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Action of anti-inflammatory steroids on the lytic action of phospholipase C and 2,4,6-trinitrobenzene sulphonic acid on lysosomes

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THE UNDERLYING biochemical mechanism of the action of anti-inflammatory steroids on lysosomal membranes is not known but work with model systems clearly indicates that steroid–phospholipid interactions are important.[1] It is less clear why the stabilising action of anti-inflammatory steroids on the membranes should fall off at high concentrations but there is some evidence that steroid–protein interactions may be involved.[2] In order to gain information on the mechanisms underlying the action of anti-inflammatory steroids on lysosomes we have investigated their effect on the action of phospholipase C (PLC) and the protein denaturing agent 2,4,6-trinitrobenzene sulphonic acid (TNBS) on lysosomes.

Lysosome-containing suspensions in 0·05 M Tris–acetate buffered sucrose (0·25 M, pH 7·4) were prepared from rabbit liver as previously described.[2] Portions (5 ml) were incubated at 37° for 90 min in 50 ml conical flasks with 100 μl of various concentrations of PLC and TNBS added in aqueous solution, or with distilled water as controls. After the incubation period the suspensions were centrifuged and the amount of lysosomal enzymes released (acid phosphatase and β-glucuronidase) determined by methods previously described,[2] using *p*-nitrophenyl phosphate and phenolphthalein glucuronide as substrates for acid phosphatase and β-glucuronidase, respectively. The results are shown in Figs. 1 and 2.

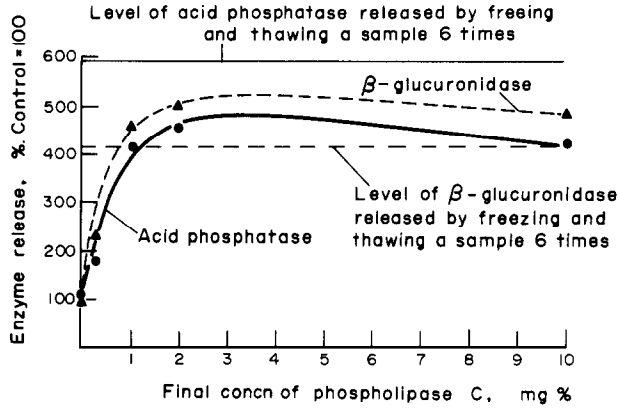

FIG. 1. Effect of phospholipase C on lysosomal enzyme release. The total amount of "releasable" enzyme was determined by freezing and thawing a lysosomal suspension six times, centrifuging and measuring enzyme concentrations in the clear supernatant. All results shown are expressed as a percentage of the controls, i.e. no PLC added. (▲) β-glucuronidase; (●) acid phosphatase.

Clearly at optimum concentrations PLC was more effective in causing lysosomal enzyme release than TNBS. It also released more β-glucuronidase than freezing and thawing but not more acid phosphatase. This could reflect differences between the binding sites of the two enzymes suggesting that β-glucuronidase is more associated with a phospholipid than is acid phosphatase.

The results with TNBS suggest that at high concentrations (10 mM) it denatured acid phosphatase and β-glucuronidase as levels found were lower than the experimental control values where no TNBS was added. In separate experiments lipolytic agents, namely saponin and lysolecithin, were found to be less effective than PLC in promoting enzyme release.

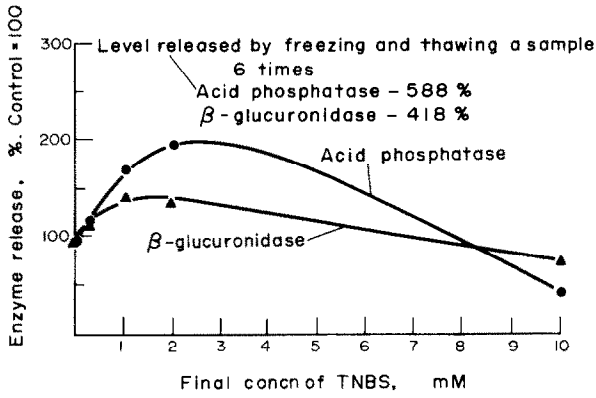

FIG. 2. Effect of 2,4,6-trinitrobenzene sulphonic acid on lysosomal enzyme release. All results are expressed as a percentage of the controls, i.e. no TNBS added. (▲) β-glucuronides; (●) acid phosphatase.

The effect of PLC on steroid-treated and untreated lysosomes was studied as follows. Portions (50 μl) of 50 mM solutions of the steroids in dimethyl sulphoxide were separately added to 5 ml portions of the standard lysosomal suspension to give a final concentration of 5×10^{-4} M. The suspensions were incubated for 60 min in a shaking reaction incubator at 37° and then PLC added in aqueous solution (100 μl) to a final concentration of 0·2 mg%.* Incubation was continued for a further 60 min after which time the suspensions were centrifuged and the free enzyme levels in the supernatant determined.

TABLE 1. EFFECT OF PHOSPHOLIPASE C (PLC) ON STEROID-TREATED (5×10^{-4} M) LYSOSOMES

Steroid	Acid phosphatase released (% of control)	β-glucuronidase released (% of control)
None (control)	100	100
Cortisone	86·1	87·3
Cortisol	84·4	84·3
Prednisone	86·1	91·7
Prednisolone	85·6	90·2
Dexamethasone	96·3	93·6
Triamcinolone	92·1	92·6

Lysosome suspensions were pre-incubated with steroids as described in the text and the effect of 0·2 mg% PLC (0·2 mg per 100 ml) on lysosomal enzyme release then determined. Results are expressed as a percentage of controls where the steroid solvent (DMSO) was added for the preincubation period. Controls released 13·6 per cent of "total" acid phosphatase and 32·7 per cent of "total" β-glucuronidase activity in the 2 hr incubation period (mean of three experiments).

