of lead encephalopathy, lead also causes vascular lesions in the neonatal rat [2, 3, 8–11] and in the suckling mouse [12]. Following a tracer dose of radioactive lead, radioactivity is localized within the cytoplasm of the endothelial cells of brain capillaries [3], and it has been shown that lead is concentrated in harvested capillaries from lead-intoxicated suckling rats [26]. It is therefore reasonable to suggest that some of the CNS toxic effects of exposure to high levels are a consequence of a direct effect by lead on the brain capillary endothelial cells (BBB), the first target organ for lead in the CNS. The endothelial cells of the brain capillaries have been shown to possess specific transport metabolic functions. The transfer of nutrients from blood into the brain is via carrier-mediated transport. There is a facilitated transport of glucose from blood into brain tissue [27], as well as of amines, hexoses, monocarboxylic acids, purines, nucleosides, neutral, basic and acidic amino acids [20, 28], and choline [29–31]. Gamma-glutamyl transpeptidase in brain capillaries has been suggested as the enzymatic basis for amino acid transport into brain [32–34]. Heavy metals, including lead, are general cytoplasmic poisons which interfere with enzyme systems in all cells of the body but particularly the brain [35, 36]. The effect of low level lead exposure on the dynamic biochemical barrier functions of the brain capillaries under asymptomatic conditions has not been thoroughly studied. It has been suggested that, since lead has no known physiological function, no threshold exists to the toxic effects of lead and that adverse biological effects exist on a continuum. If severe cellular effects are seen at high doses, then less severe, or subtle, effects must be evident at lower body burdens. We have now tested this hypothesis by studying the interaction of low level lead with the regulatory mechanism(s) of uptake into brain of two neurotransmitter precursors, choline and tyrosine.

Unfortunately, studies of CNS-related effects of lead poisoning have often been hampered by the fact that high blood lead levels (when they have been included in the reports) have been associated with retardation in body growth but that such animals have been compared to coetaneous animals of normal growth. It has been difficult to ascribe some of the reported findings to a direct effect of lead or to a confounding observation due to the interaction of lead with undernutrition [19, 34, 37]. The study of the interaction of lead and drugs with the enhanced movement of albumin across the blood–brain barrier [38, 39] employed experimental protocols similar to those used by Silbergeld and Goldberg [40], namely mice fed 2730 ppm Pb in drinking water. It is now recognized that this leads to food and fluid aversion and as much as 28% retardation in growth. Our aim in the present study was to use lead-intoxicated animals displaying normal growth characteristics but elevated blood lead values comparable to those seen in asymptomatic lead-exposed children (<60 μg/dl). In the work described in this report, growth was unaffected in lead-exposed animals that had blood lead values of approximately 15 μg/dl at 2 days of age, 30 μg/dl at 23 days of age, and 55 μg/dl at 55 days of age. The function of the blood–brain barrier was assessed in adult lead-exposed rats [24] by measuring selective penetration through the endothelial cells of the brain capillaries. In this instance, the Brain Uptake Index values for choline (83 μM) and tyrosine (2.4 μM) were 6.3 and 45%, respectively, uncorrected for blood. There was no difference between control and lead-burdened animals. The uptake of choline in normal rats has been reported to be less than 10%, depending on the dose [29–31], and that of tyrosine, 50% [24]. The BUI values found for these compounds in the present study compare favorably with those reported by others [24, 29–31]. The conclusion that low levels of lead have little effect on brain capillary endothelial cell facilitated transport into brain is suggested by two independent and contemporaneous studies on the effect of inorganic lead on cerebral capillary functions in suckling rats [41] and of organic lead in adult rats [42]. Inorganic lead had no effect on the uptake of D-glucose, L-phenylalanine, L-lysine, proline, pyruvate or uridine [41], whereas L-phenylalanine and tyrosine were unaffected by 4 weeks of intoxication with triethyl lead chloride [42]. The present findings, in conjunction with other reports [41, 42] make it reasonable to suggest that blood lead levels below that leading to increases in capillary permeability and extravasation of cells and macromolecules cause no deficits in endothelial cell facilitated transport of a number of essential metabolic substrates. If deficits do exist in relation to subtle behaviorally-linked CNS dysfunctions, as has been proposed, they are most likely linked to tissues adjacent to the blood–brain barrier, i.e. glia or neurons rather than the endothelial cell *per se*, since lead does enter the CNS [41].

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