EFFECT OF ESTRADIOL ON SPONTANEOUS SINGLE UNIT ACTIVITY IN THE ARCUATE REGION OF THE RAT HYPOTHALAMUS AT VARIOUS STAGES OF THE ESTROUS CYCLE

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Microiontophoretic injection of estradiol into the arcuate region of the hypothalamus in the overwhelming majority of experiments enhanced single unit activity. The response was more marked in diestrus-1 and diestrus-2 than in proestrus. It is suggested that changes in the predominant response of arcuate neurons to injection of estradiol in the course of the sex cycle are determined by the level of endogenous estrogens and pituitary gonadotropic hormones in the peripheral blood.

KEY WORDS: *hypothalamus*; *neurons*; *estradiol*; *microiontophoresis*; *sex cycle*.

The problem of the mechanisms of interaction between the hypothalamic centers of tonic (arcuate nucleus) and cyclic (preoptic region) regulation of pituitary gonadotropic function and the sex hormones has not been finally solved. The evidence so far available suggests that estrogens activate the function of hypothalamic neuronal formation [1, 8, 10]. The present writers' observations [2, 3] pointing to a modulating effect of estrogens on the sensitivity of single neurons in the hypothalamic arcuate region to noradrenalin and dopamine suggest that sex hormones participate in the hypothalamic regulation of pituitary gonadotropic function.

The object of this investigation was to study changes in the sensitivity of the hypothalamic arcuate neurons to estradiol (administred by microiontophoresis), in the course of the sex cycle in female rats.

EXPERIMENTAL METHOD

Experiments were carried out on 48 rats weighing 180-200 g with a stable 4-day estrous cycle. The animals were kept under standard conditions of diet and illumination (14 h of light, 10 h of darkness). Vaginal smears were taken to determine the stage of the sex cycle every morning. Single unit activity was investigated in rats immobilized with tubocurarine (0.3 mg/100 g body weight), anesthetized with ether, and fixed in a stereotaxic apparatus. Multichannel microelectrodes were used in the experiments. The central barrel was filled with 2 M NaCl solution and was used to record unit activity. The side barrels were filled with physiological saline for control treatment, with 1% estradiol solution, and with a 2% solution of $FeCl_3$ for iontophoretic labeling of the position of the microelectrode tip. Unit activity was recorded during three 120-sec periods before, during, and after the end of microiontophoresis. The strength of the iontophoretic current was chosen to be 100 nA in accordance with data in the literature [6, 9]. The duration of iontophoresis with this strength of current was such that estradiol could be injected in a dose comparable with the amount contained in 1 ml plasma in the stage of proestrus. The effect was regarded as significant if the level of single unit activity was above 125% or below 75% of the initial level. Details of the method of recording, of microiontophoresis, of analysis of spike activity, and of histological verification of the location of the microelectrode tip were described previously [2, 3].

EXPERIMENTAL RESULTS AND DISCUSSION

The total number of animals investigated and of neurons recorded at different stages of

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TABLE 1. Number of Animals and Neurons Tested in Experiments with Microiontophoretic Injection of Estradiol into Rats at Various Stages of the Estrous Cycle

	Diestrus-1	Diestrus-2		Proestrus		Estrus
		first half of day	second half of day	first half of day	second half of day	
Animals	6	7	8	9	8	10
Neurons	30	21	32	36	30	28

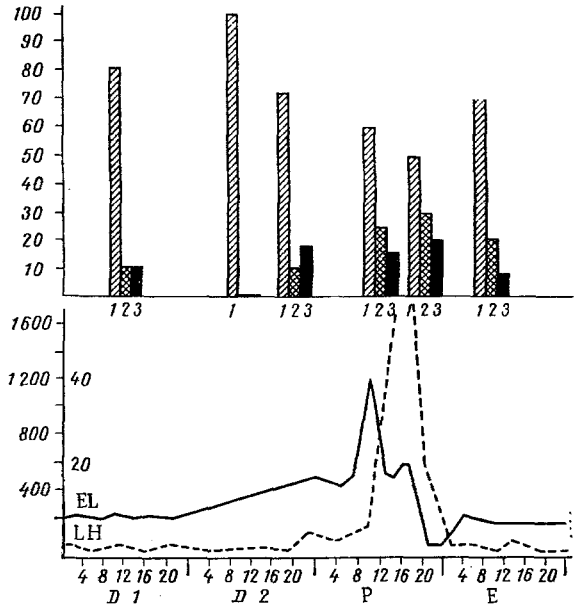

Fig. 1. Response of hypothalamic arcuate neurons to estradiol, applied by microiontophoresis, in rats during sex cycle. Ordinate: above — percentage of total number of neurons recorded at a given stage of the sex cycle; below, right — estradiol (EL) concentration [5] (in pg/ml plasma), left — content of pituitary luteinizing hormone (LH [13]) (in ng/ml plasma) in peripheral blood; abscissa, above: 1) activation, 2) inhibition, 3) no response; below, stages of sex cycle: D 1) diestrus-1; D 2) diestrus-2; P) proestrus; E) estrus.

the sex cycle in the rats is shown in Table 1. In the course of one experiment on the average 3 or 4 neurons were recorded in each animal. The latent period of response to administration of estradiol varied from several hundred milliseconds to 15-20 sec, a fact evidently attributable to differences in the original physiological activity of the units tested. The duration of the aftereffect in most experiments exceeded 800 msec but in many cases the end of the effect could not be observed during the 120-sec interval after the end of iontophoresis. The main frequency range of the discharge of the neurons tested varied from 3 to 13 spikes/sec, with a small shift of the maximum toward the higher frequency in the stage of proestrus. The frequency characteristics of spontaneous unit activity in the arcuate region of the hypothalamus in the various stages of the estrous cycle have been described previously [2]. No connection was found between the initial discharge frequency of the neurons and the direction or level of the subsequent response, which was evidently determined by the specific features of the units studied.

