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The actions of some non-steroidal drugs on lysosomes

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Several non-steroidal acidic anti-inflammatory drugs had a lytic action on lysosomes at high concentrations but no apparant action at lower concentrations. The drugs also inhibited acid phosphatase activity but only at high concentrations. High concentrations of drugs accelerated the thermal denaturation of albumin solutions but partially protected the albumin against denaturation at lower concentrations. The possibility that the denaturing action of drugs on proteins at high concentrations is related to lysosomal damage and that this damage may be associated with ulceration *in vivo* is discussed. Salicylate was found to inhibit the stabilizing action of cortisol on lysosomes.

The basis of the pharmacological action of non-steroidal anti-inflammatory drugs has been related to the property of many of them to uncouple oxidative phosphorylation (Adams & Cobb, 1958). Mizushima & Suzuki (1965) proposed that the ability of many of the drugs to stabilize proteins was a basis for their action. Other mechanisms have been reviewed recently (Whitehouse, 1968). The anti-inflammatory action of steroids has been related to their ability to stabilize lysosomes at certain concentrations (Weissmann & Dingle, 1961). Some non-steroidal drugs, for example phenylbutazone (Tanaka & Iizuka, 1968), stabilize lysosomes, but the evidence for the stabilizing action of salicylates on lysosomes is contradictory (Miller & Smith, 1966; Weissmann, 1968; Tanaka & Iizuka, 1968). Harford & Smith (1970) suggest that differences in experimental conditions may be responsible for the contradictory results reported by various authors.

It is apparent from investigations with steroids (Lewis, Symons & Ancill, 1970), that concentration and structure are important factors in the actions of these drugs on lysosomes. I have incubated non-steroidal drugs over a wide concentration range with lysosomes, and examined the effect of aromatic acids on the thermal stability of albumin and acid phosphatase (EC 3.1.3.2 orthophosphoric monoester phosphohydrolase). Aromatic acids interact strongly with proteins and it is possible that protein-drug interactions may effect the actions of drugs on lysosomes, particularly at high concentrations.

EXPERIMENTAL

The action of anti-inflammatory drugs on lysosomes

A lysosome suspension in tris-acetate (0·05M) buffered sucrose (pH 7·4) (0·25M) was prepared from rat liver (Lewis & others, 1970). An aqueous solution (adjusted to pH 7·4) of the sodium salt of the drug was prepared and portions added to 50 ml stoppered flasks and evaporated to dryness under reduced pressure. To this 5 ml of the lysosome suspension was added and the flasks were incubated for 90 min at 37° in a shaking reaction incubator. The suspensions were centrifuged at 20000 *g*

for 20 min at 4° in a Beckman Model L2 Ultracentrifuge and the supernatants examined for acid phosphatase activity (Symons, Lewis & Ancill, 1969) and protein (Lowry, Rosebrough & others, 1951). The amount of protein present in the initial lysosome suspension was also determined and the preparation was diluted, if necessary, to give a protein concentration of 5 mg/ml.

In one experiment the action of salicylate on the stabilizing action of cortisol on lysosomes was examined. The initial lysosome suspension was divided into two portions and sodium salicylate was added to one portion to a final concentration of 5×10^{-4}M. Portions (5 ml) of both the salicylate-treated and untreated lysosomes were separately added to flasks containing various amounts of cortisol. After incubation at 37° for 90 min the supernatants were examined for acid phosphatase activity and β-glucuronidase (EC 3.2.1.31 β-D-glucuronide glucuronohydrolase) activity (Symons & others, 1969).

The effect of anti-inflammatory drugs on the thermal stability of acid phosphatase and albumin

Albumin solutions. A 1% w/v solution of egg albumin (which preliminary experiments showed to behave like bovine albumin) in 0·9% sodium chloride solution was prepared and buffered at pH 5·2 with 0·1M phosphate. Aqueous solutions of the sodium salts of the drugs were prepared and 0·1 ml portions added to 5 ml of the buffered protein solution. Water alone was added to the controls. The absorbances of the solutions were measured at 420 nm. The solutions were then heated and the absorbance values at 420 nm determined at various times.

Acid phosphatase activity. A lysosome suspension in tris acetate buffered sucrose was prepared and allowed to freeze and thaw six times. The suspension was then centrifuged at 20000 *g* for 20 min at 4° to remove lysosomes and lysosomal debris. Portions (2 ml) of the solutions were added to test tubes containing 0·1 ml of an aqueous solution of the sodium salt of the drug. The control tubes contained 0·1 ml of water. After their contents had been mixed the tubes were placed in a water bath at 37° and samples examined at various times for acid phosphatase activity. The initial acid phosphatase activity of the solutions was also determined.

RESULTS AND DISCUSSION

The drugs inhibited acid phosphatase activity at 10^{-1}M but had little effect at 10^{-5}M (Table 1). Salicylate, up to 10^{-3}M, has been reported to have no effect on acid

Table 1. *Effect of non-steroidal drugs on acid phosphatase.* Only at the higher drug concentration is the enzyme inhibited.

