The hypothesis that free grafting of previously denervated muscles is possible, based on a previous investigation, was thus confirmed. However, the advanced degeneration atrophy evidently was reflected in the late results of transplantation. The elucidation of the causes of this phenomenon will be a task for future research.

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CHARACTERISTICS OF ESTRADIOL RECEPTOR SYSTEM OF THE ANTERIOR HYPOTHALAMUS AND ADENOHYPOPHYSIS IN GUINEA PIGS

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The presence of a specific estradiol-receptor system (E_2-R) with limited capacity and with a high degree of strength of formation of the E_2-R complex was demonstrated in the cytosol of the adenohypophysis and anterior hypothalamus of guinea pigs in experiments in vivo and in vitro. The physicochemical properties of the E_2-R system of the adenohypophysis and anterior hypothalamus differ in certain parameters. The E_2-R complexes of the cytosols of the adenohypophysis and anterior hypothalamus formed at different temperatures are not identical.

KEY WORDS: adenohypophysis; hypothalamus; physicochemical characteristics; receptor system.

The study of the estrogen-sensitive receptor system of structures of the CNS is one way of elucidating the mechanisms of regulation of the reproductive function.

There is extensive evidence of the presence of specific cytoplasmic and nuclear protein receptors for estradiol in the anterior hypothalamic and adenohypophyseal structures of the CNS [2, 5, 7-11]. However, because of the diversity of the experimental models and methods used, there are still no clear ideas on the properties of the estradiol-receptor (E_2-R) system of the CNS. It was therefore decided to study the basic parameters of steroid-receptor interaction in the anterior hypothalamic region and the adenohypophysis of guinea pigs under different experimental conditions.

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TABLE 1. Incorporation of ^3H-E_2-17β into Hypothalamus and Adenohypophysis of Sexually Immature Guinea Pigs Weighing 150-200 g

	Tissue investigated			
	adenohypophysis		anterior hypothalamus	
	dose of estradiol given			
	0.2 ng ^3H-E_2	0.2 ng ^3H-E_2+ 20 ng E_2	0.2 ng ^3H-E_2	0.2 ng ^3H-E_2+ 20 ng E_2
Incorporation of label (in % of incorporation of estradiol into cortex)	1580	950	230	160
Anterior hypothalamus	18	12	18	12

EXPERIMENTAL METHOD

Experiments were carried out on sexually immature female guinea pigs weighing 200-300 g in vivo and in vitro.

In the experiments in vivo, guinea pigs of group 1, anesthetized with pentobarbital, received injections initially of 20 ng unlabeled estradiol (E_2) and, 5 min later, 0.2 ng of labeled estradiol (^3H-E_2-17β) (specific activity 100 Ci/mmole; from Radiochemical Centre, Amersham), into the jugular vein. The animals of group 2 received the labeled hormone only. All the animals were killed after 1 h. Incorporation of the label in the adenohypophysis, the anterior hypothalamus,† and the occipital cortex was determined by the method of Kato and Villee [9] with slight modifications. Incorporation of the label into the tissues studied was expressed as a percentage of its incorporation into the tissue of the occipital cortex, which was used as a tissue indifferent to E_2 [9]. In the experiments in vitro, the method of obtaining the cytosol from the same tissues was the same as the scheme adopted previously [1], with a ratio of tissue: buffer of 1:5 for the hypothalamus and 1:80 for the adenohypophysis. The kinetic and thermodynamic parameters were determined and calculated by the usual methods [6, 12, 14]. When the dissociation velocity constant (k_{-1}) was determined (by the method of dissociation with a charcoal suspension), the complex was formed with 20 and 50 nmoles ^3H-E_2/ml cytosol of the adenohypophysis and hypothalamus respectively. When the equilibrium association constant (K_{ass}) was determined the specific binding was corrected by the method of excess of unlabeled hormone, with choice of concentrations of ^3H-E_2 (q*) and of E_2(q) based on estimator relationships: $q^* \leq 10K_{ass}$; $q \leq 50g^*$ [2], where K_{ass} is the expected value of the equilibrium constant. The working range of the Scatchard plot was 20-350 μmoles to 50 μl cytosol. Complex formation took place at 0 and 30°C for 18 and 5 h respectively.

A Wang-720 programmed calculator was used for the statistical analysis of the results and to calculate the physicochemical parameters, using programs developed in the writers' laboratory.

EXPERIMENTAL RESULTS

1. Incorporation of ^3H-E_2-17β into Hypothalamo-Hypophyseal Structures

The adenohypophysis and anterior hypothalamus have specific E_2-sensitive systems, as was shown by the marked decrease in incorporation of label on account of saturation of the specific combining sites after preliminary injection of a 100-fold excess of unlabeled E_2 (Table 1). Specific incorporation of ^3H-E_2 by the adenohypophysis was almost one order of magnitude (9 times) higher than in the anterior hypothalamus.

2. Physicochemical Characteristics

Steroid-receptor interaction in the CNS was studied at both 0°C and 30°C; most of the experiments, however, were carried out at 0-4°C. Data obtained in the writers' laboratory, showing the absence of dissociation of the E_2-R complex of the uterus and tubes during prolonged incubation (although dissociation of the complex of the same tissues at 30°C does take place) compleled a very cautious approach to the estimation of the condi-

†Derivation of the boundary of the hypothalamus was conducted using standard topographic parameters.

