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THE EFFECT OF SULFHYDRYL-COMPOUND ON THE LEAD ACETATE INDUCED ENDOTOXIN HYPERSENSITIVITY OF RATS

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Earlier study has shown that the sensitivity to endotoxin of rats may be increased 10,000–100,000 times above normal by a single, normally well-tolerated, intravenous injection of lead acetat [3]. Else, as compared to other animals species, rats are comparatively resistant to endotoxins [1]. According to our experiences, e.g. 5–10 mg/100 g body weight (BW) of E. coli endotoxin represents a lethal dose for Wistar origin rat usually breed in Hungary. This is far bigger than the lethal dose to the Holtzman rats used in North America [2]. On the other hand, if the rats are being treated with an intravenous injection of 5 mg/100 g BW lead acetate, then the simultaneous administration of 1–3 μg/100 g BW of endotoxin will also elicit the lethal shock. The mechanism of lead action in this phenomenon is unknown. If seems likely that the inactivation of SH groups (may be enzymes) plays an important role in lead action. The investigations reported here were designed to confirm this hypothesis experimentally. We started from the hypothesis that if a sufficient amount of compounds rich in sulfhydryl groups is given to the rats, the endotoxin hypersensitivity inducing effect of lead acetate may be prevented.

Three hundred eighty female rats of (Wistar origin) a mean body weight of 100 g (90 to 110 g) were subdivided into 19 equal groups and treated as indicated in *Table I*. Lipopolysaccharide Westphal-type endotoxin produced from strain E. coli 089 by the warm phenol-water method [4] in this laboratory. The blood pH-values of the various treated animals were determined by use Radiometer TT-1 Anstrup-micrometer (Radiometer Co, Copenhagen, Denmark). The experiment was terminated 24 hours after the injections, by killing the survivors.

As shown in *Table I*, the endotoxin lead acetate, or any tested sulfhydryl compound (doses were calculated on the basis of their molarity but are expressed more practically in milligrams, as indicated in the footnotes to Table I) in themselves did not produce any mortality. The various

combinations of endotoxin and sulfhydryl compounds and lead acetate also had no visible biological effect, and there were no significant changes in blood pH value. However, lead acetate and endotoxin together always produce about 100% mortality, as described previously. Cysteine hydrochloride inhibits this endotoxin hypersensitivity, provoking the effect of lead acetate. Glutathione and methionine also had a slight protective effect against lead acetate, but ethionine had none.

Table 1

Protective effect of various sulfhydryl compounds on lead acetate-induced endotoxin hypersensitivity

Group	Treatment	Death ratio died/total	Protective value %
1	Endotoxin	0/20	0
2	Lead acetate	0/20	0
3	Lead acetate + endotoxin	20/20	0
4	Ethionine	0/20	0
5	Ethionine + endotoxin	0/20	0
6	Ethionine + lead acetate	0/20	0
7	Ethionine + lead acetate + endotoxin	18/20	10
8	Methionine	0/20	0
9	Methionine + endotoxin	0/20	0
10	Methionine + lead acetate	0/20	0
11	Methionine + lead acetate + endotoxin	12/20	40
12	Glutathione	0/20	0
13	Glutathione + endotoxin	0/20	0
14	Glutathione + lead acetate	0/20	0
15	Glutathione + lead acetate + endotoxin	8/20	60
16	Cysteine-HCl	0/20	0
17	Cysteine-HCl + endotoxin	0/20	0
18	Cysteine-HCl + lead acetate	0/20	0
19	Cysteine-HCl + lead acetate + endotoxin	4/20	80

The agents (in 1—1 ml of water) were given simultaneously by the intravenous route, in the following order: SH compound, lead acetate and endotoxin. Amounts used were as follows: endotoxin, 3 μg/rat; lead acetate, 5 mg/rat; ethionine, 25 mg/rat; methionine, 25 mg/rat; glutathionine, 25 mg/rat; and cysteine-HCl, 25 mg/rat.

These results suggest that sulfhydryl groups are required for the inactivation or detoxification of endotoxins in macro-organisms. Maybe lead acetate inactivates some SH-enzymes (adaptive enzymes?) or other, biologically active compounds which play an important part in the inactivation or detoxification of endotoxins. For this reason, under the lead action, the organism is unprotected against bacterial endotoxins. Accordingly, even a minute amount of bacterial endotoxin will be sufficient to elicit fatal shock. According to experiences, this endotoxin-hypersensitivity

inducing effect of lead acetate could be the best prevented by cysteine hydrochloride. It may be supposed that an even better protective effect may be obtained by other sulfhydryl containing compounds as e.g. dimercaptano-propanol (BAL).

REFERENCES

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DISCUSSION

Dr. Nowotny: Does lead in vitro and in vivo neutralize endotoxin in some way?

Dr. Bertók: We tested this once in Montreal, but I cannot give you any definite answer. Lead, indeed, had some effect.

Dr. Nowotny: Did the RES get blocked upon the effect of lead acetate?

Dr. Bertók: We tried to observe the potential activity decrease of the RES on rats treated with lead acetate. Since no Indian ink of the appropriate grain size was available, we attempted the elimination of nucleated chicken red blood cells, according to Földvári's method. This was not suitable, since rats normally have a haemolysin that lyses chicken erythrocytes. We tried to use fish (carp) red blood cells, too, but there is a disturbing normal haemolysin also in that case. I can say only so much that the RES gets probably blocked, since when injecting living bacteria intravenously, we found that—as compared to the control animal—bacterial elimination gets rather prolonged, i.e. the experimental bacteraemia persists.

Dr. Gabriella Fóris: How much of cysteine or glutathione would be required to prevent endotoxin effect.

Dr. Bertók: Naturally, neither glutathione nor cysteine will prevent the effect of the usual LD_{100} endotoxin dose.

Dr. Gabriella Fóris: When did you give the SH-compounds, before or after of endotoxin administration?

Dr. Bertók: Such compounds are given always prior to the endotoxin dose. As a matter of fact, there was nothing to prevent in this particular case, since only μg doses had been given which was no more then about 1/30th of the tolerating dose. Namely, upon the effect of lead acetate sensitivity will be increased to such an extent that even 1 to 3 μg of endotoxin are sufficient to elicit the shock, that is such a small amount which has no toxic effect in itself. On the other hand, we made a test. We injected a big amount of endotoxin LD_{100}, simultaneously to cysteine but experienced no protective effect.

Dr. Gabriella Fóris: Fukuda reported his observation according to which glutathione has a protective effect in endotoxin shock. Accordingly, we tried to prevent by glutathione the effect of endotoxin on the carbo-

hydrate metabolism in rats. As per our results, glutathione was, indeep, able to prevent this effect of the endotoxin. On the other hand, glutathione was not able to prevent the lethal effect of endotoxin in rats.

Dr. Bertók: In my opinion, glutathione is able to prevent a given effect of endotoxin (e.g. hypoglycaemia) but I doubt that it would be able to eliminate its lethal effect.