Further research is required in order to determine whether the combination of inhibition of LPO and cholesterol accumulation is a specific phenomenon accompanying learning processes.

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MODULATING ACTION OF ESTRADIOL ON NORADRENALIN SENSITIVITY OF SINGLE HYPOTHALAMIC PREOPTIC AREA NEURONS

Z. I. Aivazashvili, V. Ya. Ignatkov, and V. N. Babichev

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The current view in the literature that estradiol (EST) plays an important role in triggering the preovulatory wave of luteinizing hormone (LH) and subsequent ovulation is based on the results of experiments in which estrogens were injected into the bloodstream [6, 8] or crystalline EST was implanted into various hypothalamic structures [7]. The actual triggering mechanism has been shown to be realized at the level of the preoptic area of the hypothalamus (PA), as the center regulating pituitary gonadotropic function [4, 11]. Characteristically, elevation of the blood estrogen level precedes the development of the preovulatory wave of LH [9, 10]. However, the mechanism of action of estrogens on the level of the cyclic center is not yet completely clear. This effect of sex steroids is evidently formed by several functional mechanisms, among which an important place is occupied by a change in the character of action of neurotransmitters of the monoamine series, under the influence of estrogens. Previously, when studying the sensitivity of neurons of PA to noradrenalin (NA) in the course of the estrous cycle in rats, we

Laboratory of Physiology of the Endocrine System, All-Union Endocrinology Research Center, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Changes in Character of Action of NA on Activity of Single Hypothalamic PA Neurons under the Influence of EST ($M \pm m$)

Initial response of neurons to NA	Response of neurons to NA (in %) after preliminary application of EST to them at different stages of estrous cycle											
	diestrus-1				diestrus-2 (11 a.m.)				diestrus-2 (6 p.m.)			
	n	I	N	A	n	I	N	A	n	I	N	A
I	21	19,0±3,9	47,6±5,0	33,4±4,7	23	26,1±4,4	65,2±4,7	8,7±2,8	22	18,2±3,8	54,5±4,9	27,3±4,4
N	54	16,7±3,7	57,4±4,9	25,9±4,4	55	18,2±3,8	67,3±4,7	14,5±3,5	48	20,8±4,1	54,2±4,9	25,0±4,3
A	25	12,0±3,2	60,0±4,9	28,0±4,5	22	22,7±4,2	50,0±5,0	27,3±4,4	30	13,3±3,4	56,7±4,9	30,0±4,6
Total	100	16,0±3,7	56,0±4,9	28,0±4,5	100	21,0±4,1	63,0±4,8	16,0±3,7	100	18,0±3,8	55,0±5,0	27,0±4,4
	Proestrus (11 a.m.)				Proestrus (6 p.m.)				Estrus			
		I	N	A		I	N	A		I	N	A
I	18	22,2±4,1	22,2±4,1	55,6±4,9	28	14,3±3,1	53,6±4,4	32,1±4,1	20	40,0±4,8	50,0±5,0	10,0±3,0
N	54	18,5±3,9	37,0±4,8	44,5±4,9	71	21,1±3,6	53,5±4,5	25,4±3,8	56	14,3±3,5	58,9±4,9	26,8±4,4
A	28	21,4±4,1	42,9±4,9	35,7±4,7	26	26,9±3,9	46,2±4,4	26,9±3,9	24	29,2±4,5	50,0±5,0	20,8±4,1
Total	100	20,0±4,0	36,0±4,8	44,0±4,9	125	20,8±3,6	52,0±4,5	27,2±4,0	100	23,0±4,2	55,0±5,0	22,0±4,1

Legend. n) number of neurons. I) Inhibition; N) no response; A) activation.

postulated that the time course observed is connected with the development of a wave of endogenous estrogens in the blood of the experimental animals during proestrus — the key stage of the estrous cycle [1, 2]. The aim of this investigation was to obtain objective proof of the modulating effect of estrogens on the sensitivity of single neurons of the hypothalamic center for cyclic regulation of the pituitary gonadotropic function to NA.

