EFFECT OF CHLORPROMAZINE AND STELAZINE ON HEPATIC LYSOSOMES OF RATS WITH ACUTE POISONING

V. G. Titova and T. A. Korolenko

UDC 615.9.036.11.085.
214.22 : 547.869.2

The state of the lysosomal membranes of the rat liver was assessed from the level of free activity of the lysosomal marker enzymes acid phosphatase and acid ribonuclease and also by solubilization of the enzymes in the supernatant. In a dose of 30 mg/kg chlorpromazine had a "labilizing" action on the lysosomal membranes, which was not observed after administration of stelazine (4 mg/kg). The bromsulfthalein excretion was undisturbed after administration of both phenothiazine derivatives.

In recent studies of the mechanism of action of phenothiazine drugs attention has been increasingly concentrated on their effect on biological membranes [2, 4, 9].

This paper gives the results of a comparative study of the action of two phenothiazine derivatives, chlorpromazine and stelazine, on lysosomal membranes of the rat liver during acute poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male rats weighing 150-200 g. Chlorpromazine was injected intraperitoneally in a dose of 30 mg/kg, stelazine (made in England) in a dose of 4 mg/kg. The animals were decapitated 1 h after injection of the drugs, when their concentrations in the liver were maximal [1]. Intact animals, sacrificed at the same time as the experimental, were used as the control. A 10% homogenate of the liver was prepared in 0.25 M sucrose, pH 7.2 [5, 6]. The fraction of light mitochondria containing lysosomes was isolated by de Duve's method [5], and the supernatant was obtained by centrifuging the 10% homogenate at 100,000 g for 1 h. The free and total activity of the lysosomal marker enzymes (acid phosphatase and acid ribonuclease [5, 10]) was determined in the homogenate and the lysosomal fractions, and the "nonsedimenting" enzyme activity was determined in the supernatant. Acid phosphatase was determined by the method of de Duve et al. [5], and the inorganic phosphorus split off was estimated by the method of Weil-Malherbe and Green [11]. The results were expressed in μg inorganic phosphorus split in 10 min, calculated per mg protein. Acid ribonuclease activity was determined at pH 5.8 and in an RNA concentration of 1 mg/ml. RNA and protein were precipitated with lanthanum chloride in a mixture of 80% ethanol and 1 N HCl solution. The results were expressed in optical density units (E $\times$ 1000) calculated per mg protein.

Statistical analysis of the results was carried out by the parallel line method using Student's criterion. Differences were taken as significant when $P < 0.05$. In some experiments, to monitor the detoxicating function of the liver, the bromsulfthalein test was carried out simultaneously [3].

EXPERIMENTAL RESULTS

After administration of chlorpromazine (Table 1) there was an increase in free acid phosphatase activity in the homogenate and lysosomal fraction, indicating increased permeability of the membranes toward

Central Research Laboratory, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR I. V. Toroptsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 8, pp. 77-79, August, 1973. Original article submitted November 9, 1972.

© 1974 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $15.00.

TABLE 1. Effect of Chlorpromazine on Lysosomal Enzymes of Rat Liver (M±m)

Material tested	Treatment	Acid phosphatase		Acid ribonuclease	
		total activity (in μg P in 10 min/mg protein)	free activity (in % of total)	total activity (in E × 1000 in 10 min/mg protein)	free activity (in % of total)
Liver homogenate	Intact animals	9,95±0,9	3,07±0,43	73,4±1,7	10,9±2,25
	Chlorpromazine	13,6±1,2	7,52±0,8	91,7±18,0	13,0±1,3
	P	<0,05	<0,01	>0,5	>0,5
Lysosomes	Intact animals	15,4±2,3	2,97±0,17	102,0±15,0	14,4±1,4
	Chlorpromazine	18,7±1,4	6,12±0,68	151,0±15,0	19,8±2,5
	P	0,25	<0,001	>0,5	>0,5
Supernatant	Intact animals	4,67±0,43	—	13,7±0,8	—
	Chlorpromazine	8,5±0,87	—	14,0±1,06	—
	P	<0,02		>0,5	

