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HEATING REACTIVATION OF RATS AMINOLEVULINIC ACID DEHYDRATASE IN LEAD POISONING

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SUMMARY

Heat reactivation (60°C for 5 minutes) of the red blood cells aminolevulinic acid dehydratase activity was studied in lead treated rats (0.25 to 5.0 mg/kg b.w., i.p., for 4 weeks). Complete enzyme reactivation occurs with lead blood concentration up to about 70 mcg/dl. At higher blood lead concentrations, only a part of the enzyme can be restored by heating. The different mechanisms of aminolevulinic aicd dehydratase activity inhibition by lead are discussed.

INTRODUCTION

Experiments in man (refs.1-6) and animals (ref.3) exposed to lead show that heat treatment (60°C for 5 minutes) of hemolyzed blood reactivates completely erythrocyte aminolevulinic acid dehydratase (ALA D, E.C. 4.2.1.24).

The hypothesis was, therefore, that the enzyme is inhibited by a thermolabile factor (ref.1), which is either activated or synthetized after lead exposure. This factor is induced early after lead exposure (ref.7). Gel-filtration of the erythrocyte supernatant elutes the factor with hemoglobin fractions (ref.8).

It is unknown whether the heat effect on human ALA D depends on blood lead concentration. The aim of the present paper is to measure heat reactivation of ALA D activity at different blood lead concentrations.

METHODS

Forty-eight albino, male, Wistar rats (S. Morini, S. Polo d'Enza, RE, Italy), starting weight of 200 ± 10 grams, were subdivided into 6 groups of 8 rats each. One group was considered as the control, the other 5 groups were treated i.p. with $Pb(NO_3)_2$ (C. Erba, Milano, Italy) at doses of 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg b.w. dissolved in deionized water (0.5 ml volume) for 4 weeks, once a day (5 days a week), respectively. 24 hours after the last administration, blood samples were taken from the heart under ether anesthesia. The rats were fasted overnight.

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The blood was collected in heparinized test tubes and kept in ice until measured.

ALA D activity was determined according to the standardized European method (ref.9), at pH 7.0, using ALA.HCl 0.01 M (Merck, Darmstadt, FRG) as substrate. Changes in pH were necessary for optimal heat reactivation (unpublished experiments). Heat restoration was carried out according to Gerhart and Pardee (ref.10) at 60°C for 5 minutes. Optical densities were measured with a Perkin-Elmer 550 model spectrophotometer.

Lead concentration (PbB) was measured on hemolyzed blood according to Fernandez (ref.11), using a Perkin-Elmer 305 model atomic absorption spectrophotometer with a graphite furnace HGA 76 B and background corrector.

RESULTS

ALA D activities before and after heat restoration, and blood lead concentrations are shown in Table 1. Complete heat reactivation is obtained only

TABLE 1

ALA D activity before and after heat treatment, compared to lead blood concentration

Dose mg/kg	N° of animals	PbB mcg/dl	ALA D activity (mU/ml) before heating	ALA D activity (mU/ml) after heating	% activity before heating	% activity after heating
0.0	8	3.5 ± 0.7	7.8 ± 1.0	7.1 ± 1.0	100	92
0.25	8	30.2 ± 7.8	1.6 ± 0.3	8.9 ± 0.7	20 ± 3	115 ± 9
0.5	8	53.6 ± 15.9	1.5 ± 0.6	8.6 ± 1.5	19 ± 7	110 ± 20
1.0	8	108.2 ± 43.5	1.4 ± 0.2	5.4 ± 1.9	18 ± 2	69 ± 25
2.5	8	356.9 ± 49.9	0.6 ± 0.2	2.1 ± 0.7	8 ± 3	27 ± 9
5.0	8	565.6 ± 271.6	0.4 ± 0.2	1.8 ± 0.5	6 ± 3	24 ± 6

Legend: % ALA D activity before and after heating is referred to mean value of ALA D activity of the control group before heating. Values are expressed as mean ± S.D.

with doses of 0.25 and 0.5 mg/kg lead, corresponding to a blood lead concentration up to about 70 mcg/dl. Figure 1 shows the correlation between PbB and the percentage of ALA D activity of poisoned rats before and after heat treatment of hemolyzed blood with respect to the mean value of the control group.

