36. Cytoplasmic and nuclear aldosterone receptors in frog skin, M. MOGUILEWSKY and J. P. RAYNAUD, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

The nuclear transfer of a cytoplasmic aldosterone–receptor complex has been shown to be a step in the regulation of sodium transport in the adrenalectomized rat kidney, but it is not known as yet whether sodium transport in the frog skin is regulated *via* a similar mechanism. In the present experiments, aldosterone-specific cytoplasmic and nuclear receptors have been identified by density gradient ultracentrifugation in the ventral skin of the frog (Rana esculenta). The aldosterone–receptor cytoplasmic complexes sediment in the 4–5S and 9–10S zones of a 5–35% linear glycerol gradient. Binding measured by the Dextran-coated charcoal adsorption technique is characterized by high affinity ($1/K = 0 \cdot N\ 10^{-9}$ M) and a limited number of binding sites ($N = 0{\cdot}4 \times 10^{-9}$/mg protein) and is highly specific of mineralo- and antimineralo-corticoids. The affinity of this receptor for deoxycorticosterone and progesterone is 2 to 3 times less; for cortisol, testosterone, canrenone and 9α-fluorocortisol, 10 to 100 times less. It has no affinity for either estradiol nor the highly active glucocorticoid, dexamethasone. The nuclear aldosterone receptor complex which sediments in the 3–4S zone of a 5–20% linear sucrose gradient (0·4 M KCl) has the same hormonal specificity; competition curves obtained with the above steroids in studies on the nuclear uptake of tritiated aldosterone (0·5 nM) at 25°C using crude nuclei from mucosal cells ($800\,g \times 10'$ pellet) are entirely analogous to the cytoplasmic competition curves. Further investigation of the relation between the cytoplasmic and nuclear complexes in frog skin might enable the molecular sequence of aldosterone action to be determined in a tissue, specific of mineralocorticoids, and which, moreover, is one of the simplest existing systems for the *in vitro* study of sodium transport (Ussing and Zerahn test).

37. Androgen receptors in rat testicular cytosol after perinatal treatment with HCG and an antiandrogen SCH 13521, W. KLEMM and D. GUPTA, Department of Diagnostic Endocrinology, University Children's Hospital, 74 Tübingen, Germany

Recent investigations indicated that testosterone may not only be the organizer of androgen-dependent foetal sexual differentiation, but that it also continues to play decisive role in the masculine differentiation of the hypothalamus during the first five days of life in the rat. Administration of antiserum to testosterone-3-BSA to male rats during this period of life has been seen to reduce the plasma testosterone profile as well as the sexual behaviour pattern in these animals when matured. In order to study the effect of altered circulating levels of testosterone, either increasing it with HCG or reducing it by means of a new non-steroidal antiandrogen SCH 13521 (Neri & Monahan, *Invest. Urol.* **10** (1972) 123, during this critical period of rat life, the available amount of free androgen binding sites in cytosol of rat testicular tissues was measured after a post-administration period of 14 days. Two-day-old male rats were injected either with 50 IU HCG or 5 mg SCH 13521, and sacrified on day 16 when the androgen levels are low in normal animals. Testes were removed and homogenized in TEM buffer with glycerol. A 105000 g supernatant was used as cytosol. The cytosol was incubated with labelled DHT. After incubation the free and non-specific bound DHT was removed by Dextran-coated charcoal in 16% ethanol according to Boesel *et al.* (*Biochem. biophys. Res. Commun.* **61**, (1974) 1004. In normal rats the available specific binding activity was found to be 0·25 pmol/0·2 ml supernatant. In the HCG treated animals this level was found to be lower. The animals treated with antiandrogen SCH 13521, on the other hand, showed lower testicular weight and a five times higher value for available specific binding activity found in relation to the normals. These data demonstrate whether with gonadotrophins or with antiandrogen given at the critical period of life in the male rat the production of androgens is irreversibly altered as shown by the available amount of free receptors, thereby influencing the function of the testes.

38. Molecular binding sites of the mineralocorticoids to their cytosolic receptor proteins, P. GENARD, M. PALEM-VLIERS and H. VAN CAUWENBERGE, Dept. de Clinique et de Pathologie Médicales (Professeur H. Van Cauwenberge), Hôpital de Bavière, Université de Liège, Liege, Bélgique

Three important factors influencing the steroid cellular receptors interaction: molecular conformation, electron density and polarizability of the bonds have been studied by means of nuclear magnetic resonance of ^{13}C, ^{1}H and ^{19}F on a set of natural and synthetic corticoids. Those data were compared with the dissociation constant of the complex between a steroid and its mineralocorticoid cytosolic protein receptor from the rat kidney. Our findings can be summarized as follows: (1) the high affinity of natural compounds as DOC and aldosterone is strongly dependent of the conformation and the electronic structures of the $CO\text{-}CH_2OH$ side chain at carbon 17. (2) cortisol and 9αF-cortisol have a very similar molecular conformation; however changes in electron density and polarization of the C-H are found for the carbon atoms α, β and γ to the fluorine. (3) the weaker binding of 9α-fluoro-corticoids substituted at C_{16} correspond principally to modifications of conformation and electronic structures at the ring D; the molecular active site centered on the fluorine atom at C_9 is not modified.

39. Demonstration and partial characterization of cytosol receptors for testosterone in rat kidney, submaxillary gland and skeletal muscle, AKE POUSETTE and JAN-AKE GUSTAFSSON, Department of Chemistry and Department of Germfree Research, Karolinska Institutet, S-104 01 Stockholm 60, Sweden.

Androgen uptake was investigated in several peripheral organs after administration of [1,2,6,7-^{3}H]-testosterone to castrated male rats. The animals were killed after 30 min, the organs were taken out and the radioactivity determined after tissue combustion. A relatively high accumulation of androgen was found in pancreas, adrenals, spleen, thigh muscle, kidneys and liver in addition to the classical androgen target organs, coagulation glands, seminal vesicles, prostate, preputial glands and harderian glands.

In a second series of experiments, nuclear and cytosol fractions were prepared from prostate, seminal vesicles, coagulation glands, preputial glands, spleen, submaxillary glands, kidneys and pancreas from castrated male rats given [1,2,6,7-^{3}H]-testosterone, and these fractions were then characterized by thin-layer and radio-gas chromatography with respect to their patterns of labelled steroids. Only prostate and seminal vesicles were found to contain significant amounts of nuclear [^{3}H]-5α-dihydrotestosterone. The major nuclear androgen was [^{3}H]-testosterone that was the only detectable labelled steroid in coagulation glands, preputial glands and spleen and that constituted 70% or more of the nuclear radioactivity in seminal vesicles, submaxillary glands, kidneys and pancreas. These results indicate that