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In order to determine whether human corpus luteum contains non-steroidal materials which inhibits LH receptor binding or not, these studies were carried out. Dialyzate from 30,000 g supernatant fraction of human corpus luteum homogenate using collodion bag (molecular sieve 12,400) was used as an aqueous extract (Aq.E). Addition of 100 μl Aq.E of luteal tissue exerted strong inhibition of binding of ^{125}I-hLH to human luteal receptors. Dose-dependent relationship was demonstrated between the quantity of human luteal tissue and the degree of inhibition. The Aq.E which obtained from late luteal tissue (49.3 ± 2.4%, n=4) exerted a significantly greater ($p<0.02$) ability to inhibit binding of ^{125}I-hLH as compared to the Aq.E which obtained from early and mid luteal tissue (36.7 ± 2.4%, n=3). The storage periods in frozen state (−80°) had no influence on the inhibitory activities. Incubation of Aq.E at 100°C for 20 min resulted in a slight loss of inhibitory activities, but extraction of Aq.E with diethyl ether did not result in a loss of inhibitory activities. Scatchard analysis revealed that the dissociation constant of ^{125}I-hLH binding was essentially the same in the presence or absence of LHRBI. However, addition of 100 μl Aq.E of non-luteal tissue did not exert inhibition of the binding. These results suggest that LHRBI might regulate the responsiveness of the corpus luteum to LH.

207. Electron Microscopy of Cytodifferentiation, Subcellular Steroidogenic Sites and its Localization of HCG-Receptor Complex in the Theca Cell of the Human Ovary

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The cytodifferentiation, subcellular steroidogenic sites and its localization of HCG-receptor complex in the theca cell of the human ovary was studied by electron microscopic cytochemistry and autoradiography.

Only fibroblast-like cells were seen around or near the primordial follicle. In the theca interna of the secondary and Graafian follicle however there were three different cell types: fibroblast-like cells, theca gland cells (steroid secreting cells) and transitional cells (partially or incompletely differentiated theca cells). The hallmarks of the cytodifferentiation of the theca cell were: 1) the appearance of lipid droplets, 2) a structural change of the mitochondrial cristae from lamellar to tubular form and 3) the appearance and development of smooth endoplasmic reticulum.

Reaction products of 3β-hydroxysyeroid dehydrogenase activity were localized on tubular or lamellar cristae and inner membrane of the mitochondria, and on the membrane of smooth endoplasmic reticulum in the transitional cell as well as in the theca gland cell in the secondary and Graafian follicle.

The autoradiographic localization of HCG-receptor complex seemed to be present on subcellular organelles and nuclei in the both cell types.

208. Binding of Estradiol to Purified Uterine Plasma Membranes

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The increase of cyclic AMP in the uterus has been recognized as the rapid and very early effect of estrogen, which occurs earlier than the result via mechanism of cytosolic receptor. Therefore estrogen binding sites in the plasma membrane especially of rabbit uterus were investigated. The purified plasma membrane fraction was obtained by sucrose gradient centrifugation.

Result: Steroid- and organ-specific estrogen binding site existed in the plasma membrane of rabbit uterus. The equilibrium dissociation constant (Kd) was 1.0×10^{-10} M, and maximum binding site (Bm) was 6.4 fmol/mg protein in a given case. The plasma membrane was not contaminated by cytosolic estrogen receptor. This plasma membrane fraction had the highest 5′-nucleotidase activity, the lowest glucose-6-phosphatase activity and the lowest contamination of cytosolic fraction. In human subjects, uterine endometrial plasma membranes had Kd of $(4.3 \pm 0.44) \times 10^{-10}$ M and Bm of (26.4 ± 11.3) fmol/mg protein (n=4), endometrial cancer had Kd

of (6.9 ± 2.6) × 10^{-10} M and Bm of (12.9 ± 0.6) fmol/mg protein (n=3). It was demonstrated that there were specific binding sites for estrogen in the plasma membranes of rabbit uterus, human uterus and endometrial cancer.

209. Sensibility of Uterine Myoma to Sex Steroid Hormones in View of their Receptors Levels

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Steroid receptor in cytosol and nucleus of myoma and the corresponding myometrium from human uteri was investigated empolying dextran coated charcoal and Anderson' exchange method.

Cytosol estrogen receptor level in myoma was slightly higher than in myometrium, but not statistically significant.

There was no significant difference in cytosol progesterone receptor level as well as cytosol estrogen receptor level between myoma and myometrium.

In nucleus there was no difference of estrogen receptor level in myoma and in myometrium, while progesterone receptor level in the former was lower than in the latter ($P<0.01$).

The ratio of nuclear progesterone receptor level to nuclear estrogen receptor level in myoma was lower than in myometrium ($P<0.02$), particularly in the proliferative phase.

The date from this indicate that nuclear estrogen receptor level in myoma is relatively lower than in myometrium.

Compared with the ratio of nuclear progesterone receptor level to serum progesterone level in myometrium, that was lower in myoma ($P<0.01$).

These studies suggest that the growth of myoma may be a consequence of the depressed number of progesterone receptor, an amount insufficient for regression of the tissues.

210. Cytofluorometric Measurements of Steroid Receptor and Nuclear DNA in Human Endometrial Cancer Cells

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Nuclear DNA and cytoplasmic steroid receptor levels of human endometrial cancer cells were measured by the multi-color cytofluorometry. Endometrial cancer cells incubated with 5 × 10^{-8} M estradiol or progesterone at 4°C for 1 hr were smeared and freeze-dry specimens were obtained. An indirect fluorescent steroid-antibody technique using steroid antibody raised from rabbit and FITC-labeled anti-rabbit IgG was applied to the specimens. The fluorescence intensity of FITC of individual cells on the specimens was measured by a fluorescence cytophotometer at an excitation light (γ490 nm) with a barrier filter (γ530 nm). Then, the nuclear DNA amounts of the individual cells on the same specimens were measured at an excitation light (γ490 nm) with a barrier filter (γ620 nm) after nuclear staining with propidium iodide. In a model system using endometrial nuclei incubated with various concentrations of radioactive steroid-receptor complex, a good proportionality was observed between the nuclear fluorescence intensity of FITC and the amounts of steroid-receptor complex in the nuclei measured by the radioactivity.

The results from the multi-color cytofluorometry indicated that there were three types of DNA distributional patterns in human endometrial cancer; 1) a near diploid type, 2) a hyperploid-aneuploid type, and 3) a mixed type, and that cytoplasmic receptor concentration per unit amount of DNA decreased in accordance with the ploidy of nuclear DNA in cancer cells.

211. Studies on Steroid Hormone Receptor in Ovarian Tumors

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Response of endocrine therapy, especially progestogen therapy has been recogniged for human ovarian cancers. In breast cancer, concentrations of estrogen and progestogen receptors are good indicators for the response of endocrine therapy. Therefore steroid receptors in human ovarian tumor were investigated about the biochemical characteristics and the usefulness for a response indicator. Twenty-