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Meeting Report

Steroid receptors in health and disease

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Steroid hormones are widely known to influence expression of the hormone-responsive genes in target cells. The action of steroid hormones is known to be mediated by their initial binding to specific 'receptor' proteins. The exact sequence of events, which follow hormone binding and lead to a cellular response, has remained unclear. This timely meeting has provided a forum for discussion of the recent advances in the field, which explore the structure and function of steroid receptors in health and disease.

Androgen; Glucocorticoid; Progesterone; Steroid; Estrogen; Hormone action; Receptor

1. INTRODUCTION

During the last two decades, progress in steroid hormone research has resulted in the development of new approaches to contraception as well as diagnosis and treatment of endocrine disorders and cancers. Although significant advances have been made in the purification, characterization, immunochemistry and molecular biology of steroid receptors, the precise molecular mechanism of steroid hormone action has remained obscure. The purpose of this international conference was to facilitate scientific exchange toward a better understanding of the mode of action of steroid hormones. The scientific sessions consisted of both poster presentations and state-of-the-art lectures, a brief summary of the latter is presented in this report.

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The conference opened with a keynote address by Jack Gorski (Madison) who provided a historic perspective of the field of steroid receptors, citing earlier works of Szego and Roberts (1953) and Gerald Mueller (1958) that depicted possible sites of hormonal regulation of induced proteins. Laboratories of Jensen and Gorski and others subsequently demonstrated the presence of specific receptor proteins for estrogen. These studies in the late 60's led to the 'translocation' or 'two-step' model of steroid hormone action. During the past few years the contention that unoccupied receptors are located in the cytoplasm has undergone modification and the nuclear localization of both ligand-free and liganded receptors has been defined in various systems.

2. MOLECULAR ORGANIZATION, RECEPTOR STRUCTURE AND MODIFICATION

Jean-Marie Gasc (Paris) presented evidence that under a variety of experimental conditions, an-

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tibodies to chick progesterone receptor (PR) reveal the presence of receptor molecules in the cell nuclei independent of receptor occupancy by ligands. It was proposed that the occurrence of PR in the cytosol could be due to a loss or leak of nuclear receptor during homogenization. In contrast, glucocorticoid receptor (GR) was revealed by immunostaining in both the cytoplasmic and the nuclear compartments. Furthermore, ligand administration led to intensification of immunostaining in the nucleus, as was also reported by Carlstedt-Duke and Brenner. Although these latter observations lend support to the nuclear translocation hypothesis, intensified nuclear staining of GR following hormone administration could also suggest that steroid binding allows a better and prolonged nuclear retention during experimental processing.

Results presented by Robert Brenner (Beaverton) on the immunocytochemical studies in the reproductive systems of control and spayed nonhuman primates also supported the concept of nuclear localization of estrogen receptor (ER) and PR. Spayed monkeys exhibited nuclear staining for ER, although the receptor was measurable in the cytoplasm by cell-free binding assays. Brenner suggested that different cell types in the target organs of the reproductive tract regulate their ER and PR levels differently, and that stromal cells may interact with epithelial cells by paracrine mechanisms during steroid hormone action.

In order to probe the molecular properties of the surface of ER, Hansen and Gorski (Madison) studied the partitioning behavior of ER in aqueous two-phase polymer systems and noted that both hormone binding and elevated temperature alter surface properties of ER. Partitioning of ER in biphasic polymer systems, according to Hansen, has revealed the existence of two independent conformational transitions that occur within the receptor monomer. Alteration that occurs upon hormone binding modulates the hydrophobic content of the receptor while the temperature-dependent ER transition is a hormone-independent irreversible structural change that apparently begins to occur upon homogenization and extraction of receptor into cytosol.

Steroid binding ability of uterine ER may be reversibly altered upon phosphorylation on tyrosine. Ferdinando Auricchio (Naples) discussed identification of (i) a Ca^{2+}-stimulated kinase that phosphorylates ER on tyrosine and confers on it a hormone-binding ability and (ii) a phosphotyrosine phosphatase whose action causes loss of hormone binding. Furthermore, phosphorylation on tyrosine is not a phenomenon unique to ER. Anti-phosphotyrosine antibodies were shown to recognize both ER and rat liver GR.