* The terminology mg% used in the paper refers to mg per 100 ml.

The results (Table 1) show that the steroids stabilized the membrane against enzymic attack. In further experiments lysosomes were pretreated for 60 min with prednisolone at a final concentration of 5×10^{-4} M. Then PLC was added in varying concentrations and the incubation continued for a further 60 min when the free enzyme levels were determined. The results (Table 2) show that the stabilizing effect of the steroid was lost as the concentration of PLC increased which suggests a competitive action between prednisolone and PLC.

In further experiments 5 ml portions of lysosomal suspensions were incubated at 37° for 60 min in the presence of 0·2 mg% PLC. Then prednisolone in dimethyl sulphoxide (50 μl) was added to varying final concentrations (5×10^{-5} M to 5×10^{-4} M), and the incubation continued for a further 60 min when the free enzyme levels were determined. In other experiments prednisolone (5×10^{-4} M) and PLC (0·2 mg%) were added to the lysosome suspension together at the beginning of the 2 hr incubation period, or prednisolone was added for the first 60 min followed by PLC for the second 60 min as in previous experiments. The results (Table 3) show that after pretreatment of the lysosomes with PLC the addition of steroid has increased the amount of free enzyme in the supernatant compared to controls.

TABLE 2. EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHOLIPASE C (PLC) ON ENZYME RELEASE FROM PREDNISOLONE-TREATED (5×10^{-4} M) LYSOSOMES

Treatment for first 60 min	Additional treatment for second 60 min	Acid phosphatase released (% of control)	β-glucuronidase released (% of control)
50 μl DMSO	100 ul H_2O	100 (control)	100 (control)
50 μl DMSO	0·1 mg% PLC	100	102
50 μl DMSO	0·2 mg% PLC	211	252
50 μl DMSO	0·5 mg% PLC	538	543
50 μl DMSO	1·0 mg% PLC	539	541
50 μl 50 mM prednisolone in DMSO	0·1 mg% PLC	100 (0%)*	103 (0%)*
50 μl 50 mM prednisolone in DMSO	0·2 mg% PLC	164 (22·2%)*	187 (25·8%)*
	0·5 mg% PLC	520 (3·3%)*	523 (3·7%)*
	1·0 mg% PLC	535 (0·7%)*	543 (0%)*

* These values show the percentage stabilization to PLC treatment in prednisolone-treated lysosomes as compared to control-treated lysosomes, i.e. 22·2 per cent stabilization arises from 211%–164%/211%. All results (other than those in parentheses) have been expressed as percentages of controls which received 50 μl DMSO for the first 60 min and 100 μl water for the second 60 min.

TABLE 3. EFFECT OF VARIOUS CONCENTRATIONS OF PREDNISOLONE ON ENZYME RELEASE FROM PHOSPHOLIPASE C TREATED LYSOSOMES

Treatment for first 60 min	Additional treatment for second 60 min	Acid phosphatase released (% of control)	β-glucuronidase released (% of control)
0·2 mg% PLC	50 μl DMSO (control)	100	100
0·2 mg% PLC	5×10^{-5} M prednisolone	128	117
0·2 mg% PLC	10^{-4} M prednisolone	121	113
0·2 mg% PLC	5×10^{-4} M prednisolone	117	109
0·2 mg% PLC + 5×10^{-4} M prednisolone	None	86	87
5×10^{-4} M prednisolone	0·2 mg% PLC	93	91

The results have been expressed as a percentage of controls which received 0·2 mg% PLC for the first 60 min and 50 μl DMSO for the second 60 min.

However, where the lysosomes were treated with both enzyme and prednisolone for 2 hr or by 5×10^{-4} M prednisolone for 60 min followed by PLC for 60 min the steroid has competed successfully against the enzyme for the phospholipid sites as is shown by lower levels of enzyme in the supernatant.

In a parallel set of experiments lysosomal suspensions were pretreated separately for 60 min at 37° with cortisol, cortisone, dexamethasone, prednisone, prednisolone and triamcinolone at a final concentration of 5×10^{-4} M. Then TNBS was added to a final concentration of 1 mM and incubation continued for a further 60 min. When compared to controls no stabilization against the lytic action of TNBS was found with any steroid tested.

Both PLC and TNBS caused the release of enzymes from lysosomes. Since anti-inflammatory steroids were effective in blocking the action of PLC on lysosomes, but not that of TNBS, then it appears that the stabilizing action of anti-inflammatory steroids on lysosomes is due to steroid phospholipid interactions rather than steroid-protein interactions, and that anti-inflammatory steroids will protect lysosomal membranes against lipase action.

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Inhibition of mitochondrial protein synthesis by nitrofurantoin in rat and goat liver

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THE MECHANISM of action of nitrofurantoin has not been investigated thoroughly although this and related compounds have been known for almost 43 yr.[1] Roschenthaler *et al.* examined the effect of nitrofurantoin upon RNA and protein synthesis in *E. coli* K_{12} by using the β-galactosidase system and concluded that the inhibition of the enzyme synthesis could be due either (i) to a decreased pool of nucleotide triphosphates by interference with the energy metabolism, or (ii) to disturbances of the cell membrane or (iii) to a more direct action on the protein synthesis apparatus.[2]

It is now well established that mitochondria possess their own protein synthesizing machinery[3–5] and some features of the mitochondrial protein synthesizing system resemble those of the bacterial system.[6,7] We present the results of a study of the effect of nitrofurantoin on the incorporation of ^{14}C-valine into proteins by intact mitochondria isolated from rat and goat liver.