DISCUSSION

The results show that the complete heat reactivation of ALA D activity depends upon blood lead concentration and is possible up to concentrations < 70 mcg/dl.

Sakai et al. (ref.12) suggested two types of ALA D inhibition by lead in vitro. At low metal concentrations the inhibition can be reversed by heating.

On the contrary, at high concentrations (> 1 mcM) the activity cannot be restored. This different reactivation of ALA D inhibited by lead suggests a different inhibition of the enzyme possibly related to two different binding sites of lead on molecule. At low concentrations lead binds probably to high affinity sites and the inhibition can be restored by heating. The factors found in Hb fractions (ref.8) may be, therefore, involved in the binding of lead to high affinity sites of ALA D molecule.

Our results confirm in vivo that heat treatment has different effects on ALA D reactivation depending on blood lead concentrations. According to that hypothesis (ref.12) and our results, it seems that increasing blood lead concentrations, the number of non-restorable sites of ALA D also increases. Since 20-30% of ALA D activity is always restored, this percentage might represent the high affinity sites, as suggested by Sakai et al. (ref.12).

In conclusion, the heat reactivation appears complete for lead values comparable commonly to those found in subjects occupationally exposed to lead and that justifies the results found in lead workers (refs.4,6). PbB > 100 mcg/dl are rarely found, and values > 150 mcg/dl are generally never found in subjects exposed to lead.

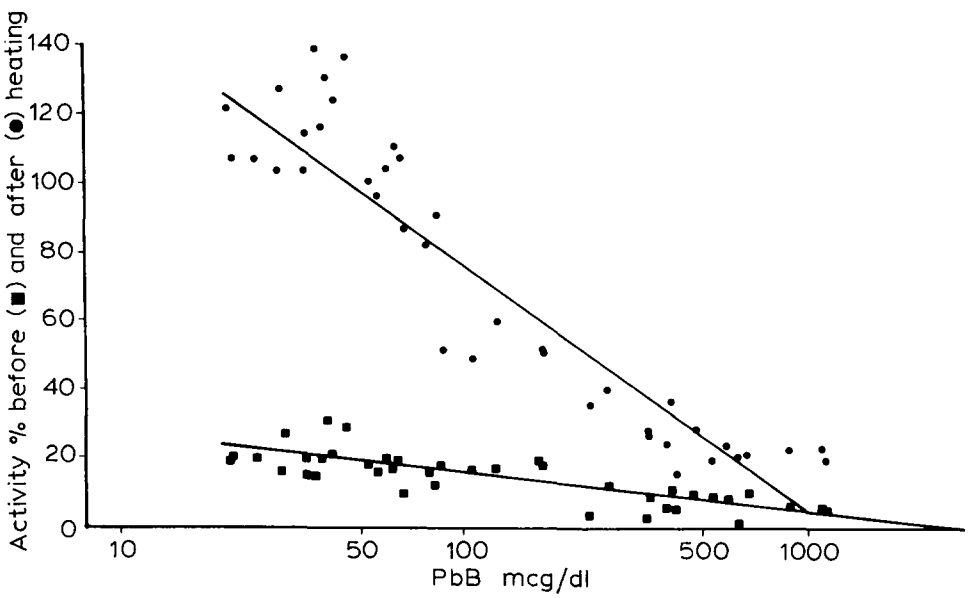

Fig. 1. Correlation between PbB and percent of ALA D activity before (y = 10.99 x + 37.27, r = -0.809), and after (y = -73.87 x + 225.65, r = -0.935) heating. For explanation see text.

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