Employing immunochemical analysis, David Toft (Rochester, MN) reported the existence of avian PR as two forms, A (79 kDa) and B (110 kDa), both of which exist as phosphoproteins and whose origin and significance remain unclear. Excessive homogenization, storage of tissue and exposure to elevated temperature are conditions detrimental to the receptor's ability to bind steroid in target tissue cytosols. Within a 5-min period of progesterone administration, a time-dependent increase in receptor phosphorylation was evident, implying that receptor phosphorylation occurs very early in progesterone action.

The native mammalian PR for human breast cancer cells, as presented by Kathryn Horwitz (Denver), consists of two independently synthesized 8 S receptors, one of which contains B-protein (120 kDa) and the other A-protein (94 kDa). Both receptor forms A and B bind to DNA and can produce biologic effects. Treatment with progesterone of $T47D_{Co}$ cells, an estrogen-resistant human breast tumor cell line in which PR is constitutively expressed, leads to down-regulation of immunoreactive A and B receptors followed by their replenishment.

The relationship of structural and functional domains of rat liver GR was discussed by Carlsted-Duke (Huddinge), who reported purification of receptor (94 kDa) in its monomeric DNA binding form. Limited proteolysis revealed that GR consists of three functional domains, namely, C-terminal steroid binding domain, a central DNA-binding domain and an N-terminal domain of uncertain function. Three amino acids, Met-622, Cys-754 and Cys-654 appear to interact with the steroid in the hormone-binding domain of GR.

3. PHARMACOLOGY AND CLINICAL CONSIDERATIONS

Detection and quantitation of both ER and PR are now used routinely in the clinical management

of breast and endometrial cancers as predictive indices of a patient's response to endocrine therapy, and as prognostic indicators of a patient's clinical course. James Wittliff (Louisville) discussed the origin and physiological significance of receptor heterogeneity observed with breast cancer patients. It was suggested that post-transcriptional modifications, such as receptor phosphorylation and association of steroid-binding peptide(s) with nonhormone-binding peptides or protein kinases may contribute to polymorphism. Erlio Gurpide (New York) suggested that the presence of receptors in breast cancer or endometrial adenocarcinoma reveals cellular properties that make the tumor more responsive, without implying receptor-mediated effects of drugs with hormonal action. It was shown that receptor levels correlate with DNA polymerase and ornithine decarboxylase activities in certain endometrial cancer cell lines and that in estrogen-nonresponsive tumor cells, the ability of ER to interact with a monoclonal anti-ER antibody is impaired.

Henry Rochefort (Montpellier) described how in ER-positive human breast cancer cell line, MCF-7, estrogens specifically increase the secretion into the culture medium of a 52 kDa glycoprotein and stimulate cell proliferation. The 52 kDa protein has been identified as a secreted precursor of a cathepsin D, and is mitogenic in vitro in estrogen-deprived MCF-7 cells. The concentration of total cellular cathepsin D is related to the proliferation of mammary ducts and to the prognosis of breast cancer. The 52 kDa protease, according to Rochefort, appears to be useful as a tissue marker for predicting high-risk mastopathies and invasive breast cancers.

4. ANTIHORMONES AND MODE OF ACTION

Antisteroid hormones compete for hormone binding at the receptor level to prevent a hormonal response. Elucidation of the mechanisms by which antiestrogens inhibit proliferation and specific protein synthesis in target cells was discussed by Benita Katzenellenbogen (Urbana). Whereas estrogen increases the rate of PR synthesis without influencing receptor degradation, progestin treatment in breast cancer cells causes both decrease in the synthesis and increase in the degradation of PR. Antiestrogens and antiprogestin RU 486 appear to favor maintenance of the ER and PR in larger aggregate forms, which afford less effective interaction with chromatin binding sites. This suggestion is consistent with recent findings of Moudgil and Hurd that the ability of calf uterine PR to undergo 8 S to 4 S transformation in vitro is impaired when the receptor is occupied by RU 486. Etienne Baulieu (Paris) proposed that a nonhormone-binding peptide, heat-shock protein of M_r 90000 (hsp 90), is involved in the oligomeric structure of steroid receptors. The latter caps the DNA-binding site of the receptor to prevent it from binding to hormone-regulatory elements (HREs) to increase the transcription of regulatory genes. A hormone agonist may induce the dissociation of the receptor oligomer to unmask the functional DNA-binding domain; RU 486-bound receptor, however, is stabilized in its 8 S form unable to dissociate from hsp 90 to elicit a hormonal response. Thomas Ruh (St Louis) provided data to reveal that ER bound by antiestrogen, H1285, interacts with antiestrogen specific sites but binds poorly to some chromatin sites which preferentially bind ER complexes. Ruh suggested that distinct nonhistone chromosomal proteins may play a role in biological actions induced by estrogens and antiestrogens.

