Steroid Binding and Stabilization of Glucocorticoid-Receptor Proteins from Rat Thymus

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Equilibrium and kinetic studies of glucocorticoid binding to rat thymus cells *in vitro* have demonstrated an obligatory role for binding to specific receptors before initiation of the effects of active steroids on glucose transport (Munck & Brinck-Johnsen, 1968; Munck & Wira, 1971). Nuclear and cytosol receptors can be extracted from cells with or without cortisol pretreatment by disruption by hypo-osmotic shock in 1.5 mM-MgCl₂; these receptors are at least in part proteins (Wira & Munck, 1970; Munck & Wira, 1971). We now report further studies of the cytosol receptors isolated without cortisol pretreatment of cells, using a dextran-charcoal assay specific for saturable binding of glucocorticoids to receptors.

The time-constant for dissociation of [³H]cortisol from the cytosol receptor at 37°C, 2.3 min, is similar to that characteristic of specifically bound cortisol in intact cells. At 3°C dissociation is slower, with a time-constant of 65 min. Measurement of maximum binding at a range of cortisol concentrations gives an apparent association constant of $1.7 \times 10^7 \text{ M}^{-1}$ at 3°C. Cortisol, dexamethasone and triamcinolone acetonide all compete with cortisol for receptor sites; both dexamethasone and triamcinolone acetonide are bound more strongly than cortisol. Cortisone does not compete. The time-constants for dissociation at 3°C are 30 min for cortisol, 322 min for dexamethasone and more than 12 h for triamcinolone acetonide.

Association of 0.02 μM -[³H]cortisol with the cytosol receptor at 3°C reaches a maximum by 90 min, but declines to 29% of the maximum by 8 h. With saturating concentrations of [³H]cortisol maximum binding is still retained at 8 h, and can be displaced by addition of an excess of non-radioactive cortisol. In the absence of steroid the receptor is rapidly inactivated, but can be partially stabilized by addition of 2.5 mM-EDTA.

With 0.02 μM -[³H]dexamethasone maximum binding is not attained until 3–4 h, and 66% of the maximum remains bound at 8 h; with 0.02 μM -[³H]triamcinolone acetonide 100% of maximum binding is retained at 8 h. We conclude that the steroid-receptor complex is stable, but that the receptor is rapidly inactivated in the absence of steroid, and that the apparent increased stabilization observed with dexamethasone and triamcinolone acetonide reflects their inability to respond by rapid

dissociation to disturbances of the equilibrium caused by inactivation of the free receptor.

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Determination of Oestradiol-17 β and Oestrone in Human Plasma

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Many hormones are bound in their target tissues by specific proteins before the expression of biological activity. These proteins can be utilized as analytical tools. For example, an oestrogen-binding 'protein' from rabbit uterus has been used in the determination of oestradiol-17 β in human plasma (Korenman *et al.*, 1969; Corker & Exley, 1970).

We report here our experiences with this oestrogen-binding 'protein' for the determination of oestrone and oestradiol-17 β in human plasma.

The method comprises: (i) addition of [³H]oestrone and [³H]oestradiol-17 β to the plasma to permit determination of manipulative losses; (ii) extraction of the plasma with ether; (iii) separation of oestrone, oestradiol-17 β and other steroids by t.l.c. on silica gel; (iv) reduction of the isolated oestrone fraction to oestradiol-17 β and rechromatography; (v) the separate determination, by competitive protein-binding assay (Korenman *et al.*, 1969), of the oestradiol-17 β fraction from the plasma and that derived from oestrone by reduction.

Recovery of added [³H]oestradiol-17 β was $76.9 \pm 5\%$ (mean $\pm$ S.D., $n = 40$) and that of [³H]oestrone was $45.0 \pm 9.3\%$ ($n = 39$).

Blanks (5 ml of tap water) were $5.5 \pm 5.6 \text{ pg}$ ($n = 23$) for oestradiol-17 β and $5.4 \pm 7.1 \text{ pg}$ ($n = 17$) for oestrone.

Precision was determined on a pool of male plasma and on samples of female plasma. The coefficient of variation (Snedecor, 1952) at the male plasma concentration was 29% for oestradiol-17 β ($n = 18$) and 30% for oestrone ($n = 13$). At concentrations above 5 ng/100 ml of plasma the coefficient of variation was 13% for oestradiol-17 β ($n = 18$) and 16% for oestrone ($n = 7$).

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