TABLE 2. Physicochemical Parameters of Estradiol-Receptor System of Hypothalamo-Hypophyseal Structures

Tissue	Incubation temperature											
	0°C						30°C					
	parameter						parameter					
	k_{-1}, sec^{-1}	experimental K_{ass}, M^{-1}	calculated K_{ass}, M^{-1}	k_{+1}, $sec^{-1}M^{-1}$	$T_{1/2}$, min	ΔG, kJ/mole	K_{-1}, sec^{-1}	$T_{1/2}$, min	experimental K_{ass}, M^{-1}	calculated K_{ass}, M^{-1}	K_{+1}, $sec^{-1}M^{-1}$	ΔG, kJ/mole
Adenohypophysis	$0,57 \pm 0,04 \times 10^{-4}$ ($n=3$)	$0,54 \pm 0,12 \times 10^{10}$ ($n=3$)	$0,60 \times 10^{11}$	$0,31 \times 10^{6}$	202	—46	$0,13 \pm 0,007 \times 10^{-4}$ ($n=4$)	103	$0,51 \pm 0,035 \times 10^{10}$ (n=3)]	$0,59 \times 10^{9}$	$0,682 \times 10^{6}$	—56
Anterior hypothalamus	$0,69 \pm 0,09 \times 10^{-4}$ ($n=3$)	$0,70 \pm 0,09 \times 10^{9}$ ($n=6$)	$0,14 \times 10^{11}$	$0,51 \times 10^{5}$	167	—41	$0,128 \pm \pm 0,002 \times 10^{-4}$ ($n=4$)	91	$0,136 \pm 0,024 \times 10^{10}$ ($n=3$)	$0,94 \times 10^{8}$	$0,158 \times 10^{6}$	—53

Legend: n) number of experiments. At least 50 animals used in each experiment.

tions of investigation of the steroid-R complex of the CNS at 0°C, having regard in addition to the unphysiological nature of these conditions.

The need for comparing the properties of individual target organs was dictated by the unification of the methods used. A comparative analysis of the kinetic and thermodynamic parameters of steroid-R interaction was accordingly undertaken, using two temperatures in order to choose the most adequate conditions.

A. Kinetics of Interaction between E_2 and the R System of Cytosols of Hypophyseo-Hypothalamic Structures. During analysis of the process of breakdown of the E_2-R complex of cytosols of the adenohypophysis and anterior hypothalamus at 30°C (the complex was formed at 30°C) rapidly and slowly dissociating components were found. During the incubation time under these conditions about 8% of receptors were inactivated. The values of k_{-1} and $T_{1/2}$ of the E_2-R complexes studied, which are given in Table 2, agree with data in the literature for animals of other species [3]. Analysis of the breakdown of the E_2-R complex (formed at 0°C) during incubation for 5 h at 0°C also revealed rapidly and slowly dissociating components. Inactivation of receptors did not take place during the incubation period at 0°C. The E_2-R complexes of cytosols of the adenohypophysis and anterior hypothalamus formed at 0°C dissociated completely in the course of 5 min at 30°C.

B. Thermodynamic Characteristics. The duration of incubation of E_2 with R of the test systems was chosen to be definitely longer than the minimal time required for equilibrium to be reached: 5 h at 30°C and 18 h at 0°C. The stability of the complex under these conditions was demonstrated. The values of the equilibrium constants and data showing the change in free binding energy are given in Table 2. The values obtained for K_{ass} of E_2 for the receptor system of cytosols of the adenohypophysis and anterior hypothalamus agree with the corresponding values for the adenohypophysis and anterior hypothalamus of rats [4, 5, 13] and are an order of magnitude lower than K_{ass} for the sheep hypophysis [15]. The number of combining sites in the tissues studied is not given, for such a calculation for them would be invalid because of the great dilution of the specific sites of the tissue by the mass of nonspecific sites.

The data given for the physicochemical characteristics suggest that during interaction between the receptor apparatus of the cytosol of the adenohypophysis and anterior hypothalamus with E_2 at both 0°C and 30°C a specific (see the values of K_{ass} in Table 2) steroid-receptor complex is formed, and in both cases its formation is determined by hydrogen bonds (see the values for the change in free binding energy ΔG). However, these complexes were not identical, as shown by differences in the values of the kinetic parameters (k_{-1}, k_{+1}, $T_{1/2}$) of the complexes investigated at the temperature of their formation. Furthermore, their nonidentity was also confirmed by the difference in behavior of the "0°C" and "30°C" complexes under identical conditions of dissociation (30°C). The significant difference between the values of K_{ass} measured at one temperature and recalculated for the other (by the equation $\ln K_{ass_2}/\ln K_{ass_1} = T_1/T_2$), is further confirmation of this state of affairs.

The disagreements noted above in the characteristics of the two complexes point to the need for a cautious approach to the comparison of data in the literature obtained at different incubation temperatures.

Comparison of the properties of the receptor systems of the adenohypophysis and anterior hypothalamus at the same temperature points to definite differences between the systems of these tissues: the rate of formation of the E_2-R complex is higher in the adenohypophysis than in the hypothalamus, for the same rates of dissociation.

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