TABLE 2. Effect of Stelazine on Lysosomal Enzymes of Rat Liver (M±m)

Material tested	Treatment	Acid phosphatase		Acid ribonuclease	
		total activity (in μg P in 10 min/mg protein)	free activity (in % of total)	total activity (in E × 1000 in 10 min/mg protein)	free activity (in % of total)
Liver homogenate	Intact animals	8,17±1,5	22,0±1,2	73,4±1,7	14,8±0,6
	Chlorpromazine	7,2±1,1	24,0±1,1	66,3±5,0	15,0±0,8
	P	>0,5	>0,5	>0,5	>0,5
Lysosomes	Intact animals	23,5±7,4	9,3±0,9	102,0±15,0	13,9±1,8
	Chlorpromazine	20,1±4,1	10,1±0,8	119,9±3,7	9,4±1,0
	P	>0,5	>0,5	>0,5	>0,1
Supernatant	Intact animals	2,69±0,43	—	13,7±0,8	—
	Chlorpromazine	2,55±0,79	—	13,8±0,8	—
	P	>0,5		>0,5	

the substrate (β-glycerophosphate), which could be regarded as evidence of injury to the lysosomal membrane. An increase in the "nonsedimenting" acid phosphatase activity was observed in the supernatant, confirming the previous hypothesis. Acid ribonuclease activity was unchanged, evidently because of differences in the bond joining the enzymes to the matrix of the lysosome.

No change in the activity of these enzymes was observed after administration of stelazine (Table 2). The rate and completeness of elimination of bromsulfthalein from the blood of all the experimental animals corresponded to that in the controls (Fig. 1).

After administration of large doses of chlorpromazine a "labilizing" action was thus observed on the lysosomal membranes of the rat liver although the liver function was undisturbed. The absence of changes in the state of the lysosomes after administration of stelazine can be attributed not only to the use of a smaller dose of the drug than of chlorpromazine, but also, probably, to differences in the method of interaction between the two phenothiazine derivatives and the lysosomal membrane. Remembering that acid lipoproteins are responsible for the concentrating of basic dyes and other cationic molecules inside lyso-

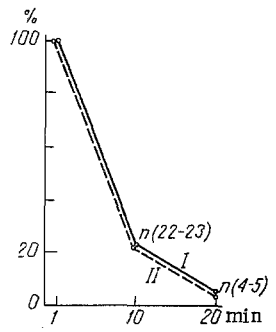

Fig. 1. Dynamics of elimination of bromsulfthalein by the liver of rats after 10 and 20 min in control (I) and experimental (II) animals (receiving stelazine): n) number of experiments. Abscissa, elimination time (in min); ordinate, percentage elimination.

somes in vivo [7], a mechanism whereby certain phenothiazine drugs are taken up by the lysosomes of the rat liver can be postulated.

LITERATURE CITED

1. V. M. Avakumov and Yu. M. Batulin, Farmakol. i Toksikol., No. 1, 14 (1970).
2. Kh. L. Gainutdinov, É. V. Narushevichus, and V. I. Tamulevichute, in: The Biophysics of Membranes [in Russian], Kaunas (1971), p. 242.
3. A. I. Evsev'eva, Pat. Fiziol., No. 3, 25 (1970).
4. M. V. Komendantova and G. N. Kravtsova, Farmakol. i Toksikol., No. 3, 301 (1971).
5. C. de Duve, B. C. Pressman, R. Gianetto, et al., Biochem. J., 60, 607 (1955).
6. R. Gianetto and C. de Duve, Biochem. J., 59, 433 (1955).
7. A. Goldstone, E. Szabo, and H. Koenig, Life Sci., 9, 607 (1970).
8. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).
9. C. Popov, Dokl. Bolgarsk. Akad. Nauk, 22,1213 (1969).
10. R. Wattiaux and C. de Duve, Biochem. J., 63, 606 (1956).
11. H. Weil-Malherbe and R. H. Green, Biochem. J., 49, 286 (1952).