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Influence of Dietary Deficiency of Nicotinamide on Lead Toxicity in Young Rats

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ABSTRACT

The influence of dietary nicotinamide deficiency on lead intoxication in young developing rats was investigated. The Pb induced an increase in brain dopamine and noradrenaline, inhibition in blood δ-aminolevulinic acid dehydratase activity, an elevation in urinary excretion of δ-aminolevulinic acid, and blood and tissue uptake of Pb were significantly more marked in animals maintained on a nicotinamide-deficient diet than those fed a nicotinamide-sufficient diet. The nicotinamide deficiency may enhance the susceptibility to Pb intoxication possibly by enhancing the absorption of Pb and altering nicotinic acid metabolism.

Index Entries: Lead; nicotinamide deficiency; neurotoxicity; biogenic amines; δ-aminolevulinic acid; rat.

INTRODUCTION

Malnutrition is common in developing countries. Nutritional status is of primary importance during brain development, and detrimental effects of under-nutrition on brain development and learning behavior have been reported (*1*). Neonatal and growing animals and children have been found to be more susceptible to lead neurotoxicity (2). Alterations in brain enzymes of Pb-exposed animals maintained on malnourished

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diet have been observed (3). The dietary deficiency of vitamin B complex (4) or thiamine (vitamin B_1) (5,6) enhanced systemic toxicity of Pb, including changes in brain neurotransmitter levels in animals. Since exposure to Pb decreases the nicotinic acid concentration of blood and urine (7), and nicotinic acid or nicotinamide is a major component of vitamin B complex, it was considered of interest to investigate the influence of its dietary deficiency on Pb intoxication, with particular reference to neurotoxic effects.

MATERIALS AND METHODS

Animals and Treatment

Thirty-two young, male, albino rats (50 ± 5 g) of the Industrial Toxicology Research Centre colony were equally divided into four groups and treated daily for 8 wk as follows. The animals were weighed every 4th d and the dose of Pb adjusted accordingly.

Group I:	Basal synthetic diet (Table 1)
Group II:	Nicotinamide-deficient diet
Group III:	Basal diet + Pb (10 mg/4 mL/kg) as Pb $(OCOCH_3)_2 \cdot 3H_2O$ dissolved in H_2O, gastric gavage
Group IV:	Nicotinamide-deficient diet + Pb (10 mg/4 mL/kg)

The animals were housed individually in metabolic cages, and a 24 h urine was collected in ice-cold polyethylene tubes. All the animals were killed by decapitation, the blood collected in heparinized vials, and the liver, kidney, and brain removed.

Biochemical Assays

Brain from each rat was divided into two halves by median sagittal section. One-half of the brain was homogenized in acidified *n*-butanol using a Potter-Elevehjem type A homogenizer with a Teflon pestle (10% wt/v). The homogenate was centrifuged (800*g*, 15 min) and the supernatant processed for the estimation of biogenic amines and their metabolites (8,9). The fluorescence was measured in a Carl-Zeiss PMQ3 spectrofluorometer. The urinary level of δ-aminolevulinic acid (ALA) was measured using a dual ion exchange chromatographic procedure (10). The activity of blood δ-aminolevulinic acid dehydratase (ALAD) was assayed according to Berlin and Schaller (11).

Lead Estimation

Lead was estimated in blood (12), liver, kidney, and brain (13) following digestion with conc. HNO_3 using a Perkin-Elmer atomic absorption spectrometer model 5000. The samples were read at 283.3 nm for Pb.

TABLE 1
Chemical Composition of the Basal Synthetic Diet[a,b]

Ingredient	As, % in diet
Casein	21
Carbohydrate	64
Oil	8
Salt mixture[c]	4
Vitamin mixture[d]	3

[a]Nicotinamide was omitted for nicotinamide—deficient diet.

[b]From American Institute of Nutrition Standards for Nutritional Studies [J. Nutr. 107, 1340, (1977)].

