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

testosterone itself may be the predominant active androgen principle *in vivo* in most androgen target organs and that conversion to 5α-dihydrotestosterone is generally not a prerequisite for androgen activity.

Using an ultrasensitive micromodification of isoelectric focusing it was possible to show that cytosol from kidney, submaxillary gland, thigh muscle and levator ani muscle and nuclei from kidney and submaxillary gland contained androgen-binding proteins with pI:s in the region 4·6–5·1 ("4·6–5·1 Complex")' This complex also formed *in vitro* after incubation of [1,2,6,7-^{3}H]-testosterone with cytosol from kidney and submaxillary gland. [1,2,6,7-^{3}H]-Testosterone was bound with high-affinity to receptor proteins in cytosol from both kidney, submaxillary gland and thigh muscle.

On the basis of these results the hypothesis is presented that a common class of testosterone receptors is present in most organs and that these receptors can be detected both *in vivo* and *in vitro* provided methods sensitive enough are utilized.

40. Glucocorticoid-protein interactions in rat liver cytosol, ÖRJAN WRANGE, JAN CARLSTEDT-DUKE, JAN-ÅKE GUSTAFSSON and SVEN A. GUSTAFSSON, Department of Chemistry and Department of Germfree Research, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

The intracellular binding of [^{3}H]-corticosterone and [^{3}H]-dexamethasone and their metabolities to macromolecules in rat liver cytosol were studied both *in vivo* and *in vitro*. After intraperitoneal injection of [^{3}H]-corticosterone to adrenalectomized rats, the radioactivity was recovered in three major steroid-macromolecular complexes in both sexes. A marked sexual difference in radioactivity bound to protein was noted with approximately ten times more in male liver cytosol. The steroid-macromolecular complexes were characterized by gel-filtration, ion-exchange chromatography, density gradient centrifugation and isoelectric focusing. The macromolecules were characterized as: (1) a steroid disulphate-binder (Stokes radius 25 Å and sedimentation coefficient 4·1S in high ionic strength; pI 8·9); (2) transcortin and (3) a corticosterone "receptor" (Stokes radius 77 Å in high ionic strength; sedimentation coefficient >10S in low ionic strength). The corticosterone "receptor" was found to be very unstable. Approximately four times as much radioactivity was bound to the "receptor" in male than in female liver cytosol after administration of [^{3}H]-corticosterone *in vivo*. The radioactivity bound to the receptor was identified as [^{3}H]-corticosterone and [^{3}H]-5-α-dihydrocorticosterone. When studied *in vitro*, [^{3}H]-corticosterone bound only to transcortin and the "receptor".

After intraperitoneal injection of [^{3}H]-dexamethasone into adrenalectomized rats, the radioactivity was recovered in two (male) or one (female) steroid-macromolecular complex(es). The steroid-"receptor" complex was found in liver cytosol from both sexes, both *in vivo* and *in vitro*. It sedimented both at 8·5S and >10S in low ionic strength and had a sedimentation coefficient of 3·8S and a Stokes radius of 66 Å in high ionic strength.

It is speculated that both corticosterone and dexamethasone may bind to the same site of a single receptor molecule but that each steroid induces different conformational changes (Stokes radii 77 Å and 66 Å, respectively) which results in different aggregation states of the binding protein. It is suggested that use of natural corticosteroids may be preferable in studies on mechanism of action of glucocorticoids in rat liver.

I. Steroids in early pregnancy, E. MENINI*, D. MANGO† and P. SCIRPA†, *Department of Biological Chemistry and Department of Obstetrics and Gynaecology, Università Cattolica, Rome, Italy

Steroid hormones play a major role in the maintenance of pregnancy and many of the maternal adjustments and physiological adaptations which occur during this period are the result of the increases in steroids produced, first by the ovary and subsequently by the placenta.

The present review will deal, mainly, with the biosynthesis, the blood levels, the metabolism, the excretion and the significance of the steroid hormones in the initial stages of normal pregnancy, complicated pregnancies, and pharmacologically induced pregnancies.

Immediately after conception, the most dramatic changes in steroid hormones production are observed in the oestradiol-17β, progesterone and 17-hydroxyprogesterone. The site of this steroidogenesis is the corpus luteum gravidarum and there is evidence that by the 7–8th week of gestation a luteoplacental shift occurs. By this time both the corpus luteum and the placenta contribute to the production of oestradiol-17β and progesterone while 17-hydroxyprogesterone is probably being synthesized mainly by the corpus luteum.

As far as we know, the enzymic reactions involved in the formation of steroid hormones in the early stages of pregnancy and in the non-pregnant female are the same, nevertheless there are differences in the utilization of precursors by the corpus luteum and the trophoblast, specially with regard to the biosynthesis of the oestrogens.

As the placenta is relatively deficient in the enzyme 17α-hydroxylase, this organ forms oestrogens to a great extent from C_{19} steroidal compounds. On the other hand the main precursors of the oestrogens in the corpus luteum are the 17-hydroxylated steroids with 21 carbon atoms. In recent years, with the advent of accurate, sensitive and practical methodology for the measurement of steroid hormones in blood and urine, many studies have been conducted with the purpose of establishing reference values for the blood levels and the urinary excretion of the main steroids implicated in pregnancy.

Hormone assays in early pregnancy are valuable, at least from two points of view. First, they contribute to the understanding of the complex interactions among the different types of hormones during this period of life and, second, they allow, in some cases, to distinguish between values which are presumptive of normal pregnancy and those which are suggestive of associated complications.

It is now well established that during the first 5–6 weeks which follow the last menstrual period, the blood and urinary concentration of oestrogens is only slightly increased or it is not increased at all with respect to the levels that are usually found in the luteal phase of a normal menstrual cycle. Oestrogens begin to increase rapidly in coincidence with the first signs of the luteoplacental shift. 17-Hydroxyprogesterone appears to be the best biochemical marker of this event. In fact the blood concentration of this compound, prevalently of ovarian origin, increases rapidly after conception and begins to decline 5–6 weeks after the last menstrual period. The 17-hydroxyprogesterone peak probably indicates the impending luteoplacental shift.

The blood levels of progesterone in the initial stages of pregnancy do also support the concept that the luteoplacental shift takes place around the 7–8th week of gestation. In fact after an initial period of 8–10 weeks in which the levels of this hormone show a plateau or in some cases a broad peak, with values of the order of those usually found in luteal phase of a normal menstrual cycle, the blood concentration of progesterone steadily