tage pulse does not reach zero in the region of values of E of about + 20 mV, whereas the curves of distribution of both types of charges reach saturation in this region. In the writers' view, this is because 10 mM tetraethylammonium chloride did not completely block the potassium channels, which were able to open by the time that these values of E were reached.

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DOPAMINE SENSITIVITY OF HYPOTHALAMIC ARCUATE NEURONS AND THEIR ROLE IN THE REGULATION OF PITUITARY GONATROPIC FUNCTION

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The effect of microiontophoretic injection of dopamine (DA) into the arcuate region of the hypothalamus on single unit activity and the plasma and pituitary levels of luteinizing hormone (LH) was investigated in rats in various stages of the estrous cycle. No significant differences in the number of neurons responding by activation or inhibition or not responding to DA could be observed in the course of the estrous cycle. However, in the first half of the day of proestrus (P) a significant increase in the number of neurons responding by activation to microiontophoretic injection of DA was observed. In all stages of the estrous cycle except the second half of P the activation response of the neurons was coupled with elevation of the plasma LH level. A significant rise in the LH level in the pituitary in response to microiontophoretic injection of DA into the hypothalamic arcuate region was observed only in stage diestrus 2.

KEY WORDS: Hypothalamus; neurons; dopamine; microiontophoresis; luteinizing hormone.

The arcuate region of the hypothalamus (ARC), the tonic center of regulation of pituitary gonadotropic function, must be regarded as a component of the neuroendocrine system in which the action of neuromediators is switched to the corresponding hypothalamic releasing factors. It has been shown that many dopamine terminals originating from higher levels of the CNS are represented in this region [2]. Dopamine (DA), as a mediator, occupies a special place in the regulation of gonadotropin releasing factors and, in particular, of luteinizing hormone releasing factor (LH-RF). Most data in the literature indicate its activating action on the liberation of pituitary luteinizing hormone (LH), mediated through LH-RF [7, 12].

This paper describes a combined study of the sensitivity of the neurons to microiontophoretic injections of DA and subsequent changes in the plasma and pituitary LH levels in rats at different stages of the estrous cycle.

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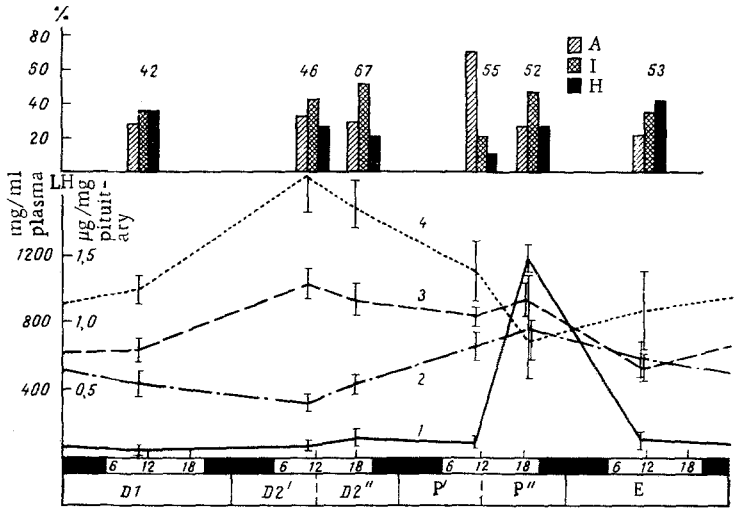

Fig. 1. Unit responses and changes in blood and pituitary LH levels after microiontophoretic injection of DA into ARC at different stages of estrous cycle in rats. Abscissa, hours and stages of estrous cycle; ordinate: above – percentage of number of neurons recorded at that stage of the cycle, below – LH levels in plasma (in mg/ml) and pituitary (in μg/mg). Numbers above columns represent number of neurons analyzed. A) Activation; I) inhibition; N) no response. 1, 3) LH levels in plasma and pituitary of control animals; 2, 4) LH level in plasma and pituitary after microiontophoresis of DA. Explanation in text.

