100. HIGHLY SELECTIVE AFFINITY LABELING OF THE ESTROGEN RECEPTOR WITH TAMOXIFEN AZIRIDINE
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Tamoxifen aziridine (TA: 1-[4-(2-N-aziridinylethoxyphenyl)]-1,2-diphenyl-1(Z)-butene), a compound known to be capable of rapid and efficient irreversible reaction with the estrogen receptor, has been prepared in high specific activity tritium-labeled form (^{3}H-TA, 17 Ci/mmol). ^{3}H-TA reacts with the estrogen receptor in cytosol and in whole cells, efficiently and with high selectivity. For example, in a rat uterine cytosol preparation containing 3.3 nM of estrogen receptor, exposure to 14 nM of ^{3}H-TA for 30 min at 25 °C results in the covalent labeling of 3.1 nM of receptor and 0.55 nM of non-receptor proteins. After exposure of MCF-7 cells to 10 nM ^{3}H-TA for 1 h at 37 °C, covalently-labeled estrogen receptor can be recovered quantitatively by salt extraction of the nuclear fraction. Non-specific labeling is low and is found mainly in the sediment fraction. Electrophoretic analysis of covalently-labeled estrogen receptor from rat uterus on SDS polyacrylamide gels reveals a major peak with a molecular weight corresponding to 70-75,000. The efficient and selective covalent labeling of the estrogen receptor that can be achieved with ^{3}H-TA in cell-free and whole-cell preparations indicates that this agent will be of wide utility in detailed receptor characterization studies, in studies on intracellular receptor dynamics and in autoradiography.

101. MONOCLONAL ANTIBODIES TO ESTROGEN RECEPTORS, Indu Parikh and Bruno Moncharmont, Wellcome Research Laboratories, Research Triangle Park, NC 27709

Monoclonal antibodies that react with estrogen receptor have been prepared by hybridization of mouse myeloma cell and spleen cells of a mouse immunized with native receptor purified from calf uterus. The receptor specific immunoglobulins secreted by the hybrids were of IgG class and were able to bind to staphylococcal protein A. The dissociation constant (Kd) of the receptor-antibody complex was determined either in solution (0.56 nM) or with immobilized immunoglobulin (0.05 nM). The ability of the antibodies to interact with the receptor was further studied by sucrose density gradient analysis. Only one antigenic determinant is present per molecule of receptor with the antibodies tested. Antibodies from some of the clones are able to form only a 1:1 complex with the 8S form of the receptor, whereas 2 receptor:1 IgG complex is formed at low antibody concentration with the high salt or nuclear form of receptor. Antibodies from two of the clones tested neither prevented the nuclear uptake of the receptor nor the extraction of the translocated receptor from the nuclei. These antibodies exhibit high affinity to the human receptor from mammary tumor tissues as well as to the receptor from MCF-7 cell lines (Kd = 0.07 nm).

102. DEVELOPMENTAL AND PATHOLOGICAL VARIATIONS OF ESTROGEN AND CORTICOSTEROID BINDING PROPERTIES OF RAT AND HUMAN SERA : EVIDENCE FOR DIFFERENTIAL MECHANISM. Nunez, E.A. and : Benassayag, C., Christeff, N., Delorme, J., Martin, M.E., Savu, L., Vallette, G. Vranckx, R., Zouaghi, H. - Faculté Médecine X. BICHAT 16, rue H. Huchard 75018 PARIS

A number of physiological circumstances such as post-natal growth or nutritional status and pathological conditions, like turpentine injection or septic shock, may provoke the decrease of the estrogen-α_1-foetoprotein interactions and of the corticosteroid-transcortin interactions.
At least three possible mechanisms could explain these decreases :
1. The plasma concentrations of the binding protein falls.
2. The conformation of the macromolecule is modified, bringing about a change of binding site.
3. The binding site is masked by endogenous or exogenous inhibitors.

We present experimental results suggesting that all these mechanisms may be operative, one or the other predominating specifically according to the biological condition considered. This evidence is essentially based on binding studies, assessment of influence of serum extractables compounds on binding, and specific immunoassay of the binding proteins.