Department of Morphology (Prof. K. Kurosumi), Institute of Endocrinology, Gunma University, Maebashi, Japan

Possible Relations between the Secretory Cycle of the Neurosecretory Cells in the Rat Paraventricular Nucleus and the Estrous Cycle

Yasue Yukitake

Received May 10, 1978

Summary. The ultrastructure of neurosecretory cells in the paraventricular nucleus of the female rat in various stages of the estrous cycle was studied by electron microscopy. On account of the secretory cycle, the neurosecretory neurons were classified into four types, and the incidence of each type was counted throughout the estrous cycle. In proestrus afternoon, neurons at the phase of protein synthesis (Type I neuron) increased in number. In estrus and metestrus, neurons at the phase of granule production (Type II neuron) and granule storage (Type III neuron) increased in number. In diestrus and in early morning of the proestrus, neurons at the phase of granule transport (Type IV neuron) and Herring bodies increased in number and in size. The secretory cycle in the paraventricular nucleus of the female rat was intimately related to the estrous cycle. We also observed the neurons in the supraoptic nucleus, but there were no changes accompanying the estrous cycle.

It has been evidenced that the plasma concentration of gonadotropins and ovarian steroids change periodically with the estrous cycle (Butcher et al., 1974). As regards oxytocin, however, in spite of its intimate relation to reproduction, it has been unknown whether the secretion or plasma concentration of this hormone changes periodically or not. Since Bargmann's first publication (1949), it has been supported by powerful experimental evidence (Hild, 1951; Stutinsky, 1951; Sloper and Adams, 1956) that the neurosecretory materials including oxytocin and vasopressin are synthesized in the perikaryon and transported to the nerve terminals by axonal flow. The classical view on the distribution of oxytocin- and vasopressin-producing cells (Hild and Zetler, 1951, 1953) had suggested that the paraventricular nucleus was predominantly or entirely responsible for oxytocin production, while the supraoptic nucleus synthesized mainly vasopressin. However, recent hormone assays and electrophysiological studies indicated the presence of both hormones in both nuclei, but relatively more oxytocin was demonstrated to be synthesized in the paraventricular nucleus and more vasopressin in the supraoptic nucleus (Burford et al., 1974). By an immunofluorescence study, on the contrary, Swaab et al. (1975) reported that the oxytocin-containing cells were 2.5 times more in the supraoptic nucleus than in the paraventricular nucleus, though both nuclei contained both hormones. We examined morphologically periodical changes of the ultrastructure of neurosecretory cells in both nuclei concurrent with the estrous cycle. At the level of light microscopy (Pascualino and Ragonese, 1958), it was already reported that the neurosecretory nucleus in the hypothalamus was closely connected with the estrous cycle. Furthermore, recent electrophysiological studies demonstrated that the frequency of
the electrical spike might change periodically accompanying the estrous cycle in the rat paraventricular neurons (Negoro et al., 1973).

In a previous paper (Yukitake et al., 1977), we reported the observations by electron microscopy of the paraventricular nuclei of male rats, and classified the neurosecretory neurons into four morphologically distinct types. We thought that they did not represent different classes of neurons but different phases of secretory activity of a single cell type. In the present paper, we will show that the secretory cycle of neurosecretory cells of the paraventricular nucleus is intimately related to the estrous cycle in the case of female animals.

Materials and Methods

Adult female albino rats of the Wistar-Imamichi strain were used. They were housed in cages in a room maintained under conditions of controlled light (light on for 14 hr. a day) and constant temperature (about 22°C), and showed a regular estrous cycle as examined by vaginal smear every noon. These rats were fed with Oriental solid chow and drinking water ad libitum. They were anesthetized by intraperitoneal injection of Nembutal (75-100 mg/kg body weight) and fixed by slow perfusion with 2.5% glutaraldehyde and 2.0% paraformaldehyde buffered to pH 7.2 with 0.1M sodium cacodylate from the left ventricle. Thin slices of the diencephalic area required were postfixed with 2.0% osmium tetroxide buffered with the same cacodylate buffer. All rats were sacrificed every 6 hrs (midnight, 6 a.m., noon, 6 p.m.) in each stage of the estrous cycle. Thin sections of Epon-Araldite embedded tissue were stained with uranyl acetate and lead citrate, and examined in a Hitachi transmission electron microscope HU-11D at an accelerating potential of 75 kV. As the ultrastructure of the neurosecretory cells did not change within a day except in proestrus, electron micrographs of materials fixed at noon in each stage of the estrous cycle were used for the morphometry. Only in proestrus, two groups of materials taken either at 6 a.m. or in the afternoon were used for counting incidences of different types of secretory neurons, because they changed remarkably within the proestrus stage. All electron micrographs were taken at 1,000 times in direct magnification and thereafter enlarged to the final magnification of 5,000 times. About one hundred secretory neurons in each stage of estrous cycle were photographed and classified into four types of secretory cycle and the numbers of neurons in each type were counted. The results were calculated to be demonstrated in percentage of all the number of secretory neurons observed in each stage of the estrous cycle.