[c]1000 g of salt mixture consisted of: calcium carbonate, 68.6 g; calcium citrate, 308.3 g; calcium biphosphate, 112.8 g; magnesium carbonate, 35.2 g; magnesium sulfate (anhydrous), 38.3 g; potassium chloride, 124.7 g; dibasic potassium phosphate, 218.8 g; sodium chloride, 77.1 g; and 16.2 g from the following mixture: cupric sulfate, 0.48 g; ferric ammonium citrate, 94.33 g; manganese sulfate, 1.24 g; ammonium alum, 0.57 g; potassium iodide, 0.25 g; and sodium fluoride, 3.13 g (=100 g).

[d]1000 g of vitamin mixture consisted of: thiamine HC1, 600 mg; riboflavine, 600 mg; pyridoxine HC1, 700 mg; nicotinamide, 3 g; D-calcium pentothenate, 1.6 g; folic acid, 200 mg; D-biotin, 20 mg; cyanocobalamine, 1 mg; retinyl palmitate or acetate, 450 mg; *d,l*-α-tocopherol acetate, 2.5 g; cholecalciferol, 2.5 mg; menaquinone, 5.0 mg; and sucrose, to make 1000 g.

Statistical Analysis

Student's *t*-test was applied to calculate the statistical difference between two comparing groups. The differences between means were considered significant if calculated p values were <0.05.

RESULTS

Retardation in growth rate and loss of fur were observed in rats maintained on a nicotinamide-deficient diet as compared to those fed the basal diet. No other abnormalities or changes in gross behavior of the animals were observed.

The exposure to Pb for 8 wk significantly increased brain levels of dopamine (DA) and noradrenaline (NE), more markedly in animals

maintained on the nicotinamide-deficient diet. The brain homovanillic acid (HVA), the metabolite of DA, also increased, but the level did not differ significantly among Pb-exposed animals fed a normal or nicotinamide-deficient diet. The Pb had no effect either on brain 5-hydroxytryptamine (5-HT) or its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) levels. The nicotinamide-deficient diet had no influence on the levels of brain biogenic amines or their metabolites (Table 2). The activity of blood ALAD was inhibited significantly, and the urinary excretion of its substrate, ALA, was consequently enhanced upon exposure to Pb. These effects were more pronounced in animals maintained on the nicotinamide-deficient diet compared to the normal-diet group (Table 3). The treatment of Pb caused significant uptake of Pb in blood, liver, kidney, and brain. The uptake of Pb was more marked in blood and liver in the nicotinamide-deficient group. The dietary deficiency of the vitamin nicotinamide had no effect on either renal or brain uptake of Pb (Table 4).

DISCUSSION

Nicotinic acid or nicotinamide is an essential dietary constituent, the lack of which leads to clinical condition characterized by signs and symptoms associated with skin, gastrointestinal tract, and central nervous system disorders. The deficiency of this vitamin may make the subject more vulnerable to the influence of Pb, including gastrointestinal absorption of the metal and its neurotoxic effects. In the present study, nicotinamide deficiency enhanced the Pb-induced increase in brain DA and NE. The effects of Pb exposure on brain catecholamine levels have not been consistent (*14*). Although some investigators observed an increase in whole brain, forebrain, and midbrain NE (*15,16*), others found either no effect or a decrease in some regions of brain (*16–18*) of Pb-exposed animals. Similarly, Dubas et al. (*16*) reported an increase, Sauerhoff and Michaelson (*17*) found a decrease, and Sabotka (*18*) observed no effect in brain DA levels related to Pb. However, the increased turnover rate of NE in Pb-treated, hyperactive rats (*19*), the increase in brain HVA in the present study, and the increased brain and urinary levels of HVA and VMA in Pb-poisoned mice (*20*) support the alterations in catecholamine metabolism observed in the present investigation. The non-influence of Pb on brain levels of 5-HT and 5-HIAA are well in agreement with certain other studies (*18–21*). This is supported by the fact that Pb had no effect on 5-HT uptake (*15*). However, the present findings clearly show the effect of Pb on brain DA and NE, which is modified by nicotinamide deficiency, regardless of brain Pb concentration.