Changes in single unit activity in the arcuate region in response to microiontophoretic injection of estradiol are shown in Fig. 1. In all stages of the sex cycle activation of most recorded neurons was observed. This was most marked in stage diestrus-1 (80%) and in the first half of the day of diestrus-2 (100%). The proportion of neurons with an activation response subsequently decreased and was minimal in proestrus. In the stage of estrus an increase in the number of neurons responding to estradiol by activation up to 75% was again observed. The decrease in the number of neurons responding by activation in the stage of proestrus was due to some increase in the number of cells with an inhibitory response.

Hypothalamic arcuate neurons are thus heterogeneous as regards their sensitivity and the direction of their response to estradiol. However, regardless of the stage of the sex cycle, an activation response was observed in most nerve cells. The proportion of such neurons varied in the course of the sex cycle to reach a minimum in proestrus. It can tentatively be suggested that this was due to functional changes in the neuroendocrine system in the course of the sex cycle and, in particular, changes in the blood level of endogenous estrogens and pituitary gonadotropic hormones. The coefficient of correlation between the plasma estrogen level (according to data in the literature [5]) and the proportion of neurons responding by activation (taking into account variations in these last changes over a period of about 10 h) was 0.772 ± 0.16 ($P < 0.01$). Under the influence of elevation of the estrogen level in the course of the sex cycle, many hitherto silent neurons may be assumed to be activated and the activity of certain neurons already functioning may be increased. This hypothesis agrees with the observed increase in the discharge frequency of the neurons in the stage of proestrus.

The estrogen level is known to begin to rise gradually in the second half of the day in the stage of diestrus-2 [4, 5, 14]. Their concentration rises sharply during the first half of the day in proestrus and then falls gradually to the basal level toward the end of the day. It was found in the present experiments that as the concentration of endogenous estrogens rose and the discharge frequency of the arcuate neurons increased under their influence, the proportion of neurons capable of responding to exogenous estradiol by an additional increase in frequency was observed to fall. However, when the concentration of endogenous estrogens fell to its initial value in the second half of proestrus, the proportion of neurons responding by activation to exogenous estradiol did not increase but continued to fall. This could be connected with the inertia of the biological system studied, and for that reason the coefficient of correlation was calculated with a shift of 10 h.

On the other hand, the writers have shown [2] that in the second half of the day in proestrus and in the stage of estrus many neurons responding by activation to microiontophoretic injection of noradrenalin can be found. The decrease in the proportion of neurons responding by activation to exogenous estradiol, accompanied by some increase in the number of neurons with an inhibitory response, in the second half of the day in proestrus is perhaps due to cessation of the discharge of some neurons and activation of others among the neuronal generation of the arcuate nucleus. This process may be determined by the sharp fall in the level of endogenous estrogens or the increase in concentration of luteinizing hormones after the end of the first half of the day in the stage of proestrus [4, 5, 11-13].

A report has recently been published [7] that after microiontophoretic injection of pituitary gonadotropic hormones approximately 80% of responding arcuate neurons are activated. The activating effect of pituitary luteinizing hormone in the second half of the day in proestrus, when the blood level of this hormone is at a maximum, perhaps prevents to some extent the additional activating effect of microiontophoretic injection of exogenous estrogens. Further investigation of the sensitivity of the hypothalamic arcuate neurons to various biologically active substances is necessary in order to confirm this hypothesis.

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THE SEX RATIO IN INBRED STRAINS OF MICE

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A study of the sex ratio in mice of inbred strains CBA and C3H and of the connection between the postimplantation embryonic mortality of mice of these strains and the sex distribution of the embryos showed that the embryonic sex ratio of these lines of mice obeys the 1:1 distribution. Data in the literature and the writer's own observations suggest that genetic differences between mice of inbred strains have no significant effect on the sex ratio of the progeny. The postimplantation embryonic mortality in C3H mice is greater than that of CBA mice (14.4 and 9.3% respectively). However, the presence of a balanced sex ratio in the mice of these strains is evidence of absence of selective death of embryos of either sex during embryogenesis.

KEY WORDS: *sex ratio; embryonic mortality; inbred strains of mice.*

The investigation of the mechanism controlling the sex ratio in progeny is of great interest. Information in the literature on the effect of genetic differences between mice of different strains on the sex distribution in the progeny is highly contradictory. Some workers have found that genetic differences between inbred strains have no marked effect on the sex ratio [4], whereas others consider that their effect is insignificant and they point to an unbalanced sex distribution of fetuses of the mice of certain strains [3, 6].

The object of this investigation was to study the sex ratio in mice of inbred strains CBA and C3H and to analyze the connection between the postimplantation embryonic mortality of mice of these strains with the sex distribution of the embryos.

EXPERIMENTAL METHOD

Mice of strains CBA and C3H obtained from the Rappolovo nursery were studied. Females were crossed with males of the same strain and the day of discovery of a vaginal plug was taken as the first day of pregnancy. The females were killed on the 18th day of pregnancy. Embryos at the 18th day of development were removed from the uterine cavity and, after laparotomy, their gonads were examined with the MBS-1 microscope. The ratio between the number of males and the number of females was determined.

EXPERIMENTAL RESULTS

The postimplantation embryonic mortality was determined from the 18th day of pregnancy. As Table 1 shows, the postimplantation mortality of the C3H embryos was higher than that of the CBA embryos.

A study of the sex distribution of the embryos (Table 2) showed that both in the C3H and the CBA mice the sex ratio of the embryos obeyed the 1:1 distribution.

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