Seasonal Changes of the Ultrastructure of the Pars tuberalis of the Hypophysis of *Rana temporaria*

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Summary. In the pars tuberalis of the hypophysis of *Rana temporaria*, which shows the ultrastructural characteristics of a polypeptide hormone secreting endocrine gland, seasonal changes of the ultrastructure are described. In accordance with the literature, these seasonal changes of ultrastructure are interpreted as the morphological expression of seasonal changes of endocrine activity of the pars tuberalis.

Key words: Pars tuberalis — *Rana temporaria* — Seasonal changes of ultrastructure — Electron microscopy.

Introduction

In a previous paper, the structure and the vascularization of the pars tuberalis of the hypophysis of *Rana temporaria* were described. It was concluded that the pars tuberalis presents the general structural and cytological characteristics of a polypeptide hormone secreting endocrine gland. Electron microscopy showed that all pars tuberalis cells have the same general structure. They are elongated cells with long branching processes ending on the external basement membrane of the pericapillary space. The pars tuberalis cells contain spheroidal and rod-shaped secretory granules which are apparently formed in the Golgi apparatus. In the perikaryon only a small number of secretory granules is present. They accumulate in the pericapillary endings of the cell processes. The accumulation of numerous secretory granules in these endings, of which the plasma membrane is in close contact with the pericapillary space, strongly suggests a release of secretory products into the blood capillaries of the pars tuberalis. Electron microscopy pleads for an asynchronism of secretory activity between the individual cells of one and the same pars tuberalis (Dierickx *et al.*, 1971a). In this paper, ultrastructural evidence of an annual secretory cycle of the pars tuberalis is described.

Material and Methods

Adult male frogs of the species *Rana temporaria* were used. The frogs were of about the same length and weight.

From July 1970 till August 1971, each month, two animals were killed by decapitation. The lower jaw and the basis of the skull were immediately removed, so that the brain, which remained in situ, was easily accessible to fixatives.

For transmission electron microscopy the brain, together with the remaining parts of the skull, was briefly washed in a modified Millonig phosphate buffer (pH 7.4) and then fixed for 90 min with 3.9 per cent glutaraldehyde in phosphate buffer. The fixation was followed by washing for 30 min in phosphate buffer. During this washing period, of each animal, the pars tuberalis of the hypophysis was removed bilaterally, together with the caudal end of the pars...
ventralis tuberis cinerei, under a Zeiss stereomicroscope. The tissue pieces were post-fixed for 1 hour in 2 per cent osmium tetroxide. After post-fixation, the tissue pieces were washed in phosphate buffer, dehydrated in an ethanol series and embedded in Epon 812.

For general orientation studies, sections of 1 micron were made. After elimination of the Epon, the sections were stained with Harris hematoxyline (Aparicio et al., 1969).

Then, with LKB Ultrotomes III, uninterrupted serial sections of 600 Å were made. The sections were stained with uranyl acetate and lead citrate (Reynolds, 1963). Of these serial sections, alternatively, the first 30 sections were placed on three 300 mesh grids and the following 20 sections on two grids with rectangular opening 1 x 2 mm, coated with a formvar film (0.5 per cent in chloroform).

For transmission electron microscopy, a Jeol Jem 7 A electron microscope was used. Of each animal, about six composite micrographs, with many secretory granule-containing perivascular cell processes, were made. For ease of comparison, these micrographs were made at the same magnification (×15000). The measurements of the secretory granules were performed on the cell processes of these composite micrographs.

Quantitation of Electron Microscopic Data. On electron micrographs, three-dimensional granules generate two-dimensional profiles. With our semi-automatic equipment (Vandesande and Goossens, 1973), the major and the minor axes of these profiles were measured. The measures were directly printed in Ångström. As the arithmetic mean is very sensitive to outliers and as the mode is influenced by the choice of the class-limits, we calculated the median and the quartiles.

The karyometric measurements were done on the semi-thin sections of 1 micron, with the same semi-automatic equipment used for the measurements of the secretory granules. Since the nuclei are seldom perfectly spherical, but have rather an ellipsoidal shape, we measured the major and the minor axes and we calculated the volume according to the following formula:

\[ V = \frac{1}{6} \pi LB^2 \]

(Palkovits and Fischer, 1968).

For the statistical treatment of the measures, the Kolmogorov-Smirnov two-sample test was used (see Keeping, 1962).

As the shape of a large number of the secretory granules measured was irregular, the measures of the major and minor axes of Fig. 9 are not completely representative for the volume of the granules. Nevertheless, the result of the statistical treatment of these measures strongly supports the subjective quantification of the volumes indicated in Table 1.

Observations

As known, the hibernation and the reproduction play an important role in the annual life cycle of *Rana temporaria*. Therefore, in accordance with the literature (see Barthelemy, 1930; Dierickx et al., 1960, lit.) we divide the annual life cycle of *Rana temporaria* into four periods: (1) the hibernation period extending from the beginning of November till February; (2) the reproduction period (copulation, ovulation): during March and April; (3) the early summer period: during May, June and July; (4) the late summer period; during August, September and October.

The ultrastructural changes occurring in the pars tuberalis of the hypophysis (cf. Figs. 1–7) during these four periods are summarized in Table 1.

The ultrastructural features of the pars tuberalis of the hibernation period, the reproduction period and the late summer period described in Table 1 apply for the large majority of the pars tuberalis cells. But, it has to be added that, in the hibernation period, a minority of pars tuberalis cells tend to show the ultrastructural features of the pars tuberalis cells of the early summer period and that the number of these cells progressively increases during the reproduction period. On the other hand, in the beginning of the late summer period, a minority of pars