5. HORMONES AND BEHAVIOR

Gonadal steroids are secreted in response to signals emanating from the brain and are known to influence animal behavior. Bruce McEwen (New York) studied the mechanism by which steroids regulate lardosis (mating) behavior of female rats in response to estradiol and progesterone. Induction of lardosis involves hormonal activation of the genome of a small group of neurons in the ventromedial hypothalamus (VMN). Exposure of the VMN to testosterone early in life renders the animal defeminized. McEwen demonstrated that since the VMN contains the same density of ER in male and female rats, factors other than receptor must contribute to sex differences.

6. ONCOGENES AND STEROID RECEPTORS

Normal cellular genes have the ability to induce neoplastic growth. These 'oncogenes' or 'proto-

oncogenes' were the theme of the presentation of Inder Verma (San Diego), who discussed the transcriptional and post-transcriptional regulation of proto-oncogene *fos*. Proto-oncogene *fos* is an inducible gene whose expression is influenced by growht factors and stress. The resultant *fos* protein is susceptible to modification post-transcriptionally mainly by phosphorylation. Another proto-oncogene, ras^H, is involved in tumorigenesis of human breast cancer cells. Edward Gelman (Bethesda) investigated the influences of ras^H expression levels and activating mutations on tumorigenesis by these cells by transfecting into MCF-7 cells isogenic constructs of the ras^H gene with only single point mutations. Of the ras^H gene mutants, only the V-ras^H homologue conferred an increased incidence of tumor formation on the MCF-7 cells in the absence of estrogen, but estrogen was required for maximal tumor growth.

7. CLONING AND EXPRESSION OF STEROID RECEPTOR GENES

It is widely acknowledged that steroids influence cellular function by regulating gene expression. The steroid receptor structure-function analysis has been facilitated by recent success in the cloning and expression of complementary DNAs (cDNAs) corresponding to the estrogen, glucocorticoid and progestin receptors. In an attempt to define functional domains of the human androgen receptor (AR) involved in gene regulation, Govindan (Quebec) isolated cDNA clones encoding AR from human testis λ-gt-11 cDNA library. The cDNA clones were inserted into a bacterial expression vector. The protein product of the clones bound [^{3}H]DHT with high affinity and specificity. To elucidate mechanisms by which steroids control target tissue gene expression, Olli Janne (New York) reported AR-mediated androgenic regulation of the ornithine decarboxylase (ODC) genes. Androgen treatment of female mice increased ODC mRNA accumulation and ODC protein concentration. Although antiandrogens exhibited a dose-dependent accumulation of androgen receptors in the renal nuclei, receptor-antagonist complexes failed to influence gene expression.

Hinrich Gronemeyer (Strasbourg) demonstrated that amino acid sequences of ER from human and chicken reveal three highly conserved regions: A (AA 1–38), C (AA 180–263) and E (AA 302–553). All steroid receptors and V-erb A/thyroid receptor contain sequences homologous to regions C and E. Region E represents the steroid-binding domain, and region C, which contains the putative DNA-binding 'fingers', is required for tight nuclear binding. Both regions C and E are required for efficient activation of the HREs of various genes studied.

Edwin Milgrom (Paris) presented data on the cloning of rabbit and human PR genes. Using electron microscopy to observe PR binding to regulatory regions of uteroglobin and mouse mammary tumor virus genes, Milgrom and colleagues demonstrated the binding of PR oligomers at two DNA sites. The interaction results in DNA-loops when the HREs were at a distance from one another. The data suggested high-affinity interaction between steroid receptors and discrete regions of DNA. An understanding of regulatory sequences in the rat prolactin and growth hormone genes has permitted Michael Rosenfeld (La Jolla) and co-workers to define cooperative interactions in the functional effects of the transcriptional activators, estrogen and T3. He discussed the relationship between the above homeostatic regulators and tissue-specific transcriptional factors, noting that the regulatory proteins and the transcription factors differ in their DNA-binding properties.

The conference presentations have provided further impetus to examine and scrutinize receptor structure closely, the genes responsible for their synthesis and cellular factors which potentially modulate both gene expression and receptor activity. Future work in the molecular biology of steroid receptors should aid in understanding the precise structure-functional relationship, and allow in vitro mutagenesis to locate the inter-receptor binding regions and correlate them with a function or biological activity. As these mysteries unfold, society will benefit distinctly from the clinical applications of this information.