Results

Four types of neurosecretory cells described in the paraventricular nucleus of male rats as reported in the previous paper (Yukitake et al., 1977) were also found in the same nucleus of the female rats. The ultrastructural characteristics of each type were identical with those described on the male material (Yukitake et al., 1977) and are briefly as follows: Type I (phase of synthesis) is characterized by dilated cisternae of RER and a few secretory granules, Type II (phase of granule production) by collapsed cisternae of RER and moderate numbers of granules in the Golgi area, Type III (phase of granule storage) by collapsed cisternae of RER and a large number of granules throughout the cell, and Type IV (phase of granule transport) by collapsed cisternae of RER and few secretory granules in the perikaryon.
The incidence of occurrence of each type of neurons that corresponds to each stage in the secretory cycle in the paraventricular nuclei of the normal male rats was shown in Table 1. It was probable that this result was caused by the difference in length of time for each phase in the secretory cycle. Namely, the time for Type II or Type III seemed to be longer than that of Type I or Type IV stages. In the female rats, however, the neurosecretory cells in the paraventricular nuclei were so variable from time to time of the estrous cycle, that it was very difficult to find a definite law. According to the observation at every 6 hrs throughout the entire period of the estrous cycle, morphological changes of the neurosecretory cells were not related to the circadian rhythm, but to the estrous cycle except in proestrus. As it was found that the morphological characteristics of neurosecretory cells in proestrus morning were quite different from those in the afternoon, we took the material both in the morning and afternoon.

1) In proestrus afternoon: Most neurosecretory cells showed characteristics of Type I (Fig. 1), but the time for the stage of Type I seemed to be very short, because it was rare to observe such many neurons of type I at noon of proestrus. We observed the cases in which most neurons indicated the characteristics of Type I, in two out of six rats which were sacrificed from noon to 5 p.m. However, the incidence of occurrence of Type I neurons showed the highest in every case of this period. As we found many neurosecretory cells classified in Type I at this period, the secretory cycle of paraventricular neurosecretory cells seemed to start at a certain time in the afternoon of proestrus.

2) In estrus: The highest incidence in this period was shown by Type II and the next was Type I (Table 1). The large number of Type II neurons in this stage indicated that many of the Type I neurons found in proestrus afternoon had changed their structure to become Type II neurons (Fig. 2). As Type II neuron corresponds to the phase of granule formation, most secretory granules may probably be formed in the estrus stage in the female rat.

3) In metestrus: The neurosecretory cells in this period were less in variation and looked similar to those in estrus. But the number of secretory granules in the cytoplasm increased to some extent (Fig. 3), and typical Type III neurons suggested to be the phase of granule storage were observed in some places. In this period, Type I neurons were very seldom found and few Type IV neurons were observed either. Almost all the neurosecretory cells in this stage contained more or less secretory granules in the cytoplasm. Hence, this period seemed to be the phase which

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proestrus after</td>
<td>14.2</td>
<td>38.3</td>
<td>29.1</td>
<td>18.0</td>
</tr>
<tr>
<td>Estrus</td>
<td>25.5</td>
<td>42.6</td>
<td>16.3</td>
<td>17.1</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metestrus</td>
<td>8.2</td>
<td>52.7</td>
<td>26.7</td>
<td>15.5</td>
</tr>
<tr>
<td>Diestrus</td>
<td>5.1</td>
<td>11.3</td>
<td>52.6</td>
<td>31.9</td>
</tr>
<tr>
<td>Proestrus morn</td>
<td>21.2</td>
<td>15.2</td>
<td>15.9</td>
<td>48.2</td>
</tr>
</tbody>
</table>

The incidence of occurrence of each type of neurons that corresponds to each stage in the secretory cycle in the paraventricular nuclei of the normal male rats was shown in Table 1. It was probable that this result was caused by the difference in length of time for each phase in the secretory cycle. Namely, the time for Type II or Type III seemed to be longer than that of Type I or Type IV stages. In the female rats, however, the neurosecretory cells in the paraventricular nuclei were so variable from time to time of the estrous cycle, that it was very difficult to find a definite law. According to the observation at every 6 hrs throughout the entire period of the estrous cycle, morphological changes of the neurosecretory cells were not related to the circadian rhythm, but to the estrous cycle except in proestrus. As it was found that the morphological characteristics of neurosecretory cells in proestrus morning were quite different from those in the afternoon, we took the material both in the morning and afternoon.

