The uptake of anti-inflammatory steroids by lysosomes

Recently, Lewis, Symons & Ancill (1970) showed that the concentration of steroids was a critical factor in their action on lysosomes, and at high concentrations (10^{-3}M) the stabilizing action of the steroids was lost. It was assumed that at the higher concentrations a structural change was produced in the membrane. We now report the uptake of cortisol and cortisone by our sub-cellular preparation.

Lysosome suspensions in 0·05M tris-acetate buffer (pH 7·4) sucrose (0·25M) were prepared from rabbit liver (Symons, Lewis & Ancill, 1969) (1 ml ≡ 1 g of liver) and 1·5 ml portions transferred to dialysis bags, which were placed in stoppered test tubes containing 10 ml of [^{3}H]labelled steroid solutions prepared in the same sucrose buffer. The steroid was omitted in some tubes and in others the lysosome suspension was replaced by the sucrose-buffer. The tubes were rotated at 1 rev/min for 4 h at 37°. After 4 h, the observed time for maximum values, portions (0·1 ml) were removed from the dialysis bags and added to 15 ml of scintillation fluid [0·1 g 1,4-di-2-(4-methyl-5-phenyloxazolyl)-benzene; 5 g 2,5-diphenyloxazole in 1 litre of toluene and 500 ml methanol]. After being corrected for quenching, the amount of steroid taken up by the organelles was calculated after subtracting control values, and related to the protein concentration (Lowry, Rosebrough & others, 1951). Free

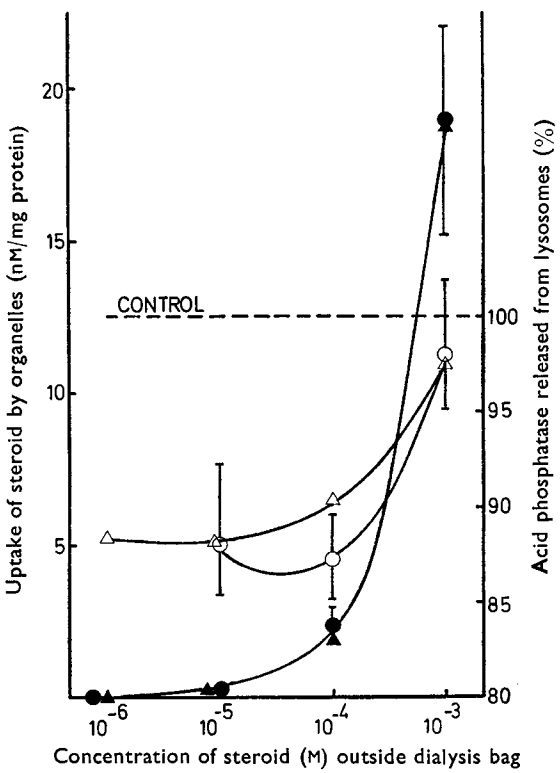

FIG. 1. The effect of cortisone and cortisol concentration on the uptake of the steroids by the organelles in our homogenate and the release of acid phosphatase from lysosomes.

Cortisone results are indicated with triangles and cortisol with circles. Open symbols represent enzyme release, and closed symbols uptake. Acid phosphatase released in the presence of the steroid has been expressed as % of that released in control experiments without steroid. In six experiments the enzyme released in controls was 49·3% ± 4·2% (s.d.) of the "total" released by freezing and thawing the homogenate four times. Each point is the mean of three experiments. Ranges are shown for cortisol results.

acid phosphatase was measured in the supernatant obtained from centrifuging the contents of the dialysis bag at 20 000 *g* for 20 min at 4° (Symons & others, 1969).

The subcellular fraction concentrated the steroids from the surrounding medium and the amount of steroid taken up was proportional to the steroid concentration of the medium (Fig. 1). Also, it seems that our previous assumption was correct in that a high concentration of steroid within the lysosome led to a loss of stability of its membrane and higher levels of free acid phosphatase. At maximum values the amount of steroid taken up by the fraction was equivalent to 0·1 mg/g fresh liver.

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A comparison of the β-adrenoceptor stimulant properties of salbutamol, orciprenaline and soterenol with those of isoprenaline

The discovery of different structure-activity relations for the actions of catechol-ethanolamines at β-adrenoceptors in different tissues led Lands and co-workers (Lands, Arnold & others, 1967; Lands, Luduena & Buzzo, 1967) to suggest the existence of two types of β-receptors, namely β_1 and β_2. In the present work we have examined the activities of four β-adrenoceptor agonists on tissues thought to contain β_1 or β_2 adrenoceptors. The drugs used were isoprenaline, orciprenaline, salbutamol and soterenol.

Brief details of experimental methods are given in Table 1. Full dose-response curves were obtained for isoprenaline and for one of the other drugs on each preparation. The activities of the drugs were compared at 50% of the maximum effect or, if this was not possible, at suitable equi-effective dose-levels.

The dose-ratios for the β-adrenoceptor agonists compared with isoprenaline are given in Table 1. As shown in the Table the activities of the four drugs on guinea-pig ileum and colon have not been included because it was found that a major proportion of the response to isoprenaline is mediated through stimulation of α-receptors (Farmer & Levy, 1970b).

Salbutamol was selective in its actions, being much more active at β–2 than at β–1 receptors. The difference in the mean dose-ratios for activity in the β–1 and β–2 groups of adrenoceptors was highly significant ($P = 0{\cdot}002$). Some separation of effects in the β–1 group is indicated in the relatively greater action of salbutamol on rate than on force in isolated rat atria. The mean of the activities for orciprenaline at β–2 receptors was seven times greater than at β–1 receptors and this difference was just significant ($P = 0{\cdot}015$). Soterenol had high activity at β–2 adrenoceptors