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EFFECTS OF VASOACTIVE DRUGS ON LYSOSOMAL STABILITY *IN VITRO**

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Abstract—The effects of methylprednisolone, chlorpromazine, phenoxybenzamine and phentolamine on thermolabilized lysosomes were studied by determining the unsedimentable, fragile lysosomal and total lysosomal activities of β-glucuronidase and acid phosphatase in centrifuged fractions. At concentrations of 10^{-5}–10^{-3} M, methylprednisolone stabilized the lysosomes. Chlorpromazine and phenoxybenzamine at 10^{-3} M had a labilizing effect. The changes in the enzyme activities due to phentolamine could not be explained as changes in membrane permeability. The glucocorticoids appear to protect the cell membranes directly and thus counteract the adverse effects of circulatory shock. The same mechanism may explain the salutary effects which chlorpromazine at low concentrations reportedly possesses. The improvement of microcirculation in shock after the administration of phenoxybenzamine or phentolamine is due to their α-adrenergic blocking activity; this in turn leads to vasodilatation and indirectly to a membrane protection.

PROTECTION against circulatory shock by various pharmacological agents is due to vasodilatation, decreased resistance to blood flow and, thus, improved capillary perfusion. Different mechanisms of action may exist behind this apparently similar effect. Stabilization of the lysosomal membrane[1,2] and α-adrenergic blockade[3] have been considered as protective mechanisms. The anti-inflammatory steroids have been shown to stabilize the lysosomes both *in vivo* and *in vitro*.[1] On the other hand, their effect in circulatory shock has been interpreted as vasodepression,[2,4] i.e. inhibition of vasoconstricting stimuli. Chlorpromazine has been reported to stabilize the lysosomes[5] and to block neurogenic peripheral vascular control.[6] In the present study, the effects of methylprednisolone and chlorpromazine on the enzyme release from thermolabilized lysosomes are compared with the effects of two α-adrenergic inhibitors, phenoxybenzamine and phentolamine.

MATERIALS AND METHODS

Animals. White male rats (original strain Sprague–Dawley) weighing from 125 to 180 g were used. From eight to 12 animals were used in each experimental group. The liver samples (0·5 g) were removed under ether anesthesia and washed with 0·25 M sucrose at 4°.

Chemicals. The chemicals were reagent grade. The following test drugs were used: methylprednisolone (donated by the Upjohn Co, Kalamazoo, Mich.), chlorpromazine (Dumex, Copenhagen, Denmark), phenoxybenzamine (Smith, Kline & French Labs., Philadelphia, Pa.) and phentolamine (Ciba, Basel, Switzerland).

Fractionation of lysosomes. Rat liver lysosomes were fractionated according to Hsu and Tappel.[7] The tissue samples were processed in a 0·25 M sucrose solution containing

* A portion of the results were presented at a Symposium at Brook Lodge, Augusta, Mich., 1–3 June, 1971; *Shock in Low- and High-Flow States* (Eds. R. C. LILLEHEI, B. K. FORSCHER and S. S. STUBBS), p. 263. Excerpta Medica, Amsterdam (1972).

10^{-3} M tetrasodium salt of EDTA and adjusted to pH 7·0. In the experimental groups, the test drug (methylprednisolone etc.) was added to the sucrose medium prior to the adjustment. The tissue was homogenized in a cold chamber in 10 ml of the medium by six up-and-down strokes of a Potter–Elvehjem homogenizer (Thomas B) driven at 900 rev/min. After the separation of the nuclear fraction at 480 *g* for 10 min (Fig. 1),