1) In proestrus afternoon: Most neurosecretory cells showed characteristics of Type I (Fig. 1), but the time for the stage of Type I seemed to be very short, because it was rare to observe such many neurons of type I at noon of proestrus. We observed the cases in which most neurons indicated the characteristics of Type I, in two out of six rats which were sacrificed from noon to 5 p.m. However, the incidence of occurrence of Type I neurons showed the highest in every case of this period. As we found many neurosecretory cells classified in Type I at this period, the secretory cycle of paraventricular neurosecretory cells seemed to start at a certain time in the afternoon of proestrus.

2) In estrus: The highest incidence in this period was shown by Type II and the next was Type I (Table 1). The large number of Type II neurons in this stage indicated that many of the Type I neurons found in proestrus afternoon had changed their structure to become Type II neurons (Fig. 2). As Type II neuron corresponds to the phase of granule formation, most secretory granules may probably be formed in the estrus stage in the female rat.

3) In metestrus: The neurosecretory cells in this period were less in variation and looked similar to those in estrus. But the number of secretory granules in the cytoplasm increased to some extent (Fig. 3), and typical Type III neurons suggested to be the phase of granule storage were observed in some places. In this period, Type I neurons were very seldom found and few Type IV neurons were observed either. Almost all the neurosecretory cells in this stage contained more or less secretory granules in the cytoplasm. Hence, this period seemed to be the phase which
Fig. 1. Low magnification electron micrograph of secretory neurons from the paraventricular nucleus in proestrus afternoon. Most neurons are Type I having well-developed RER with dilated cisternae. ×1,500
was shifted from granule formation to granule storage.

4) In diestrus: In this period, the number of the secretory granules in the cytoplasm increased more and more (Fig. 4). Many typical Type III neurons were observed, and Type IV neurons and, in their vicinity, Herring bodies were found in some places. In this period, the neurosecretory granules seemed to be stored in the cytoplasm, and partially transported to the axons.

5) In proestrus morning: In this period, most neurosecretory cells showed Type IV or Type I, and many axon-swellings filled with numerous secretory granules were observed (Fig. 5). They were Herring bodies and were probably formed during this period in the female rat paraventricular nucleus. Type II neurons were also observed, though they were very few.

6) The supraoptic nucleus: In paraffin sections, both the supraoptic and para-
ventricular nuclei were intensely stained with Gomori’s aldehyde-fuchsins. But the neurons in the paraventricular nucleus were stained differently from one period to another in the estrous cycle, while the neurons in the supraoptic nucleus were stained uniformly. Herring bodies in the paraventricular nucleus always appeared near the perikarya and were relatively large in size, whereas those in the supraoptic nucleus appeared at a distance from perikarya and were not so large. Careful observation could not reveal any morphological changes accompanying the estrous cycle in the supraoptic nucleus.

Discussion

One of the purposes of this paper is to evidence the secretory cycle of the neurosecretory cells in the rat paraventricular nucleus, which was postulated in our previous paper (Yukitake et al., 1977). We agreed with Zambrano and De Robertis (1966) who stated that the perikarya of the neurosecretory cells in the supraoptic
nucleus have a secretory cycle. Our previous results, however, differed from theirs in two points. We could not observe the intracisternal bodies in the paraventricular nucleus and we suggested that one Herring body might be formed at the end of each secretory cycle. Theirs and ours were both observations at one time section of the progressing changes of individual neurosecretory neurons and the argued secretory cycle was nothing more than a speculation. The present observations are dynamic though asynchronous, because the estrous cycle is the physiological periodic changes of female animals. The results are consistent with the idea that each type of neurosecretory cell represents a different stage in the specific secretory cycle rather than a difference in neuron grouping. Neurosecretory cells in various types except for some of Type IV are easily identified to be secretory neurons, because they contain more or less characteristic secretory granules. However, some cells that we classified into Type IV contained almost no granules and there was a possibility that some of them might be neurons of another group. Recent studies using horseradish peroxi-
dase evidenced the presence of tracts from the hypothalamic nuclei to the spinal cord (Kuypers and Maksy, 1975). According to such studies, horseradish peroxidase injected into the upper spinal cord was demonstrated in the neurons with poor secretory granules in the paraventricular nucleus (Saper et al., 1976). The horseradish peroxidase labelling, however, was shown in the neurons at the parvocellular part of the paraventricular nucleus, and probably not in the magnocellular part. The latter contains exclusively neurosecretory neurons and our observation was limited to this part. It seems thus safe to say that most of the Type IV neurons here classified are not the neurons connected to the spinal cord, but one of the paraventriculohypophyseal tract of secretory nature.

