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

By K. G. M. M. ALBERTI and G. W. G. SHARP.
(Departments of Medicine and Pharmacology,
Harvard Medical School and Massachusetts General
Hospital, Boston, Mass., U.S.A., and Nuffield
Department of Medicine, Radcliffe Infirmary, Oxford)

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 (poly-methyl 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

By HENRI ROCHEFORT, AUDREY ALBERGA, HÉLÈNE TRUONG and ETIENNE-EMILE BAULIEU (introduced by K. GRIFFITHS). (*Lab Hormones, 94-Bicêtre, France*)

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

By SUSAN L. RUSSELL, B. R. D. MACDOUGALL and G. H. THOMAS (introduced by J. M. CROCKER). (*Department of Anatomy, University of Birmingham*)

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

& Tait, 1968). The present work was undertaken to show that the biological effects of oestrone on rat and rabbit uterus *in vitro* can be related to the ability of the tissue to convert oestrone into oestradiol.

The effects of oestrone and oestradiol on rat and rabbit uterus were assessed initially by examining the histology of explants cultured for 3 days in Eagle's medium at 37° with a gas phase of N₂ + O₂ + CO₂ (25:70:5). The most sensitive indicator of oestrogenic action under these conditions was the state of the epithelial cells of the glands and lumen. Oestradiol was able to maintain the structure of both rat and rabbit uterus, 0.1 µM being the optimum concentration of hormone in the medium. Oestrone (1.0 µM) was as effective as oestradiol in maintaining the uterus of the rabbit.

These conclusions were supported by results from a second set of experiments in which the biochemical viability of the explants was determined by their ability to incorporate labelled glucose into acid-soluble nucleotides, lipids, RNA, DNA and proteins. Both oestrone and oestradiol caused significant stimulation in the glucose metabolism of rabbit uterus cultured for 3 days, compared with material cultured in the absence of oestrogen. In the rat only oestradiol had a stimulatory effect on glucose metabolism; there was no significant difference between material cultured with oestrone and in the absence of hormone.

This species difference in the response of uterine tissue to oestrone can be related to the presence of oestrone 17β-reductase in rabbit uterus but not that of rat (Macartney & Thomas, 1969). Analysis of the labelled steroids in explants cultured in the presence of [4-¹⁴C]oestrone and [4-¹⁴C]oestradiol indicates that the biological effects of oestrone are derived from its conversion into oestradiol in the rabbit uterus.

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Effects of Thyroid Hormones on Oxidative Phosphorylation and on 11β-Hydroxylation of Ox Adrenal-Cortex Mitochondria

By R. MICHEL, J. BOUENIK and O. MICHEL.
(*Endocrinologie, Faculté de Pharmacie and Biochimie, Collège de France, Paris, France*)

Previous studies showed that 2,4-dinitrophenol uncouples oxidative phosphorylation and decreases corticosterone synthesis from deoxycorticosterone by adrenal-cortex mitochondria (Guerra, Péron

& McCarthy, 1966). Thyroid hormones exhibited uncoupling properties on mitochondria from several tissues (Hoch, 1966; Michel & Leblanc, 1969), but, in contrast with 2,4-dinitrophenol, whose inhibitory doses in state 3 are higher than those that stimulate state 4, their opposite effects were obtained with the same doses. Our work was performed in order to study the effects of thyroid compounds on the functions of adrenal-cortex mitochondria.

The ox particles were prepared as described by Cammer & Estabrook (1967). Iodothyronines affected the oxidative phosphorylation of adrenal-cortex mitochondria in the same manner as those of other organs; with succinate or malate in state 4 the respiration increased at low doses but decreased at high doses. In state 3 the inhibition was observed at any concentration. Thyroid hormones also decreased the rate of oxygen consumption in the presence of deoxycorticosterone and simultaneously lowered the synthesis of corticosterone. In the same conditions 2,4-dinitrophenol increased respiration but lowered the 11β-hydroxylation. The respiratory action of L-thyroxine was dependent on the substrate concentration, but the responses differed in the presence of both ADP and deoxycorticosterone. The plot of the reciprocal of the oxygen consumption rate against the reciprocal of the substrate concentration showed that the curves obtained with or without L-thyroxine were straight lines converging on the ordinates with deoxycorticosterone and on the abscissa with ADP.

It was known that the steroid conversion required NADPH. In one hypothesis Purvis, Battu & Péron (1968) considered that the energy would be generated by a transhydrogenase. The results we obtained with 2,4-dinitrophenol and L-thyroxine may support this suggestion. However, according to another hypothesis (Simpson & Estabrook, 1969), 2,4-dinitrophenol would prevent NADPH synthesis by inhibiting the mitochondrial 'malic' enzyme. The quantitative experiments performed with different substrate concentrations showed that inhibition by L-thyroxine was in accordance with the Michaelis law with both ADP and deoxycorticosterone. The effect was uncompetitive with the phosphate acceptor but competitive with the steroid. The inhibition of corticosterone synthesis probably takes place mainly on the mitochondrial 'malic' enzyme, whereas the action of thyroid hormones on oxidative phosphorylation seems to be more complex.

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