EFFECT OF BENZENE AND LEAD ON RELATIONSHIP BETWEEN Δ-AMINOLEVULINIC ACID AND BRAIN MYELIN PROTEINS

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Δ-Aminolevulinic acid (ALA), the first metabolite in the system for synthesis of porphyrins and heme, and essential for the formation of cytochromes and other hemoproteins [1], is synthesized in cells of the CNS. Many toxic substances inhibit heme formation at different stages after ALA formation, with the result that its synthesis is increased as a compensatory reaction to heme deficiency. The writers showed previously [2] that in experimental lead and benzene poisoning disturbance of heme synthesis in the nervous system is combined with demyelination. In many people who are in contact at work with these toxic substances, disturbances of porphyrin metabolism are accompanied by changes in CNS activity [2].

The aim of this investigation was to study binding of ALA by brain myelin under normal conditions and under the influence of lead and benzene.

EXPERIMENTAL METHOD

Male rabbits of the same breed and age and roughly the same weight, kept on an ordinary diet, were used. The animals were divided into three groups: group 1) control; group 2) lead poisoning caused by injections of lead acetate in a dose of 40 mg/kg 5 days a week for 3 weeks; the animals were used in the experiments 20 days after these procedures; group 3) subcutaneous injection of benzene into the animals 5 times a week for 5 months in a dose of 0.1 ml/kg body weight. The degree of poisoning was determined from the characteristic changes in the peripheral blood picture.

Rabbits of all three groups were anesthetized and then given an intracranial injection of ^{14}C-ALA (50 μCi) in physiological saline in a volume of 0.25 ml. After 24 h the animals were killed and the gray and while matter of the brain removed.

The myelin fraction was obtained by differential centrifugation [4] and the ^{14}C-ALA in it was assayed on an LKB liquid scintillation counter (Sweden).

Electrophoresis of the myelin proteins was carried out in polyacrylamide gel in the presence of sodium dodecylsulfate [3]. The gel was stained with Coomassie Blue P-250 and cut into transverse strips. Pieces of gel were introduced into vials, 1 ml of solvent was added to them, and the vials were incubated for 1 h at 55°C. Scintillation fluid was added, and the presence of radioactivity determined on the counter. Proteins were identified by the method in [6]. The protein content was determined by Lowry's method [5].

EXPERIMENTAL RESULTS

Benzene and lead poisoning caused an increase in the ALA content in cells of the CNS. As the writers' previous investigations showed [2], under the influence of benzene endogenous ALA accumulates in the brain cells in much larger quantities than under the influence of lead. By determining the quantity of exogenous ^{14}C-ALA bound with myelin, we found (Table 1) that myelin of white matter contains more of the acid than myelin of the gray matter. The difference in the quantity of radioactive label in these two regions of the brain can be explained by the fact that the injections were given deep into the subcortical regions, from which the ALA penetrated into the surrounding structures, to reach the cerebral cortex.

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TABLE 1. Binding of ^{14}C-ALA (in cpm/mg protein) by Brain Myelin under Normal Conditions and in Benzene and Lead Poisoning (M ± m; n = 6)

Brain substance	Control	Poisoning by	
		Lead	Benzene
Gray mater	711±30	593±41*	324±46*
White matter	3948±254	2997±365*	1358±422*

TABLE 2. Relative Percentage of Basic Proteins of Myelin in Rabbit Brain under Normal Conditions and in Benzenes and Lead Poisoning (M ± m; n = 6)

Protein composition of myelin	Normal	Poisoning by	
		Benzene	Lead
Basic proteins	43±5	56±7*	54±5*
Proteins of proteolipids	34±3	17±5*	19±2*
Wolfgram's proteins	13±3	18±3*	16±4*

Legend. Here and in Tables 2 and 3, *P < 0.05 compared with control.

TABLE 3. Binding of ^{14}C-ALA by Brain Myelin Proteins under Normal Conditions and in Benzene and Lead Poisoning (M ± m)

Protein composition of myelin	Control	Poisoning by	
		Benzene	Lead
Basic proteins	1268±82 / 543±27	441±23* / 247±16*	422±25* / 228±21*
Proteins of proteolipids	880±49 / 299±18	593±36* / 101±22*	587±41* / 111±24*
Wolfgram's proteins	2009±98 / 261±13	1674±84* / 268±21	1494±93* / 269±29

Legend. Numerator — cpm/mg protein of each group; denominator — cpm/mg total protein of myelin.

In lead and, in particular, in benzene poisoning binding of exogenous ALA with myelin was reduced. In fact, whereas more ALA was formed in the brain tissue during the action of benzene than during the action of lead, more of it bound with the myelin, and exogenous ALA was now being incorporated into myelin in smaller quantities.

Quantitative analysis of the protein composition showed that the relative percentages of the main groups of proteins in rabbit brain myelin did not differ in normal animals from those established previously [6], but in benzene and lead poisoning, there were significant changes (Table 2). The content of basic proteins and of Wolfgram's proteins was increased, the content of lipoproteins was reduced. These changes were more marked in the case of benzene poisoning.

In all cases of benzene and lead poisoning, and also in healthy animals, radioactivity of the main groups of brain myelin proteins was determined. The presence of ^{14}C-ALA was found in the isolated myelin proteins (Table 3). Benzene and lead poisoning caused a decrease in ALA binding by basic proteins, by Wolfgram's proteins, and by myelin lipoproteins. Binding of ALA with basic proteins was reduced particularly sharply. This can be explained on the grounds that under the influence of benzene and lead large quantities of ALA are formed in nerve tissue cells and accumulate there. Some of the ALA binds with myelin proteins, and after injection of an additional dose of ALA, binding reaches saturation.

Table 3 gives data on binding of ^{14}C-ALA calculated per milligram of each group of myelin proteins isolated. These data are convenient for comparison of the ability of labeled ALA to bind with the same quantities (1 mg) of proteins of each group. Comparison of the isolated groups of proteins shows that both under normal conditions and in benzene and lead poisoning, Wolfgram's proteins have the greatest ALA binding capacity. However, the relative percentages of the proteins in myelin differ and vary depending on the type of poisoning studied in the experiments. For instance, according to our data, in myelin of the rabbit CNS 1 mg total protein consists of 43% of basic proteins, 34% of proteolipid proteins, and 13% of Wolfgram's proteins. Data on the true quantity of radioactive label of ALA bound with each of the main groups of myelin proteins, depending on their content in myelin, are therefore interesting (Table 3).

It was found that although Wolfgram's proteins have the greatest capacity for binding ^{14}C-ALA compared with the other groups of myelin proteins, almost half the quantity of this acid is bound in myelin with basic proteins. Benzene and lead poisoning caused considerable decreases in binding of the label by basic proteins and by proteolipid proteins. Under these circumstances the quantity of acid bound with Wolfgram's proteins remains unchanged.

Thus in benzene and lead poisoning, accompanied by ALA accumulation in brain cells, intensive binding of the acid with myelin proteins takes place. This incorporation of ALA into myelin in bound to affect the charge, stability, and functions of myelin proteins, and it may perhaps be one cause of the disturbance of CNS activity.

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