

16. Carbonic anhydrase activity in the receptive rat uterus induced by 17- α -hydroxy progesterone and estradiol.

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Combined treatment of estradiol (E_2) and progesterone (P) produces uterine receptivity. The effect of sex hormones on carbonic anhydrase (CA) of the rat uterus was studied. E_2 decreases the uterine enzyme concentration of ovariectomized rats, while P does not. Different combinations of 17- α -hydroxy-progesterone (17- α -OH-P) and E_2 were administered to induce a receptive uterus in ovariectomized rats and CA was determined in such preparations. Long-Evans adult female rats were ovariectomized and divided in five groups, each one receiving a single 100 μ l s.c. injection containing: Group I, corn oil; Group II, 100 ng E_2 ; Group III, 5 mg 17- α -OH-P; Group IV, E_2 + 17- α -OH-P; and Group V, 17- α -OH-P + E_2 . Simultaneous experiments were made with fifth day pregnant rats to obtain both implantation sites (IS) and non-implantation sites (NIS) in the uterine horns. In all uteri CA was determined. The greatest activity was obtained in Group IV that resembled the hormonal conditions of the receptive uterus. The pregnant rats presented an increase in enzyme activity in the NIS areas (P 0.05), where a greatest concentration of progesterone has been found. In conclusion CA is an essential enzyme participating in capacitation (Collado et al. Biol. Reprod. 20:747, 1979) fertilization and implantation processes.

17. GLUCOCORTICOID RECEPTOR INTERACTION WITH DNA: INACTIVATION BY AURINTRICARBOXYLIC ACID. Moudgil, V.K. and Caradonna, V.M.-Department of Biological Sciences, Oakland University, Rochester, Michigan 48063 U.S.A.

Effects of aurintricarboxylic acid (ATA), a triphenylmethane dye, were examined on the DNA binding properties of rat liver glucocorticoid receptor. Incubation of receptor preparations with 0.1-0.5 mM ATA for 30 min at 4°C completely abolished their DNA-cellulose binding capacity. Heat activated receptor appeared to be more sensitive to ATA presence. The half-maximal inhibition (I.D.50) in the DNA binding of [3 H]triamcinolone acetonide (3 H-TA)-receptor complex was observed at 40- and 130 μ M ATA depending upon whether the inhibitor was added prior to or following the receptor activation. Furthermore, the DNA-cellulose-bound [3 H]-TA-receptor complex from control samples could be completely extracted by an incubation with 20-100 μ M ATA. The amount of available [3 H] TA-receptor complexes remained unchanged under these conditions. The inhibition in the DNA binding of glucocorticoid receptor in ATA-treated preparations was not due to complexing of ATA to the affinity resin. The inhibitory effects of ATA could be completely reversed upon a thorough dialysis of receptor preparations. The effects of ATA appear to result from its interaction with the receptor protein and its use should provide an interesting chemical probe for analysis of domains of glucocorticoid receptor involved in interaction with the nuclear components.
(Supported by NIH grant AM 20893)

18. CHARACTERIZATION OF ANDROGEN BINDING TO NUCLEAR ENVELOPES FROM THREE VARIANT CELL LINES OF THE SHIONOGI MOUSE MAMMARY CARCINOMA
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To determine whether the translocation of steroids across the nuclear envelope plays a role in modulating steroid responsiveness of tissues, we are investigating uptake of androgens into nuclei and binding of androgens to nuclear envelopes prepared from an androgen-dependent and two androgen-independent cell lines of the Shionogi mouse mammary carcinoma. Nuclei from the three tumour cell lines displayed similar affinity for dihydrotestosterone (DHT) but the two androgen-independent cell lines had less than one-quarter the number of uptake sites. Purified nuclear envelope fractions were prepared from the three cell lines using a high pH/DNase treatment. The nuclear envelopes are relatively free of DNA and contaminating cytoplasmic tags as determined by chemical, enzymatic, and morphological analyses. Characterization of specific binding by the nuclear envelopes revealed two classes of binding sites: one of high affinity and low capacity and the other of low affinity and high capacity. At DHT concentrations of 10nM, nuclear envelopes from the androgen-dependent cell line bound two-fold more DHT than the unresponsive cell lines. Salt extraction, using 0.4M KCl, did not remove the DHT specifically bound to the nuclear envelopes. In conclusion, nuclear envelopes prepared from the three cell lines have binding sites for DHT but the androgen-independent cell line nuclear envelopes possess less sites than those of the androgen-dependent cell lines.