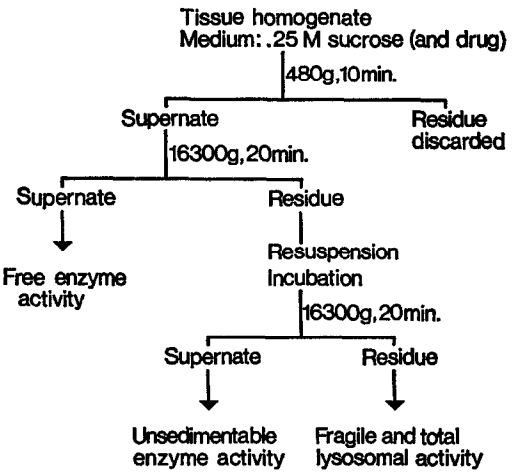

FIG. 1. Lysosome fractionation scheme.

the supernatant was centrifuged at 16,300 *g* for 20 min. The free enzyme activity was determined in the 16,300 *g* supernatant. The effect of the test drug on the enzyme determination was judged by the enzyme activity of this fraction. The large granular fraction was resuspended in the medium and incubated at 30° for 2 hr. Unincubated samples were studied to evaluate the labilizing effect of the incubation on the lysosomal enzymes. The supernatant after centrifugation was used for the determination of "unsedimentable enzyme activity". In the residue, the "fragile lysosomal activity" was assayed, while the "total lysosomal enzyme activity" was determined after the addition of 0·1% Triton X-100 to the residue. Methylprednisolone, chlorpromazine, phenoxybenzamine or phentolamine was added to the medium at concentrations of 10^{-5}, 10^{-4} and 10^{-3} M.

Enzyme assays. β-glucuronidase was determined according to Gianetto and de Duve[8] with phenol-β-D-glucuronide as substrate. Acid phosphatase was determined by the method of Berthet and de Duve[9] with β-glycerophosphate as substrate. The samples were incubated at 37° for 10 min. In an earlier study,[10] the amount of protein per fresh wt of liver varied insignificantly in the tissue fractions. Therefore, the enzyme activities were calculated per fresh weight of tissue. In order to compare the results of different determinations, the activities were also expressed as percentages of the corresponding control group mean values.

Statistical analysis. Student's *t*-test was used. The significance level was set at $P < 0{\cdot}01$.

RESULTS

Methylprednisolone, phenoxybenzamine and phentolamine did not alter the free enzyme activities. Chlorpromazine, at 10^{-5} M decreased the free acid phosphatase activity and at 10^{-3} M decreased the free β-glucuronidase activity by 23 per cent ($P < 0.001$).

The labilizing effect of the 30° incubation on the large granular fraction was demonstrated by a significant increase in the fragile lysosomal activities of both acid phosphatase (30 per cent) and β-glucuronidase (42 per cent) as compared with the unincubated samples ($P < 0.01$) (Tables 1 and 2).

TABLE 1. DRUG EFFECTS ON THE β-GLUCURONIDASE OF THERMOLABILIZED LIVER LYSOSOMES

Drug*	Unsedimentable activity†		Fragile lysosomal activity†		Total lysosomal activity†	
Unincubated sample	368 ± 41		712 ± 80	$P < 0.01$	1071 ± 180	
Incubated control	450 ± 21		1017 ± 70		1085 ± 122	
Control	450 ± 21	$P < 0.01$	1017 ± 70		1085 ± 122	
Methylprednisolone	357 ± 22		1423 ± 141		1403 ± 145	
Control	370 ± 23	$P < 0.001$	510 ± 28	$P < 0.001$	678 ± 30	$P < 0.001$
Chlorpromazine	757 ± 40		708 ± 33		360 ± 41	
Control	450 ± 21		1017 ± 70		1085 ± 122	
Phenoxybenzamine	358 ± 36		1169 ± 97		928 ± 44	
Control	880 ± 36	$P < 0.001$	1012 ± 127	$P < 0.001$	1883 ± 126	$P < 0.001$
Phentolamine	519 ± 39		2394 ± 120		2680 ± 133	

* Drug concentration in the medium 10^{-3} M.
† μg/g fresh liver tissue/10 min, mean ± S.E., 10–12 samples in a group.