Lead disturbs nicotinic acid metabolism, resulting in decreased body concentration (*7,22*), either by blocking the synthesis of nicotinic-acid-related coenzymes or by enhancing the degradation of nicotinic acid (*23*). The administration of inosine and adenosine phosphate, the products of

TABLE 2
Effect of Nicotinamide Deficiency on Lead-Induced Alterations in Brain Biogenic Amines and Their Metabolites in Rats[a]

Fresh tissue, μg/g	Basal diet	Nicotinamide-deficient diet	Basal diet + lead	Nicotinamide-deficient diet + lead
DA	0.84 ± 0.03	0.84 ± 0.02	0.95 ± 0.04*	1.11 ± 0.06*,**
NE	0.35 ± 0.02	0.35 ± 0.03	0.43 ± 0.02*	0.52 ± 0.005*,**
5-HT	0.44 ± 0.01	0.45 ± 0.01	0.43 ± 0.02	0.40 ± 0.02
HVA	0.11 ± 0.01	0.11 ± 0.007	0.17 ± 0.01*	0.16 ± 0.004*
5-HIAA	0.55 ± 0.02	0.53 ± 0.01	0.52 ± 0.03	0.60 ± 0.10

[a]Each value is mean ± SE of six animals.
*$p < 0.05$ compared to basal diet group.
**$p < 0.05$ compared to basal diet + lead group.

TABLE 3
Effect of Nicotinamide Deficiency on Lead-Induced Alterations in Blood δ-Aminolevulinic Acid Dehydratase Activity and Urinary δ-Aminolevulinic Acid Excretion in Rats[a]

	Basal diet	Nicotinamide-deficient diet	Basal diet + lead	Nicotinamide-deficient diet + lead
Blood, ALAD[b]	5.82 ± 0.70	5.02 ± 0.23	2.30 ± 0.05*	1.60 ± 0.10*,**
Urinary, ALA[c]	0.10 ± 0.005	0.11 ± 0.003	0.43 ± 0.01*	0.57 ± 0.01*,**

[a]Each value is mean ± SE of six animals.
[b]nmol δ-ALA/min/mL erythrocyte.
[c]mg/100 mL.
*$p < 0.05$ compared to basal diet group.
**$p < 0.05$ compared to basal diet + lead group.

TABLE 4
Effect of Nicotinamide Deficiency on the Uptake of Lead in Blood and Tissues of Rats[a]

	Basal diet	Nicotinamide-deficient diet	Basal diet + lead	Nicotinamide-deficient diet + lead
Blood[b]	8.34 ± 1.16	7.97 ± 0.71	134.40 ± 5.99*	161.50 ± 7.57*,**
Brain[c]	0.38 ± 0.07	0.41 ± 0.03	3.99 ± 0.30*	4.89 ± 0.15*
Kidney[c]	1.41 ± 0.15	1.50 ± 0.28	12.95 ± 0.51*	14.76 ± 0.44*
Liver[c]	1.70 ± 0.30	1.57 ± 0.11	10.45 ± 0.47*	14.18 ± 0.73*,**

[a]Each value is mean ± SE of six animals.
[b]μg/100 mL.
[c]μg/g, fresh tissue.
*$p < 0.05$ compared to basal diet group.
**$p < 0.05$ compared to basal diet + lead group.

nicotinic acid, have been shown to partially reverse Pb-induced anemia (*24,25*). Thus, it appears that nicotinic acid has a modulating role in alleviation of Pb intoxication. Conversely, nicotinic acid deficiency may enhance the susceptibility to Pb, as reflected by the response of Pb-sensitive parameters, blood ALAD, urinary ALA, and increased uptake of tissue Pb in exposed animals maintained on a nicotinamide-deficient diet. This observation is supported by the fact that nutritional status is one of the important factors in the expression of Pb-induced changes, including encephalopathy (*26*).

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