The results are evidence that conditioned reflex formation is connected with the formation of definite relationships between activity of the upper and lower layers of the cortex in the frequency band of the conditioned stimulus. In the initial stage of reflex formation the main changes in activity in this frequency band take place in the upper layers of the cortex. In the stage of the consolidated reflex, changes in activity in the lower layers of the cortex in the hemisphere contralateral to the side of reinforcement are dominant. The dynamics of changes in the ECoG potentials thus revealed confirms the view that the mechanism of temporary connection closure is located at many different levels in the thickness of the cortex and that the lower cortical layers play the leading role in realization of the conditioning function of the brain.

LITERATURE CITED


IDENTIFICATION AND CHARACTERIZATION OF THE BINDING PROPERTIES OF STEROID HORMONE RECEPTORS IN THE NUCLEAR FRACTION OF ADENOMAS OF THE HUMAN PROSTATE GLAND

Ts. I. Gerasimova, A. N. Smirnov, and A. F. Bunyatyan

Benign tumors of the prostate are among the most widespread diseases of the sexual organs of elderly men, which is due, as is generally believed, to imbalance of the secretion and metabolism of the sex hormones [1-3]. Moreover, it has been established that in the adenomatous tissue itself, the metabolism of androgens is greatly changed [4, 5]. Possibly the sensitivity of the adenoma of sex steroids is also changed.

According to the modern concepts, the action of steroid hormones on cells is determined by the intracellular protein receptors, which are initially localized in the cytoplasm and are translocated in complex with the hormone into the nucleus, where the biological effect of the hormone is exerted [6]. Proteins that specifically bind androgens, estrogens, and progestins have been detected in the soluble fraction of cells of prostate tumor tissue [7, 8], which may be considered as evidence of the potential ability of the adenoma to respond to the action of the corresponding steroids. However, such investigations cannot give an unambiguous answer to the question of whether this possibility is realized under conditions of the intact organism of the patient. Evidently information on the functional activity of the receptor apparatus of the adenoma can be obtained by investigating the content of steroid receptors in the nuclear fraction of the tissue. A necessary condition for the successful performance of such an investigation is the development of methods of identification and quantitative measurement of the receptors of various groups of steroids.
Fig. 1. Dynamics of the exchange of [3H]-steroids with endogenous hormone receptor complexes of nuclei of human prostate adenomas at various temperatures. a: 1) DHT at 23°C, 2) at 5°C [as in Russian original]; 3) DHT at 5°C; 4) DHT at 0°C; 5) E2 at 5°C; b: 1) total binding; 2) nonspecific binding; 3) specific binding. Along y-axis: bound [3H]-E2 at 17°C.

MATERIALS AND METHODS

Adenomas of the prostate were obtained during the operation of adenomectomy in the urology clinic of the I. M. Sechenov First Moscow Medical Institute. Fresh tissue was immediately placed in a thermos with dry ice and delivered to the laboratory where it was stored at -20°C until the investigation. The frozen tissue was pulverized, homogenized in a 1:2 ratio in buffer A (0.02 M Tris-HCl, 0.003 M MgCl2, 0.32 M sucrose, 10% glycerol, 0.001 M dithiothreitol, pH 7.5) in a blender (type 302, Poland), 10 times for 15 sec periods, at 45 sec intervals. The homogenate was diluted fivefold with buffer A and filtered through three layers of gauze. The filtrate was centrifuged at 800g for 15 min, and the precipitate washed three times with the same buffer. All the operations were performed at 0-4°C. The nuclear receptors were extracted from crude chromatin by suspension in buffer B (0.02 M Tris-HCl, 0.001 M EDTA, 10% glycerol, 0.4 M KCl, 0.001 M dithiothreitol, pH 7.5), freezing of the suspension for 18 h at -10°C, followed by thawing and centrifugation for 1 min at 40,000g. Aliquots of the nuclear extract (0.5 ml) were incubated with 0.02-5 nM labeled steroids, dissolved in 0.2 ml of buffer B without KCl. We used [1,2,4,5,7-3H]-5α-dihydrotestosterone (DHT), specific activity 109 Ci/m mole; [1,2,6,7-3H]progesterone (P), specific activity 101 Ci/m mole; [2,4,6,7-3H]estradiol-17β(E2), specific activity 101 Ci/m mole; [1,2-3H]cortisol (F), specific activity 44 Ci/m mole; [3H]dexamethasone (D), specific activity 28 Ci/m mole (Amersham); dimethyl-19-nor-pregna-4,9-diene-3,20-dione-(17α-methyl-[3H]) (R5020), specific activity 87 Ci/m mole (New England Nuclear). To change the level of nonspecific binding of [3H]-hormones, a 400-fold excess of the corresponding unlabeled hormone was added to the samples. The separation of protein-bound and free hormone was performed for 5 min at 0-4°C by the method of solid phase adsorption on activated charcoal, coated with dextran (final concentration of the charcoal 1%), as described earlier [9]. The content of radioactivity was measured in scintillation liquid [10] in a MARK II B-scintillation counter (Nuclear Chicago). The count efficiency was 25%. The amount of specifically bound [3H]-hormone (B9) was calculated as described earlier [9]. The values of the measurable equilibrium constant of association (K9) and the concentration of binding sites (N) for receptors were calculated for B9 by the Scatchard method [11]. The hormonal specificity of the property of the receptors was evaluated according to the ability of various steroids to induce 50% inhibition of the specific binding of the [3H]-hormone [12]. In the latter case, as well as in experiments on the study of the dynamics of the binding of [3H]-hormones, we used saturating concentrations of the latter. The protein content in the samples was determined according to Lowry [13].

RESULTS AND DISCUSSION

Androgen Receptors. It was established that, as a rule, most of the nuclear receptors for steroid hormones are complexed with endogenous steroids. Therefore, for the detection and quantitative evaluation of the content of receptors in the nuclear material, it is necessary first of all to determine the conditions in which complete exchange between labeled and endogenous steroids occurs. In Fig. 1a it is evident that the reaching of a maximum level of B9 of [3H]-DHT at 23°C occurs far more rapidly than at 0 and 5°C. However, the stability of the complexes decreases substantially when the temperature is increased. B9 remains con-