TABLE 2. DRUG EFFECTS ON THE ACID PHOSPHATASE OF THERMOLABILIZED LIVER LYSOSOMES

Drug*	Unsedimentable activity†		Fragile lysosomal activity†		Total lysosomal activity†	
Unincubated sample	73·9 ± 6·2		179·0 ± 9·2	$P < 0.01$	1069 ± 22·4	
Incubated control	77·0 ± 6·4		232·0 ± 10·8		1044 ± 36·8	
Control	77·0 ± 6·4		232·0 ± 10·8		1044 ± 36·8	
Methylprednisolone	65·1 ± 9·9		180·7 ± 16·5		943 ± 64·2	
Control	122·6 ± 6·2	$P < 0.001$	179·9 ± 11·4	$P < 0.001$	1146 ± 29·7	$P < 0.001$
Chlorpromazine	631·4 ± 22·1		631·6 ± 21·3		735 ± 25·5	
Control	77·0 ± 6·4	$P < 0.001$	232·0 ± 10·8	$P < 0.001$	1044 ± 36·8	
Phenoxybenzamine	122·7 ± 5·5		557·9 ± 30·7		1073 ± 48·6	
Control	125·0 ± 12·9		162·8 ± 24·6	$P < 0.01$	1131 ± 87·3	
Phentolamine	118·3 ± 4 4		312·4 ± 47·2		1307 ± 87·5	

* Drug concentration in the medium 10^{-3} M.
† μg P/g fresh liver tissue/10 min, mean ± S.E., 10–12 samples in a group.

Effects of methylprednisolone. The level of unsedimentable β-glucuronidase activity was significantly lowered by 10^{-3} M methylprednisolone ($P < 0.01$) (Table 1).

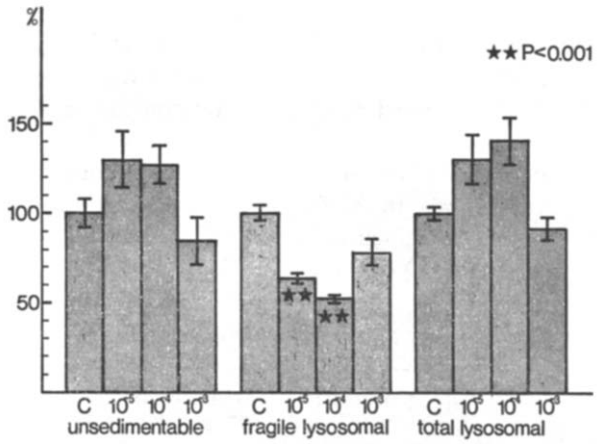

FIG. 2. The effects of methylprednisolone at concentrations of 10^{-5} to 10^{-3} M on the acid phosphatase activity of thermolabilized liver lysosomes. The P values are derived from a comparison with the respective control group mean values (C). The vertical bars represent S.E. Eight to 12 rats were used per group.

10^{-4} M and 10^{-5} M methylprednisolone significantly lowered the fragile lysosomal levels of acid phosphatase activities ($P < 0{\cdot}001$) (Fig. 2).

Effects of chlorpromazine. The levels of unsedimentable and fragile lysosomal β-glucuronidase activities were significantly increased by 10^{-3} M chlorpromazine ($P < 0{\cdot}001$) (Table 1); the total lysosomal β-glucuronidase was significantly diminished ($P < 0{\cdot}001$). 10^{-3} M chlorpromazine had the same effect on acid phosphatase (Table 2 and Fig. 3). At 10^{-4} M it had no significant effect but at 10^{-5} M it significantly decreased the unsedimentable acid phosphatase activity ($P < 0{\cdot}01$).