	% Activity of acid phosphatase preparation remaining after incubation in the presence of added drugs										
Incubation period (min)	None (control)	• Ibufenac		† Ibuprofen		‡ Fluprofen		2-*p*-(2-Methyl biphenylyl)-propionic acid		Salicylic acid	
		10^{-1}M	10^{-5}M	10^{-1}M	10^{-5}M	10^{-1}M	10^{-5}M	10^{-1}M	10^{-5}M	10^{-1}M	10^{-5}M
0	100	—	—	—	—	—	—	—	—	—	—
90	66 ± 4	15 ± 3	61 ± 3	9 ± 4	64 ± 3	9 ± 2	63 ± 2	9 ± 1	64 ± 2	31 ± 3	57 ± 5
210	47 ± 3	5 ± 2	46 ± 1	2 ± 1	42 ± 2	1 ± 1	42 ± 4	2 ± 2	46 ± 4	15 ± 2	43 ± 2
330	30 ± 3	2 ± 2	31 ± 5	1 ± 1	30 ± 4	1 ± 1	31 ± 2	2 ± 2	32 ± 1	7 ± 1	27 ± 4

Each result is the mean value ± standard deviation of four experiments.
Abbreviations: •4-isobutylphenylacetic acid; †2-(4-isobutylphenyl)propionic acid; ‡2-*p*-(2-fluorobiphenyl)propionic acid.

phosphatase activity (Robinson & Willcox, 1969). Since the drugs inhibited the action of acid phosphatase, this enzyme could not be used to measure lysosome stability at high drug concentrations. However, the amount of enzyme released from lysosomes incubated with drugs over a concentration range of 10^{-4}–10^{-7}M was similar to the control values. The drugs do not appear to have stabilized the lysosomes at these concentrations. The amounts of protein released from the lysosomes after incubation with the drugs also showed no significant difference from control values at drug concentrations below 10^{-3}M but above this the results show the drugs to have a lytic action on lysosomes (Table 2). Although these high concentrations are not usually of physiological significance, it is possible that local high concentrations of the drugs occur after oral administration. A high concentration would certainly be present in the liquid film coating particles of the drugs in the stomach or elsewhere. It is therefore possible that gastrointestinal ulceration, a side-effect of these drugs, may be associated, at least in part, with lysosomal damage

Table 2. *Release of protein from lysosomes by anti-inflammatory drugs.*

	Amount of protein (as % with control value adjusted to 100) in supernatant after treating the lysomes with the drugs below					
Drug concn. (M)	Aspirin	Ibufenac	Ibuprofen	Fluprofen	2-p-(2-Methyl biphenylyl) propionic acid	Salicylic acid
None (control)	100	100	100	100	100	100
5×10^{-1}	—	—	—	—	—	208 ± 10
10^{-1}	385 ± 2	—	—	—	—	—
5×10^{-2}	—	183 ± 16	170 ± 2	175 ± 6	156 ± 5	104 ± 2
10^{-2}	210 ± 6	—	—	—	—	—
5×10^{-3}	—	99 ± 7	105 ± 4	130 ± 6	125 ± 2	94 ± 5
10^{-3}*	118 ± 4	—	—	—	—	—

* Concentrations of drug below this (to 10^{-8}) have no effect on protein release.

Table 3. *Effect of non-steroidal drugs on the thermal denaturation of albumin*

Concentration (M)	Aspirin (at 54°)	Ibuprofen (at 57°)	Ibufenac (at 58°)	Salicylic acid (at 55°)
None (control)	147 ± 3	185 ± 1	210 ± 2	162 ± 4
10^{-1}	1000 ± 50	—	—	1200 ± 200
5×10^{-2}	—	—	—	244 ± 15
$2{\cdot}5 \times 10^{-2}$	—	1209 ± 80	428 ± 10	—
10^{-2}	158 ± 3	—	—	—
5×10^{-3}	—	—	—	172 ± 2
$2{\cdot}5 \times 10^{-3}$	—	216 ± 4	220 ± 12	—
10^{-3}	144 ± 5	—	—	—
5×10^{-4}	—	—	—	158 ± 2
$2{\cdot}5 \times 10^{-4}$	—	179 ± 2	196 ± 4	—
10^{-4}	138 ± 2	—	—	—
5×10^{-5}	—	—	—	155 ± 3
$2{\cdot}5 \times 10^{-5}$	—	170 ± 3	178 ± 6	—
10^{-5}	140 ± 3	—	—	—
5×10^{-6}	—	—	—	164 ± 2
$2{\cdot}5 \times 10^{-6}$	—	178 ± 2	172 ± 9	—
10^{-6}	141 ± 2	—	—	—

The values represent the percentage increase in the absorbances of the solutions after heating for 15 min at various temperatures.

induced by high concentrations. Further experiments showed that salicylate and ibufenac caused a rapid release of protein from gut and stomach tissue suspended in oxygenated Ringer–Tyrode solution. However, it was not possible to determine the cellular source of this protein.

The drugs stabilized albumin against thermal denaturation at the lower concentrations but rapidly increased the rate of denaturation at high concentrations (see Table 3). It is possible that a denaturing action by the drugs on lysosomal membrane proteins may be responsible for the lytic actions of these drugs at high concentrations. Cortisol stabilized lysosomes over a concentration range of 10^{-3}–10^{-8}M but induced lysis at 10^{-2}M. The stabilizing action of cortisol was abolished in the presence of salicylate. At a concentration of 10^{-2}M some cortisol was taken up by the lysosome, despite the presence of salicylate, since some stability was conferred on the organelle. It would appear that salicylate blocked the uptake of cortisol at the lower concentrations. At a cortisol concentration of 10^{-2}M sufficient cortisol was taken up by the lysosome to stabilize it but insufficient to induce lysis.

This result may have some clinical interest since salicylates and steroids are occasionally used together in the treatment of rheumatoid arthritis.

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