

stantiated by their differing ability to activate RNA polymerases and replenish the cytosolic estradiol and progesterone receptors. Both estrogens, estradiol and moxestrol, increase the number of polymerase molecules involved in transcription and also increase chain elongation, whereas the antiestrogen, RU 16117 would appear to have an inhibitory effect.

11. Microfluorimetric method to detect specific oestradiol receptors in different cell types or cell suspensions from target tissues, D. BECCATI, G. LANZA, I. NENCI and A. PIFFANELLI, Istituto di Anatomia Patologica, Università di Ferrara, 44100 Ferrara, Italy

Fluorescent antibody localization of oestradiol-17 β by immuno-fluorescence sandwich technique has been developed and applied to the demonstration and quantitation of specific oestradiol receptors in different cell types on cell suspensions from target tissues. Using this method the quantity of high affinity receptors occupied or unoccupied by endogenous oestradiol can be measured. Methodological details are given.

So far it has been possible to investigate, *in intact cells*, the temperature-dependent two-step mechanism suggested for the interaction of oestradiol with target cells, the intranuclear attachment and, lastly, the fate of oestradiol-receptor complexes.

Moreover, applying this technique to the study of cell populations from breast and endometrial tumours it is possible to differentiate the heterogeneous cell populations containing cytoplasmic receptor within a single tumour.

The quantitation of these hormone-independent mixed cell clones might be useful to predict the response of each tumour to endocrine therapy.

Defects in the transformation and subsequent translocation of cytoplasmic receptor or in its final binding to chromatin (which may well occur in the presence of abundant cytoplasmic receptors) have also been revealed in these preparations of intact cells.

D. Biosynthesis of steroid-hormone receptors, A. HUGHES, P. SZENDRO, CECILIA TERAN, JANET KIELHORN, W. SIERRALTA, G. STONE, M. LITTLE and P. W. JUNGBLUT, Max-Planck-Institut für Zellbiologie, D 294 Wilhelmshaven, Postfach 1009, Germany

The responses of tissues to steroid hormones are mediated by interaction with specific cytoplasmic receptors and subsequent transfer of the steroid-receptor complexes into the nucleus. In consequence, a depletion/replenishment cycle of cytoplasmic receptors is a common phenomenon in the course of action of every steroid hormone. This cycle has been studied for the soluble cytoplasmic estradiol receptor in rat uteri, and for both the soluble and structure-bound receptor in pig uteri [1].

Following the injection of estradiol into ovariectomized, estrogen-primed rats ($4 \times 1 \mu\text{g}$ E-2 in oil, every second day, last injection 3 days before experiment), there is a rapid fall in estrogen receptor levels (depletion), compared to control values, followed at 5–6 h after injection by a replenishment phase. Such a response follows a subcutaneous, intraperitoneal or, in the present case, an intrauterine injection of estradiol. This last technique involves injection into the uterine lumen *via* the cervixes. The bi-cornuate structure of the rat uterus allows for a treated and a control horn in the same

animal. Actinomycin D-injected 2 h earlier or together with estradiol, had no effect on the control (zero time) levels or on the initial depletion step, but definitely inhibited, although not completely, the replenishment phase. Puromycin, injected 2 h before estradiol, induced a small drop in the zero time receptor levels, but had no effect on the replenishment phase unless injected 5 h after estradiol, resulting in a temporary arrest of the replenishment phase. Both sets of experiments show that replenishment does represent resynthesis. The puromycin-induced fall in control levels further indicates that receptor synthesis persists in animals which are deprived of their main hormonal sources. This latter point was confirmed by a 3 months study of uterine estradiol receptor levels in ovariectomized or ovariectomized/hypophysectomized rats kept under controlled conditions [1]. Not only was estradiol receptor present throughout this period, there were also considerable, but irregular fluctuations in receptor levels. Similar results were obtained with ovariectomized/adrenalectomized rats.

The response of the pig uterine soluble cytoplasmic E-2 receptor to estradiol is similar to that of the rat. Following the extraction and characterisation of specific estradiol receptors from pig uterine microsomes, the existence of a biosynthetic receptor sequence became apparent [1]. Uterine microsomes, extracted with low ionic strength buffer containing estradiol, and analyzed by density gradient centrifugation and argargel electrophoresis, contain a "basic" 3-5S receptor and an "acidic" 4-5S receptor. The interrelationship of these receptor forms with the soluble cytoplasmic receptor was established following a series of experiments using the intrauterine injection technique *via* the cervical route. Ovariectomized, pre-treated pigs, with one horn disconnected from the corpus uteri (control horn), were injected with estradiol and then killed at various times after injection, when the levels of the microsomal and cytosol receptor were determined. The soluble receptor, as expected, showed an initial rapid depletion. Replenishment started about 5 h after injection. In contrast, the concentration of basic 3-5S receptor extracted from the microsomal fraction rose steeply between 60 and 90 min after the intrauterine application of estradiol. The control horn levels of all receptors are, by comparison, unchanged. The data from these and similar experiments strongly suggest that the basic 3-5S receptor is an early product of receptor biosynthesis.

This small receptor can be dimerised to a basic 4-5S molecule on warming. Both this dimer and the extracted acidic 4-5S molecule are reversibly dissociated by protonation into 3-5S basic and 3-5S acidic subunits respectively, the latter subunit probably being the authentic *in vivo* component, which dimerises during the extraction procedure [2]. The formation of stable dimers requires the presence of estradiol. Comparison of these data for the microsomal receptor with those available for the soluble cytoplasmic receptor forms shows certain similarities. Uterine cytosol extracts, extracted at pH 7.5 with low ionic strength buffer, and run in gradients containing buffered 0.4 M KCl, display only acidic receptors sedimenting at 4S and 5S. The radioactivity sedimenting at 5S can be shifted to the 4S position by protonation, the reverse shift is accomplished by proton withdrawal and accelerated by warming. Similar to the estradiol requirement for the formation of stable microsomal 4-5S dimers, stable cytosol 5S-estrogen complexes are derived from estradiol charged 4S molecules but not from estrone complexes. On the basis of such evidence, the major receptor biosynthetic pathway *in vivo* is presumed to be: basic microsomal 3-5S \rightarrow acidic microsomal 3-5S \rightarrow cytosol 4S \rightarrow cytosol 5S. The commonly observed 8–10S cytosol receptor is thought to be a storage form, which can be activated when necessary. Although the bio-