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Stabilising action of anti-inflammatory steroids on lysosomes

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STRONG evidence has implicated lysosomes as mediators of inflammation.[1] Weissmann *et al.*[2–5] have shown that glucocorticoids have a stabilising action on lysosomes and this probably accounts, at least in part, for the anti-inflammatory action of these compounds. A large number of steroids have been synthesised in recent years and several of these compounds have been shown to have a great anti-inflammatory potency on a weight basis than the naturally occurring hormones. In this investigation a number of synthetic steroids have been investigated for possible stabilising action on lysosomes.

Experimental

Lysosomes were prepared from livers excised from freshly killed rabbits.[5] The rabbits were deprived of food overnight prior to killing. The lysosomes were suspended in 0·05M Tris-acetate buffer (pH 7·4)

TABLE 1. EFFECT OF STEROIDS ON RELEASE OF ACID PHOSPHATASE AND β-GLUCURONIDASE FROM LYSOSOMES

Steroid added	% Release of enzyme compared with control. The mean values of four experiments are given ± S.D. Acid phosphatase	β-Glucuronidase
Cortisol	88·0 ± 3·5	92·4 ± 0·7
Cortisol acetate	82·5 ± 1·8	88·0 ± 3·4
Cortisone	92·2 ± 2·0	94·1 ± 1·5
Cortisone acetate	89·3 ± 3·2	91·4 ± 2·9
Corticosterone*	89·9 ± 3·5	94·4 ± 1·9
Corticosterone acetate	85·8 ± 4·1	93·0 ± 1·6
Dexamethasone	88·4 ± 1·9	94·3 ± 1·8
Etiocholanolone	108·8 ± 1·8	113·7 ± 4·6
9α-Fluorocortisol	83·1 ± 3·7	91·3 ± 1·8
9α-Fluoroprednisolone	81·2 ± 3·9	90·3 ± 1·2
Fluoxymesterone	88·0 ± 2·2	91·6 ± 1·3
β-Methasone alcohol	89·8 ± 4·5	93·5 ± 2·5
Methylprednisolone acetate	87·7 ± 2·3	96·1 ± 0·9
Prednisolone	86·5 ± 2·3	88·9 ± 2·5
Prednisolone stearoyl glycolate	80·8 ± 6·7	89·2 ± 3·5
Prednisone	90·9 ± 1·9	92·1 ± 2·0
Triamcinolone acetonide	88·8 ± 5·8	92·9 ± 2·3

*Weissmann[4] showed stabilisation with corticosterone at 20° but lysis at 37°.

-sucrose (0·25M) and the protein concentration determined.[6] In previous reports steroids have been added to lysosome suspensions in solution, or in suspension e.g. in 1,4-dioxan,[4] or ethanol.[4] In preliminary experiments we have found that ethanol, propylene glycol and 1,4-dioxan have a lytic action on lysosomes. In this investigation a satisfactory procedure was devised in which the steroid was dissolved in 1,4-dioxan and added to a 50 ml stoppered conical flask, The 1,4-dioxan was removed by evaporation under reduced pressure leaving the steroid deposited as a thin film. In the control flasks the steroid was omitted. The lysosome suspension (5 ml) was added to give a final steroid concentration

of 5×10^{-4} M. The flasks were incubated in a shaking Reaction Incubator (Gallenkamp) for 90 min at 37°. The lysosomes were removed by centrifuging at 20,000 *g* for 20 min in a Beckman Model L2 Ultracentrifuge. The supernatants were examined for the presence of acid phosphatase (EC 3.1.3.2 orthophosphoric monoester phosphohydrolase) and β-glucuronidase (EC 3.2.1.31β-D-glucuronide glucuronohydrolase). Acid phosphatase was determined by incubating 0·1 ml of the supernatant with 0·5 ml of *p*-nitrophenyl phosphate[7] (0·015M) (Sigma) and 0·5 ml of citrate buffer (0·09M) pH 4·8, for 30 min at 37°. The reaction was stopped by the addition of 5 ml of 0·1M sodium hydroxide and the liberated *p*-nitrophenol determined at 410 mμ. β-Glucuronidase was determined by incubating 0·1 ml of the supernatant with 0·5 ml of phenolphthalein glucuronide[8] (Sigma) (0·0015M) and 0·5 ml of acetate buffer (0·2M) pH 4·5,[8] for 30 min at 37°. The reaction was stopped by the addition of 5 ml of glycine buffer (0·2M) pH 10·4. The amount of phenolphthalein liberated was determined at 540 mμ.

The "total" acid phosphatase and β-glucuronidase activity of the lysosomes was determined by freezing and thawing a sample of the lysosome suspension six times. The lysosome debris was removed by centrifuging and the acid phosphatase and β-glucuronidase activity of the supernatants determined.

Results and discussion

The results in Table 1 compare the percentage release of acid phosphatase and β-glucuronidase from lysosomes in the presence of steroids. Some naturally occurring steroid hormones have been included for comparison with the synthetic steroids. Etiocholanolone, a steroid with a lytic action on lysosomes, has also been included. Steroids have been listed by their generic names for convenience. The control values have been fixed arbitrarily at 100 per cent and values below this figure represent a protective action by the steroids. Values greater than 100 per cent represent a lytic action. "Total" activity values were found to be 213 per cent for acid phosphatase and 185 per cent for β-glucuronidase. The protein concentration of the final suspension, using bovine serum albumin as a standard, was 5·31 ± 0·6 mg/ml. The results clearly show that the synthetic anti-inflammatory steroids share the common property with the naturally occurring hormones of stabilising lysosomes. The list extends considerably the number of anti-inflammatory steroids known to have a stabilising action on lysosomes. It also provides further evidence that an important property of the steroids is the protective action of these compounds on lysosomes.

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