EXPERIMENTAL METHOD

Experiments were carried out on 69 rats weighing 180-200 g with a stable 4-day estrous cycle. The animals were kept under conditions of a standard diet and illumination (14 h of light). Single unit activity was investigated in rats anesthetized with ether and immobilized with tubocurarine (0.3 mg/100 g body weight). Microelectrodes, oriented according to de Groot's atlas [6], were introduced to coordinates L = 0.5, H = 3.5, A = 5.4. Multiple-barreled glass microelectrodes were used; the central barrel was filled with 2 M NaCl solution and was used to record spontaneous single unit activity in ARC. The side barrels were filled with physiological saline for the control test, with 2 M DA hydrochloride solution (pH 7.2), and with 2 M $FeCl_3$ solution for iontophoretic tagging of the position of the microelectrode tip. The resistance of the central barrel was 2-5 MΩ and of the side barrels 200-300 MΩ. Unit activity was recorded during three 120-sec intervals before, during, and after the end of microiontophoresis. An iontophoretic current with a strength of 100 nA was used in accordance with data in the literature. The effect of DA was considered to be significant if the level of unit activity was above 125% or below 75% of its initial value. Only those experiments in which microiontophoretic injection of physiological saline by a current of 100 nA did not affect spontaneous ARC unit activity were subjected to analysis. The details of recording, microiontophoresis, analysis of spike activity, and the histological control of the location of the microelectrode tip were described previously [1]. Of the 69 experimental animals, the LH level in the pituitary and blood was investigated in 50 by a radioimmune method [10] 20 min after the end of microiontophoretic injection of DA into ARC.

EXPERIMENTAL RESULTS AND DISCUSSION

In the course of the experiment activity of altogether 315 ARC neurons was recorded in animals in different stages of the estrous cycle. The latent period of response of the neurons to injection of DA varied in these experiments from a few hundreds of milliseconds to a few seconds. Changes in the sensitivity and direction of response of the ARC neurons after microiontophoretic injection of DA are illustrated in Fig. 1. The figure shows that in stage diestrus 1 (D1) among the discharging ARC neurons there were no significant differences in the representation of cells responding by activation or by inhibition or not responding to DA. However, in the course of the day diestrus 2, in its first (D2') and second (D2") halves an increase in the representation of neurons responding by inhibition to DA was observed. Although the differences found did not reach statistical significance, a marked tendency for their development was found (χ^2 for D1-D2 0.941, for D2-D2" 0.013, for D1-D2" 3.412). At the stage of the first half of the day of proestrus (P') when, according to data

in the literature, the level of endogenous estrogens reaches a maximum [3, 4], marked predominance of cells responding by activation to DA was observed among the ARC neurons (χ^2 for D2"-P' 20.3). At the stage of the second half of the day of proestrus (P"), cells responding by inhibition again predominated among the discharging ARC neurons, just as in stages D1 and D2 (χ^2 for D2"-P" 0.557). The changes arising during proestrus were significant (χ^2 for P'- P" 19.09). In the stage of estrus (E), among the ARC neurons a small majority did not respond to microiontophoretic injection of DA, although the differences between P" and E were not significant (χ^2 for P"-E 2), as was also the case when E and D1 were compared (χ^2 for E-D1 0.546). As the results show, the most significant changes in the sensitivity of the ARC neurons to DA took place in the stage P'. In this stage of the estrous cycle, which differs from the resting in having the maximal peripheral blood estrogen concentration, a marked and statistically significant change was found in the predominant response of the ARC neurons to DA, namely the manifestation of its activating effect.

The next step was to analyze changes in the blood and pituitary levels of LH of the animals after the end of microiontophoresis in relation to the direction of the unit responses. Analysis of the data showed that in 32 of the experiments with microiontophoresis of DA the LH level rose. In 21 of these experiments (65.6%) this was combined with an activating effect of DA on the ARC neurons, in 10 (31.2%) with an inhibitory action of DA, and in one case the LH level was increased whereas DA had no effect on the activity of the ARC neurons. DA, incidentally, lowered the LH level in only four of the 50 experiments, and this effect of DA, moreover, was observed only in stage P". As data in the literature [3, 4, 14] and experiments on the control animals (Fig. 1) showed, this phase of proestrus is characterized by the development of a preovulatory LH wave in the blood. Because of the sharp differences in the initial LH level in P" compared with the other stages of the estrous cycle, the results of these experiments could not be subjected to statistical analysis in conjunction with the data as a whole. Analysis of the data for all stages of the estrous cycle except P" showed that in 21 of the 23 experiments in which DA stimulated activity of the ARC neurons the plasma LH level was raised, whereas in two it was unchanged. The action of DA was inhibitory, elevation of the LH level was observed in nine and no effect in six experiments. Of the three experiments in which no change was observed in the activity of the ARC neurons in response to DA, the plasma LH level was raised in one and unchanged in two. Statistical analysis of the results, with calculation of the index of differences (χ^2) led to the conclusion that differences were significant in the alternative groups ($\chi^2 = 6.957$).