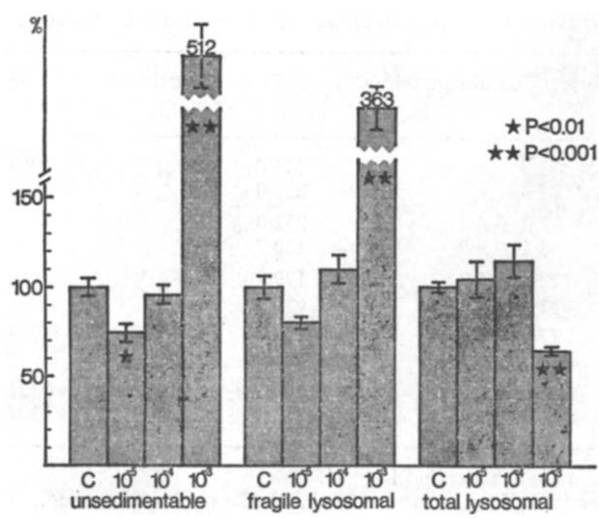

FIG. 3. The effects of chlorpromazine at concentrations of 10^{-5} to 10^{-3} M on the acid phosphatase activity of thermolabilized liver lysosomes. The P values are derived from a comparison with the respective control group mean values (C). The vertical bars represent S.E. Eight to 12 samples were used per group.

Effects of phenoxybenzamine. No changes in the β-glucuronidase activities were observed. At a concentration of 10^{-3} M, phenoxybenzamine increased the unsedimentable and fragile lysosomal acid phosphatase significantly ($P < 0{\cdot}001$) (Table 2 and Fig. 4). At lower concentrations, no significant changes were observed.

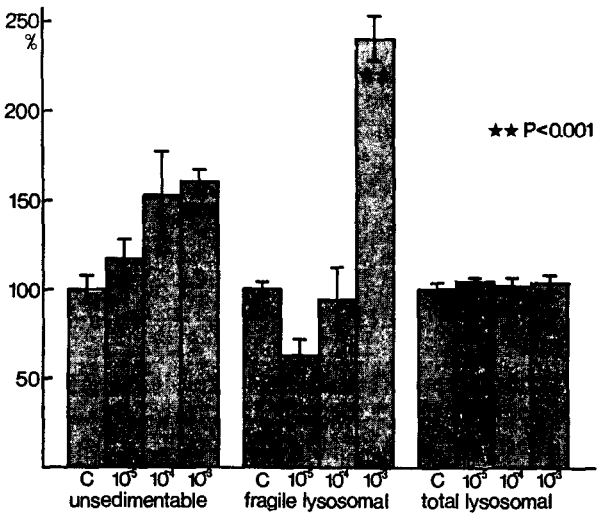

FIG. 4. The effects of phenoxybenzamine at concentrations of 10^{-5} to 10^{-3} M on the acid phosphatase activity of thermolabilized liver lysosomes. The P values are derived from a comparison with the respective control group mean values (C). The vertical bars represent S.E. Eight to 12 animals were used per group.

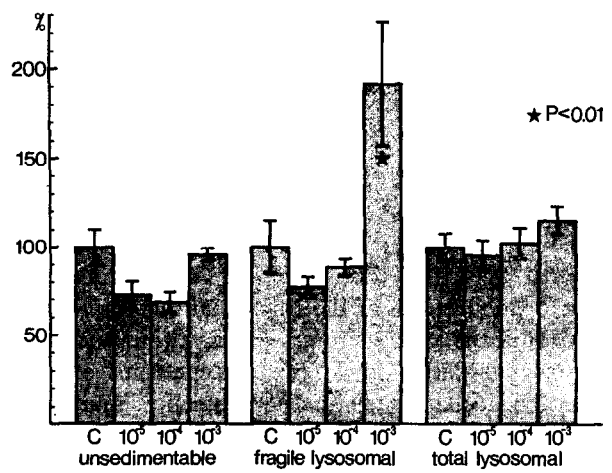

FIG. 5. The effects of phentolamine at concentrations of 10^{-5} to 10^{-3} M on the acid phosphatase activity of thermolabilized liver lysosomes. The P value are derived from a comparison with the respective control group mean value (C). The vertical bars represent S.E. Eight to 12 rats were used per group.

