The dopamine sensitivity of individual neurons of the midbrain preoptic region as a center of the cyclic regulation of hypophysial gonadotropic function was investigated under conditions of dopamine microiontophoretic delivery during various stages of the estrous cycle in rats. It was shown that most neurons do not respond to dopamine during the diestrus-1 stage. The first half of the day of the diestrus-2 stage was characterized by a high representation of neurons with an activation response. Cells with an inhibitory response to dopamine were observed to increase among the spontaneously active neurons of the preoptic region starting from the second half of the day of this stage of the estrous cycle. The greatest quantity of neurons with an inhibitory response was observed during proestrus.

KEY WORKS: preoptic region; neurons; dopamine; microiontophoresis; estrous cycle.

The preoptic region of the midbrain plays an exceptionally important role in the mechanism of central regulatory influences in the neuroendocrine system, being the center determining the cyclic character of hypophysial gonadotropic function [9, 15]. The presence of luteinizing hormone in the preoptic region is now known [12, 17], and a direct connection has been demonstrated between luteinizing hormone and the arcuate regions of the hypothalamus and median eminence, through which gonadotropic function is directly controlled by the overlying segments of the central nervous system [13]. However, a number of the fine mechanisms of the process of cyclic control of gonadotropic function by the preoptic region and, in particular, the significance of various neuromediators remain insufficiently clarified. Earlier we studied the sensitivity of neurons of the midbrain preoptic region to noradrenaline during the estrous cycle in rats [4]. The data obtained indicated the releasing role of noradrenaline in effecting the preovulatory surge of luteinizing hormone in the blood and were consistent with the results of other authors [14]. However, there are data in the literature indicating a rather high concentration of dopamine in the preoptic region. Of the midbrain regions, this region is second in dopamine level only to the median eminence and the arcuate nucleus [16]. At the same time the presence of a specific sequence of changes in the content of this monoamine in the preoptic region has been noted during the estrous cycle, with a minimum in the evening hours of proestrus [10]. The foregoing facts apparently permit the hypothesis that this mediator also plays a definite role in regulating the gonadotropic function of the hypophysis at the level of the cyclic center. The present paper studies the sensitivity of neurons of the preoptic region to dopamine under conditions of its microiontophoretic delivery during various stages of the estrous cycle in rats.

METHOD

The experiments were conducted on 77 female rats of 200-230 g mass with a stable four-day estrous cycle. The animals were maintained under standard conditions of nutrition and illumination (14 h light, 10 h darkness). Animals immobilized with tubarine (0.3 mg per 100 g mass) under conditions of urethane narcosis (100 mg per 100 g mass) were transferred to artificial respiration and secured in an SEZh-2 stereotactic apparatus. Extracellular recording and the microiontophoretic delivery of dopamine were done using multichannel glass microelectrodes. The central stem of the electrode used to recorded the impulse activity of single preoptic-region neurons was filled with 2% pontamine azure in 2 M NaCl and had a resistance of 3-10 MΩ. The side stems were filled with 2 M dopamine hydrochloride and 0.15 M...
TABLE I. Distribution of Number of Animals and Recorded Neurons of Preoptic Region in Different Stages of the Estrous Cycle

<table>
<thead>
<tr>
<th>Investigated Stages of estrous cycle</th>
<th>diestrus-1</th>
<th>diestrus-2</th>
<th>proestrus</th>
<th>estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Neurons</td>
<td>30</td>
<td>36</td>
<td>43</td>
<td>40</td>
</tr>
</tbody>
</table>

NaCl to control for the effect of the electrical current on the investigated structure and the application of the compensating potential during dopamine microionophoresis to individual preoptic-region neurons. The resistance of the side stems comprised 30-100 MΩ. Microelectrodes oriented in accordance with the atlas [11] were inserted into the brain at the coordinates H = 0.0 (±1.5), AP = 7.8, L = 0.4. Dopamine microionophoresis was done using a multichannel microionophoreometer. The strength of the iontophoretic current was 120 nA; a 100-sec duration was provided for with a precision of up to 1 msec with a quartz VA-G-120 generator. Neuronal activity was recorded using a UBP-03 biopotential amplifier, SI-16 oscillograph supplied with an FOR-2 photorecording apparatus, and a seven-channel NO-36 magnetograph. The amplitude–frequency–time analysis of the impulse activity of individual neurons was done using a Nokis LF-4840 multichannel analyzer, the information from which was fed to an ES-1022 computer for subsequent analysis by a special program. Neuronal activity was recorded during 100-sec time intervals before, during, and after the cessation of the microionophoresis of 0.15 M NaCl or dopamine. After recording the activity through the central stem, filled with 2% pontamine azure in 2 M NaCl, dye iontophoresis (10 μA, 20 min) was done to localize the position of the tip of the microelectrode. The marker was found in brain sections of 30 μ thickness prepared with a freezing microtome and stained with hematoxylin eosin.

RESULTS AND DISCUSSION

The spontaneous activity of 256 neurons in the preoptic region was investigated in the course of 77 experiments. Table 1 presents the distribution of the quantity of investigated animals and the number of neurons studied in the animals at various stages of the estrous cycle. In evaluating the dopamine sensitivity of preoptic-region neurons, it was found that 62 of the neurons in the entire pool of measurements, i.e., 24.2%, showed an activation response to dopamine microionophoresis, 103 neurons (40.2%) were inhibited, while 91 neurons (35.6%) did not respond to dopamine. It should be noted that the inhibitory response of neurons of the preoptic region was of a more stable character by comparison with the activation effect, while in eight instances the latter effect was of the character of brief surges of an increase in the frequency of spontaneous activity on the subsequent frequency histogram. The distribution of the frequency characteristics of background activity of spontaneously active neurons in the preoptic region did not differ appreciably in the present series of experiments from data published by us earlier.

The experimental results obtained upon analysis of the character of the response of preoptic-region neurons to microiontophoretic dopamine delivery during various stages of the estrous cycle are presented in Fig. 1. As apparent from the figure, the character of the distribution and the predominant representation of neurons with a specific response to dopamine changed during the estrous cycle. The majority of the cells (41.0 ± 7.9%) among the spontaneously active preoptic-region neurons did not respond to dopamine during the diestrus-1 stage of the cycle, 28.2 ± 7.2% gave an activation response, while 30.8 ± 7.2 were inhibited. Thus, there was no appreciable difference in the representation of neurons showing activation or inhibition in response to dopamine delivery during the diestrus-1 stage, and in addition to this a pronounced unresponsiveness of the given structure with respect to dopamine was demonstrated. An increase in the representation among the spontaneously active preoptic-region neurons of cells with an activation response to dopamine (47.2 ± 8.3%) was observed later during the estrous cycle, during the morning hours of the diestrus-2 stage. At the same time the quantity of inhibitory neurons did not change appreciably (25.0 ± 7.2%), but the excitability of the structure increased, as indicated by the decline in the representation of cells not responding to dopamine (27.8 ± 7.4) by comparison with the diestrus-1 stage (41.0 ± 7.9). An