The results are evidence that the activating effect of DA on ARC neurons leads most frequently to a significant increase in the blood LH level.

The blood LH concentration at different stages of the estrous cycle in the control animals (curve 1) and the animals with an activating effect of DA on the discharge of the ARC neurons (curve 2) is shown in Fig. 1. It is an interesting fact that in some experiments the elevation of the blood LH level was combined with an inhibitory effect of DA on the ARC neurons. A functioning system of neurons blocking the liberation of RF-LH of the peptidergic neurons of the mediobasal hypothalamus evidently exists in ARC. This blocking effect is probably mediated through inhibition of peptidergic neurons, resembling the Renshaw type of recurrent inhibition of spinal motoneurons. The inhibitory effect of DA on the nerve cells included in the system of recurrent inhibition of peptidergic neurons must lead to facilitation of the liberation of releasing factors into the pituitary portal system. The existence of a system of recurrent inhibition has been reliably proved for the neurohormonal cells of the supraoptic and paraventricular nuclei of the hypothalamus [9]. The results showing that microiontophoretic injection of DA in the phase of the preovulatory wave of LH liberation (P") can induce a decrease in the blood LH level are also interesting. The effect of DA on liberation of the releasing factors of the gonadotropic hormones may perhaps be determined not only by the modulating effect of endogenous estrogens [13], but also by the blood LH concentration. Pituitary gonadotropic hormones themselves are known to be able to change the level of spontaneous activity of ARC neurons [8]. This may evidently account for the contradictory data in the literature on the effect of DA on the release of LH [5, 7, 11, 12].

As Fig. 1 shows, in the control animals the pituitary LH level reached a minimum in E. In the course of D1 it gradually increased, and the increase became statistically significant in D2. Microiontophoretic injection of DA led to a significant increase in the pituitary LH level only in stage D2. The process of accumulation of LH in the pituitary, which is under the control of hypothalamic structures, may perhaps be mediated to a certain degree by DA. However, the direction of the effect of DA on the activity of ARC neurons cannot be connected with changes in the pituitary LH level, for the value obtained for the index of differences showed them not to be significant in the alternative groups ($\chi^2 = 2.63$).

The results indicating that DA participates in the process of hypothalamic regulation of pituitary gonadotropic functions thus suggests that the stimulant effect of DA on LH release may be connected with both its ac-

tivating and its inhibitory action on ARC unit activity. However, the first mechanism evidently plays the decisive role.

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CORRELATION BETWEEN ACTIVITY OF THE KALLIKREIN, PLASMIN, AND THROMBIN SYSTEMS OF THE BLOOD DURING INTENSIVE PHYSICAL EXERTION

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Changes in the level of precursors of kallikrein, plasmin, and thrombin and of their inhibitors were studied by a combined method developed by the authors. The blood plasma of healthy athletes and of athletes with a syndrome of myocardial overstrain was investigated at rest and during intensive work on a bicycle ergometer. The results of the test and of correlation analysis reveal functional correlation between the components of the "Hageman factor system." In athletes with a syndrome of overstrain, the functional capacity of the humoral systems of vascular control was impaired, as reflected in a reduced level of proenzyme activity and a decrease in the values of the inhibitors. Correlation between the above-mentioned indices at rest and during exertion was disturbed in these subjects.

KEY WORDS: kallikrein–kinin system in man; physical exertion.

Adaptation of the hemodynamics to the factors of physical exertion takes place with the participation of the kallikrein system of the body. As a result of continuous physical training, the human kallikrein–kinin system undergoes modifications that reflect its increasing perfection as one of the regulatory mechanisms of the circulation [3, 4].

In the modern view, the kallikrein–kinin system of the blood is looked upon as being in functional unity with the fibrinolytic and clotting systems, as an important component of the mechanism of vascular control [1, 5, 7]. Changes in the activity of the fibrinolytic and clotting systems of the blood under the influence of physical exertion have been studied previously [6, 8].

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