Effects of phentolamine. The unsedimentable β-glucuronidase activity was significantly decreased by 10^{-3} M phentolamine ($P < 0{\cdot}001$) (Table 1). The fragile and total lysosomal β-glucuronidase activities were significantly increased ($P < 0{\cdot}001$) as well as the fragile lysosomal acid phosphatase activity ($P < 0{\cdot}01$) (Table 2 and Fig. 5). Lower concentrations of phentolamine did not change the acid phosphatase activities as compared with the control samples.

DISCUSSION AND CONCLUSIONS

α-Adrenergic-blocking agents appear to be salutary in circulatory shock.[3] These drugs dilate the constricted vascular beds especially in the splanchnic viscera. Chlorpromazine, which in many ways acts differently, has been demonstrated to have adrenergic blocking activity and the ability to protect the experimental animals from the adverse effects of shock.[6,11]

Glucocorticosteroids in large doses appear to resemble both α-adrenergic blocking drugs and chlorpromazine in lowering the vascular resistance of the viscera,[4] in improving the oxygen consumption and in decreasing tissue acidosis during experimental shock.[12,13]

Glucocorticoids stabilize the lysosomal membrane.[1] The labilizing effect of circulatory shock may be reverted by cortisone.[2] Chlorpromazine has been shown to inhibit the lysosomal enzyme release at low concentrations both *in vitro* and *in vivo*, whereas high concentrations appear to increase the lysosomal membrane permeability.[5]

In the present study, the effects of methylprednisolone, chlorpromazine and two α-adrenergic blocking agents, phenoxybenzamine and phentolamine on lysosomal enzyme release were compared. The drugs, with the exception of chlorpromazine, did not interfere with the determination of acid phosphatase and β-glucuronidase activities. The inhibitory action of chlorpromazine has to be taken into consideration when interpreting the results.

In agreement with earlier reports,[1] methylprednisolone inhibited the release of enzymes from the thermolabilized lysosomes. Chlorpromazine at a concentration of 10^{-3} showed a labilizing effect; the unsedimentable and fragile lysosomal enzyme activities increased with a diminution of the total lysosomal activities. The decrease in the unsedimentable acid phosphatase activity caused by 10^{-5} M chlorpromazine cannot be interpreted as lysosomal stabilization in face of a similar change in the free enzyme fraction. Phenoxybenzamine at 10^{-3} M appeared to labilize the lysosomes; both unsedimentable and fragile lysosomal enzyme activities were augmented. Phentolamine at 10^{-3} M decreased the unsedimentable β-glucuronidase activity and increased the fragile lysosomal β-glucuronidase, the fragile lysosomal acid phosphatase and the total lysosomal β-glucuronidase activities. These effects defy a simple explanation of stabilization or labilization of the lysosomal membrane.

The present study suggests that the improvement of microcirculatory perfusion in shock by various drugs is based on different mechanisms of action. The effect of glucocorticoids may be primarily membrane protection and maintenance of functional[12,13] and structural (including vascular) integrity.[14] Chlorpromazine also appears to have a direct effect on the lysosomal membrane by stabilizing at low concentrations and labilizing at high concentrations.[5] Only the latter effect was demonstrated in the present study. The biphasic action may explain the dual hemodynamic effects reported in a clinical study.[15]

Phenoxybenzamine and phentolamine do not appear to have membrane stabilizing effects. In a study of endotoxin shock,[16] phenoxybenzamine prevented increases in both plasma and tissue acid hydrolase levels, a sign of lysosomal protection. The effect of α-adrenergic inhibitors during shock is vasodilation.[3] The membrane protection is most likely an indirect consequence of the improved blood flow.

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