Another purpose of this study is to search for the controlling factors of the secretory cycle in the paraventricular neurons. We performed morphological and morphometric analysis on the terminal boutons abutting on the surface of the neurosecretory cells (Kurosuni and Yukitake, 1977). The incidence of the occurrence of

Fig. 5. Electron micrograph of secretory neurons from the paraventricular nucleus in proestrus morning. Most neurons are Type IV and Herring bodies are increased in number and size. H Herring bodies. ×1,500
different types of the boutons as well as the ratio of the surface area of the perikarya covered by the boutons were almost constant among different stages of the secretory cycle of the paraventricular neurosecretory neurons. The results indicated that these types of neurosecretory cells represented different stages in the cyclic change of secretory function. But the synaptic vesicles in the terminal boutons abutting on the secretory perikarya clearly increased in number as the stages of the neurosecretory cycle advanced from Type I to Type IV. It is suggested that these terminal boutons may transmit some nervous impulses controlling the secretory or synthetic functions of the paraventricular secretory neurons. Since it is well known that the sex steroid might act on the neurosecretion in the hypothalamus (Flament-Durand and Desclin, 1968; Stumpf, 1968), it might be suggested that neurosecretory cells were intimately related to the estrous cycle. By electrophysiological experiments, Negoro et al. (1973) reported that the frequency of the spike changed periodically with the estrous cycle in paraventricular neurons. According to them, frequency of the spike in paraventricular neurons showed the highest value in proestrus and the lowest in diestrus. Furthermore, a biochemical quantification by radioimmunoassay

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Proestrus afternoon</td>
<td></td>
<td></td>
<td>-25%</td>
</tr>
<tr>
<td>Estrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metestrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diestrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proestrus morning</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. Graphic representation of results shown in Table 1. Arrows with solid line indicate the shift of the peak, while those with broken line indicate the parallel shift of the bottom.
demonstrated in rats that the plasma concentration of estrogen was the highest in proestrus morning and that of progesterone in proestrous afternoon (Butcher et al., 1974). Although physiological or biochemical results do not always correspond to those of morphology, it is interesting that in proestrus the neurosecretory cells of the paraventricular nucleus are most remarkably changed also in morphology. The results of electrophysiology and also of morphology showed the periodicity only in the hypothalamus but indicated that plasma concentration of vasopressin or oxytocin did not change periodically accompanying the estrous cycle. It is probable that hormone synthesis in the hypothalamus and hormone release in the posterior pituitary are controlled by different factors. Kazawa (1976) showed changes in the numerical and volume densities of the secretory granules accompanying the estrous cycle and aging in the median eminence. As far as we observed, however, the neurosecretory granules in the posterior pituitary did not change with the estrous cycle. The fact found in the present study is that the estrous cycle, which is intimately related to sex steroid, is one of the factors controlling the secretory cycle in the female rat paraventricular nucleus, but not effective for the release of hormones from the posterior pituitary.

Fig. 7. Diagram showing probable correlation between secretory cycle and estrous cycle in female rat paraventricular nucleus. Type I neuron increase in number at proestrus afternoon and the secretory cycle may start at this period. As the estrous cycle proceeds, the neurons may change in structure in accordance with secretory cycle. Herring bodies may be formed in the early morning of proestrus. M midnight, N noon.
As shown in Table 1 and Figure 6, the functional phases of neurosecretory cells in the paraventricular nucleus in the female rat regularly shift concurrently with the advancement of the estrous cycle. It is suggested that most but not all the secretory neurons in this nucleus are changed in their structure and function concomitantly with the estrous cycle, because a small percent of the secretory neurons do remain in the opposite phase of estrous cycle, shown as bottoms in the curves of Figure 6. The peak values are between 50 to 60%, while those at the bottom are 10–15%. It might be conjectured that at least 60% of the secretory neuron population in the female paraventricular nucleus are strongly affected by the reproductive steroids or gonadotropins. They are probably concerned either directly or indirectly with the production of oxytocin, but neurosecretory cells of population between 10 to 40% might be associated with the production of vasopressin. These results are in accordance with those of Burford et al. (1974) but not with the results of Swaab et al. (1975). The settlement of this debate must await further studies with many different means of biological techniques.

References