EXPERIMENTAL METHOD

Experiments were carried out on female rats weighing 200-250 g with a stable 4-day estrous cycle. The rats were kept under standard lighting conditions (daylight from 6 a.m. to 8 p.m.) on a pellet diet. Spontaneous activity of single neurons was recorded and EST and NA were applied microiontophoretically to them with the aid of multichannel glass microelectrodes. The central barrel was filled with a 2% solution of Pontamine azure 6BX in 2 M NaCl solution, and the side barrels with a 0.3 M solution of EST (pH 8.8), a 1 M solution of NA bitartrate, and 0.15 M NaCl solution. A six-channel microiontophorometer of our own design, made jointly with the Experimental Design Workshops (No. 1) of the Academy of Medical Sciences of the USSR, whereby several biologically active substances could be tested in series and in parallel, using iontophoretic, compensating, and holding currents, was used in the experiments. The strength of the iontophoretic current was 30 nA and its duration 20 sec. In the course of the experiment a control injection of NaCl, followed by NA and then EST, was given, and 20 sec later a second microiontophoretic application of NA was given. The time intervals between the different applications was 100 sec. Microelectrodes with a tip 3-5 μm in diameter, and with a resistance of the central barrel of 2-7 MΩ and of the side barrels of 30-100 MΩ were used. For the operations the rats were anesthetized with urethane (0.8 g/kg), immobilized with tubocurarine (0.3 mg/kg), and fixed in a type SEZh-2 stereotaxic apparatus. The electrode was inserted into the medial PA at coordinates $L = 0.2$, $H = 0$ (−1.5), AP = 7.8, using the atlas [5]. To verify exact positioning of the microelectrode, after the electrophysiological tests Pontamine azure was injected through the central barrel for 30 min, using an anodal current of 20 μA. Neuronal activity was recorded on a standard electrophysiological apparatus described by the writers previously [1, 3]. The results were analyzed on a Nokia LP-4840 analyzer, connected to an ES-9092 information store, for subsequent processing by an ES-1022 computer.

EXPERIMENTAL RESULTS

Activity of 625 PA neurons was studied in rats in different stages of the estrous cycle. During evaluation of the total pool of test neurons, in a high percentage of cases EST changed the sensitivity and direction of the original response of neurons of the cyclic center for hypothalamic regulation of gonadotropic function to NA. Of 338 neurons initially not responding to NA, 45.3 ± 2.7% acquired ability to respond to it after preliminary injection of EST. Of this number, 91 cells (26.9 ± 2.4%) were activated, but 62 (18.4 ± 2.1%) were inhibited by the monoamine. Thus, after estrogenization, previously insensitive PA neurons were activated significantly more often ($p < 0.01$) by this neurotransmitter. Moreover, 27.3 ± 3.9% of cells responding to NA by inhibition, acquired the ability to give an activating response after administration of the estrogen. As a result of all redistributions in the groups it was found that after preliminary microiontophoresis of EST, testing with NA induced an activation re-

sponse of PA neurons significantly more often (27.4 ± 1.8%) than an inhibitory response (19.8 ± 1.6%, $p < 0.001$). Of 287 neurons responding in one way or other to application of NA, in 50.5 ± 2.9% of cases they lost their sensitivity to the neurotransmitter.

The results of experiments carried out at different stages of the estrous cycle are given in Table 1. They show that among neurons with an initial inhibitory response, the highest percentage (55.6 ± 4.9%) of transformation into activating was observed on the morning of the proestrus stage. Meanwhile, the largest number of cells was observed among spontaneously active PA neurons which, while not responding initially to NA, acquired as a result of estrogenization the ability to respond to application of this monoamine by activation (44.5 ± 4.9%). It is interesting to note that it was at this time of the estrous cycle that the ability of EST to convert neurons responding in one way or another to NA into nonresponding cells was weakest. Whereas in other stages of the cycle the percentage of these neurons relative to the total number of cells fluctuated from 52 on the evening of the proestrus stage to 63 on the morning of diestrus, on the morning of proestrus we observed this effect in only 36.0 ± 4.8% of cases. Characteristically, this applied to cells giving both an activating (42.9 ± 4.9%) and an inhibitory response to NA (22.2 ± 4.1%).

The changes discovered in the morning of the proestrus stage can hardly be explained by a circadian rhythm of sensitivity of the PA neurons to NA. Comparison with the morning of the diestrus-2 stage indicates an essential difference between the character of the changes brought about by estrogens in the sensitivity and direction of the response of the test cells to NA ($p < 0.001$). This difference was most probably connected with the processes of change in the rats' endocrine status in the morning of the proestrus stage, when the blood levels of endogenous estrogens in female rats rise sharply. This signal to the brain from the periphery, brought about by increased excretion of sex steroids by the ovaries, reports on the level of readiness of the ripening oocytes for ovulation. Activation of PA neurons under its influence, and an increase in their sensitivity to NA for responding by activation are essential for this cyclic center to exert its triggering function, with excitation of its peptidergic LHRH-producing neurons and potentiation of LHRH translocation into the region of the mediobasal hypothalamus. All these provide the necessary basis for development of the preovulatory wave of LH in the blood on the evening of the proestrus stage, that is necessary for ovulation of a ripe oocyte within the next few hours. It has to be pointed out that our data on the modulating effect of EST on the sensitivity of PA neurons to NA are in full agreement with our results published earlier and with our conclusions regarding the character of the dynamics of sensitivity of single neurons of the cyclic center regulating the gonadotropic function to NA in the course of the estrous cycle [1, 2].

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