

sol and nuclei was investigated. Kinetic analysis of cytosol revealed the presence of specific binding sites with a K_D of 11×10^{-9} M and a receptor site concentration of 0.32 pmol/ng protein. The complex was labile at a temperature of 45 °C. The specificity was indicated in a competition study using unlabelled competing steroids at 10, 100, 1000-times the molar concentration of radioactive hormone. The inhibition was similar for both cold testosterone and DHT (80%), methyltestosterone (66%), androstenediol (64%), DHA (54%), androstenedione (42%), E_2 (40%), E_3 (3%). The specific binding protein has a sedimentation coefficient of 5S. Purified nuclei or whole tissue incubated with [3 H]-T or [3 H]-DHT at 35 °C for 1 h gives a nuclear radioactivity that is completely inhibited by cold competitor and with approximately the same nuclear binding sites/DNA respectively for [3 H]-T and [3 H]-DHT. Our data support the presence of an androgen receptor in human placental cytosol the role of which needs further investigation.

77. Estrogen receptors in lactating mammary gland of the rat

MEHTA, R. G., PARK, D. C., BOYD, P. A. and WITTLIFF, J. L., Department of Biochemistry, University of Louisville Medical School, Louisville, KY, U.S.A.

Kinetic and molecular properties of estradiol receptor (ER) in cytosol were characterized by titration analyses, DNA-cellulose binding, ion-exchange chromatography and density gradient centrifugation. The rate constants for association and dissociation at 0 °C were $2-3 \times 10^{-7}$ M $^{-1}$ min $^{-1}$ and $2-4 \times 10^{-3}$ min $^{-1}$, respectively. These data and those of Scatchard analyses indicated binding sites with high affinity ($K_D = 10^{-10}$ M). Only estrogenic compounds with 3 and $^{17\beta}$ hydroxyl functions, as well as unsaturation of A ring, were bound. The 8S ER complex chromatographed as 2 components on DEAE-cellulose columns. ER binding to DNA-cellulose was increased significantly if charged receptor complex was warmed at 28 °C for 30 min; whereas the presence of 1 mM EDTA during activation reduced binding to DNA-cellulose by 40-50%, an effect that can be reversed by divalent cations. Activation of charged ER was a prerequisite for the translocation of ER into nuclei. Activated ER stimulated Mg $^{2+}$ dependent RNA polymerase activity 3-fold without altering Mn $^{2+}$ dependent activity. These results support the notion that activation of steroid receptors is essential for their translocation into nuclei and subsequent stimulation of nuclear synthetic activity.

78. Androgen and progesterone binding in human testis cytosol

STOA, K. F., DAHL, O., HEKIM, N. and HOISAETER, P. A., Hormone Laboratory and Departments of Oncology and Surgery, University of Bergen School of Medicine, Bergen, Norway

The present report describes an attempt to detect, characterize and measure in human testis cytosol proteins corresponding to the androgen receptor which has been found to be present in the testis of hypophysectomized rats. The methods used have been exchange assay with [3 H]-methyltrienolone ([3 H]-R 1881), studies of dissociation rates and heat sensitivity, as well as sucrose gradient centrifugation. In normal human testis cytosol, a concentration of 119 ± 59 fmol/mg protein receptor-like androgen-binding activity, was found. In only two out of five tumour specimens (seminomas) a relatively low (23-33 fmol/mg protein) exchange activity could be detected. Receptor affinities for R 1881 and testosterone were found to be about equally high, whereas the affinity for progesterone was slightly

lower. There was only negligible binding inhibition by oestradiol. Gradient centrifugation revealed two peaks, corresponding to 3S and 4S, respectively. Progesterone affinity could be demonstrated solely for the 4S peak. The results indicate that two types of high affinity binding proteins are present in human testicular cytosol, one with affinity for both androgens and progestins, and the other with specific androgen-binding activity.

79. Estrogen induction and functional importance of carrier proteins for riboflavin and thiamine in the rat during gestation

ADIGA, P. R. and MUNIYAPPA, K., Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

During gestation in higher animals thiamine (B_1) and riboflavin (B_2) are preferentially transported across the placental barrier and concentrated by the foetus. The mechanisms of such facilitated transport are unknown. We have shown in the hen that vitamin transport for the embryonic development is mediated through B_1 and B_2 carrier proteins. We report here that in the pregnant rat (but not in the male or immature female) similar proteins with immunological cross reactivities to chicken B_1 and B_2 carrier proteins do exist. The hormone responsible for induction of these proteins was estrogen (E) since (1) specific induction of these proteins in the male and in ovariectomized adult female rats could be elicited by E (2) the blood levels of these proteins alter in concert with the changing E in cycling female and pregnant rats. Passive immunization of pregnant rats (4-16 d) with antibodies to chicken vitamin carrier proteins (but not to ovalbumin) resulted in foetal resorption/abortion showing functional importance of carrier proteins for embryonic development and survival.

80. Influence of prolactin on testosterone production and action in the male rat

PRASAD, M. S. K., Department of Biochemistry, Indian Institute of Science, Bangalore, India

Prolactin (PRL) facilitates testosterone (T) action on male accessory sex organs of rodents, but the mechanism is not clear. A temporal relationship between circulatory levels of PRL and T in developing rats was observed. In 90-day old rats, neutralization (for 5 days) of PRL by specific antiserum (A/S) significantly reduced the serum T and weights of ventral prostate and seminal vesicles, whereas injection of PRL (1 mg/100 g.b.wt. \times 5d) to such rats significantly enhanced serum T levels and the weights of the accessory organs. However, their testes responded to saturating levels of LH to a similar extent in terms of T production *in vitro*. Injection of PRL enhanced the ability of the prostate and seminal vesicles to concentrate [3 H]-T *in vitro*, whilst A/S treatment had no significant influence. PRL treatment enhanced the binding of [125 I]-LH to testicular membrane preparations whilst A/S was without any effect. Neither PRL nor A/S modulated the binding of [125 I]-PRL to membranes.

81. Protein induction and estrogenic potency

VON SCHOULTZ, B., DAMBER, M.-G., SANDSTRÖM, B. and STIGBRAND, T., Department of Obstetrics and Gynecology and Physiological Chemistry, University of Umeå, S-901 87 Umeå, Sweden

Estrogen treatment has become more frequent during the last years. There are few methods to quantify and compare the estrogenic effect of various preparations. Animal