Four Classes of Steroid Identified by their Interaction with Mineralocorticoid Receptors in the Toad Bladder

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Aldosterone stimulates sodium transport in the toad bladder (Crabbé, 1961; Sharp & Leaf, 1964; Porter & Edelman, 1964). It is thought to act on the cell nucleus, stimulating specific protein synthesis (Williamson, 1963; Edelman, Bogoroch & Porter, 1963). More recently specific mineralocorticoid-receptor complexes have been isolated from toad bladder mucosal-cell nuclei (Alberti & Sharp, 1968).

Steroids have been classified into three types by virtue of their mineralocorticoid effects. These are: active steroids, inactive steroids and competitive inhibitors. The present study defines these three types and describes a fourth type of interaction. Aldosterone and hydrocortisone, testosterone, cortisone, and cortexolone have been used as model steroids.

The stimulation of sodium transport by steroids in toad bladder was measured in Lucite (polymethyl methacrylate) double chambers by the short-circuit current technique (Ussing & Zerahn, 1951). Steroid-receptor interactions were studied by isolating radioactively labelled steroid-protein complexes from toad bladder mucosal-cell nuclei after incubation of the tissue with [^3H]aldosterone, [^3H]hydrocortisone or [^3H]cortisone alone or with other unlabelled steroids.

The results define four types of steroid interaction with mineralocorticoid receptors. First, aldosterone and hydrocortisone combined with the specific receptors and stimulated sodium transport. Secondly, steroids such as testosterone did not bind to the receptors (as tested by their ability to displace [^3H]aldosterone from the binding sites). They have no effect on sodium transport and did not inhibit aldosterone. Thirdly, a class of compounds typified by the spirolactones, which bound to the receptors, had no effect on sodium transport alone but inhibited sodium transport stimulated by aldosterone. Cortisone was found to belong to this group, inhibiting aldosterone-stimulated sodium transport at a competition ratio of 500:1. It displaced bound [^3H]aldosterone when present in 1000-fold excess, but not at 100-fold excess. Fourthly, a new class, illustrated by cortexolone, which bound to the mineralocorticoid receptors, weakly stimulated sodium transport alone at $10\mu\text{M}$ but then inhibited the action of $0.02\mu\text{M}$ -aldosterone. A 1000-fold excess of cortexolone,

like cortisone, displaced bound aldosterone, but no displacement was found with 100-fold excess.

The structure-activity relationships of these four classes may increase our understanding of the mechanism of action of steroids.

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Studies on Oestradiol Receptors in Uterus

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The cytoplasmic '8s' receptor of the rat uterus is reversibly dissociated into '6s' and '4s' protein entities, according to the ionic strength, in the presence or the absence of oestradiol. The 'nuclear' '4-5s' receptor cannot be reverted back to an '8s' configuration. Its formation depends on the presence of the cytoplasmic receptor and a soluble heat-sensitive nuclear factor extracted by 0.5M-KCl from uterus nuclei. This '4-5s' nuclear receptor can be obtained during incubation *in vitro* of uterine tissue even without oestradiol (whereas it is not formed *in vivo* in animals deprived of the hormone).

In purified nuclei obtained from calf uterus, not having been exposed to oestradiol, preliminary results indicate the possibility of a specific protein binding system of very high affinity for oestradiol (K_A approx. 10^{11}M^{-1}), very few number of sites per cell and small size. It is found in the acidic protein fraction of a chromatin preparation.

Effects of Oestrone and Oestradiol on Rat and Rabbit Uterus in Organ Culture

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Studies on blood production rates and secretion rates of the sex hormones and their precursors suggest that steroid metabolism within the target tissue may be a mechanism for the local regulation of hormonal potency (Baird, Horton, Longcope