97. CONTROL OF GLUCOCORTICOID RECEPTOR ACTIVITY BY CALCIUM IONS

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Binding of steroids to the glucocorticoid receptor is influenced by calcium ions. This was investigated in cultured rat hepatoma (HTC) and human lymphoblastoid (IM-9) cells. In HTC cytosol calcium decreased 3-fold the association rate constant for dexamethasone binding to the receptor, without change in the dissociation rate constant or in receptor site concentration. This occurred between 0.1 and 1 μM free calcium, i.e. concentrations at which this ion exerts its second messenger functions inside living cells. Inhibition of dexamethasone binding by calcium was not prevented by five antagonists of calmodulin, a ubiquitous intracellular calcium receptor protein. Upon purification, the receptor retained its calcium sensitivity. The calcium effect was reversible and independent of receptor-protecting agents such as Na_2MoO_4 and dithiothreitol; it was not mimicked by other divalent cations. Dexamethasone binding to the human glucocorticoid receptor was also sensitive to calcium. However, binding of some steroids that behave as glucocorticoid antagonists was not affected. The rate of dexamethasone binding to intact HTC cells was decreased by the Ca^{2+} ionophore A23187, indicating that calcium may act as a physiological modulator of glucocorticoid hormone action at the receptor level.

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98. A NEW METHOD FOR ASSAYING OCCUPIED AND UNOCCUPIED ANDROGEN RECEPTORS IN THE PROSTATE USING CONTROLLED PORE GLASS BEADS.

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It is thought that prostate tumours can be controlled by endocrine means if functional hormone receptors are present, but most assay techniques depend on exchange methods at temperatures which may allow protein degradation. This method, modified from that of Hospelhorn and Jensen (personal communication), enables distinction between occupied and unoccupied receptor sites using temperatures of 4°C.

Small columns of controlled pore glass beads (0.2 ml)were made in Pasteur pipettes blocked with glass wool and a glass fibre filter. All solutions contained 15% glycerol and the homogenisation buffer also included 5 mM Na_2Mo_4 to stabilise the receptor. Endogenous bound hormone may be displaced on the columns by 1.25 mM $AgNO_3$ in buffer before overnight incubation with 5 nM [^{3}H] 5α-dihydrotestosterone (5α-DHT); non-specific binding was estimated by adding 1 mM unlabelled 5α-DHT. Bound radioactivity was eluted with ethanol after 20 min at room temperature.

Binding by rat prostate cytosol was suppressed by excess 5α-DHT and the antiandrogen, cyproterone acetate, while castration for 24 h decreased the number of occupied sites and increased the unoccupied ones. No binding occurred with human plasma, i.e. sex hormone binding globulin. Human prostate studies are in progress. This simple, reproducible assay should prove a useful clinical tool.

99. NUCLEAR TESTOSTERONE RECEPTORS IN THE LAMB TESTIS

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Nuclear androgen receptors were demonstrated and characterized in the lamb testis by a testosterone exchange assay. Nuclei were isolated from the testicular parenchyma and partially purified through 1.5 M sucrose. A protamine sulphate precipitation was applied directly to the nuclear suspension. The precipates were incubated with (^{3}H)-testosterone at 4°C for 16h, after which steroid-receptor complexes were separated from free steroid by repeated washings of the precipitates.

The amount of receptors is linear between 0.1 and 0.9 mg of DNA per ml of incubation buffer. Their relative affinities for steroids are dihydrotestosterone > testosterone > estradiol > cyproterone acetate > progesterone > 5α-androstanediol. They bind testosterone with high affinity ($Kd \sim 2 \times 10^{-9}$ M) and limited capacity (40-600 fmoles per mg DNA). When measured in 25 to 100 days - old lambs, the androgen receptor concentration per weight of testis tissue decreases abruptly at the age of 70 days, i.e. when germinal cells appear in the seminiferous tubules. This suggests that androgen receptors are localized essentially in somatic cells of the testis.