Structure and Innervation of the Pineal Gland of the Rabbit, *Oryctolagus cuniculus* (L.)

II. An Electron Microscopic Investigation of the Pinealocytes

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Summary. In the rabbit pineal gland a cortex and a medulla can be distinguished, based on the distribution pattern of two cell types. The medulla consists of so-called light and dark pinealocytes, whereas in the cortex only light ones occur. The cytological distinction between the two cell types is principally due to the exclusive presence of abundant pigment granules in the dark pinealocytes. The light pinealocytes have long offshoots. Their club-shaped terminals are crowded with smooth endoplasmic reticulum from which grey vesicles are pinched off. In the medulla, these offshoots run for the most part into the direction of blood vessels to terminate in close topographical relationship with dark pinealocytes which are mainly arranged around these vessels. The ultrastructure of both the light and the dark pinealocytes is described.

Key words: Pineal gland (Rabbit) — Light and dark pinealocytes — Ultrastructure.

Introduction

In the literature, only scanty data are found concerning the ultrastructure of the rabbit pineal gland. Wartenberg and Gusek (1965) were first in using the electron microscope for looking at its overall cell structure, while Leonhardt (1966, 1967) investigated secretory phenomena in the pinealocytes. The present electron microscopic study deals with the topography and fine structure of the rabbit pinealocytes. The autonomic pineal innervation will be discussed in a next paper.

Material and Methods

Adult grey or black male rabbits, F₁ bastards of the Alaska and the White Vienna strains, weighing 2000–3000 g, were used. The animals were kept separately under normal laboratory conditions. Perfusion via the aorta of anaesthetized animals with a mixture of glutaraldehyde and formaldehyde in phosphate buffer of pH 7.38 (Karnovsky, 1965) was performed as a standard fixation procedure. In contrast to the original prescription, the concentrations of glutaraldehyde and formaldehyde were reduced to 2.5 % and 2 %, respectively. The time of aldehyde fixation varied from 30-45 min during which the temperature was lowered from 37°C to 4°C. Next, the pineal gland was quickly dissected and cut into small pieces, which were postfixed by immersion in 1 % osmium tetroxide in phosphate buffer (Millonig, 1961) at 4°C for 1.5 h without previous rinsing. During this time the tissue was allowed to adapt to room temperature. Following dehydration in ethanol and propylene oxide and embedding in Epon 812, ultra-thin sections were cut on a LKB ultratome. The sections were doubly stained with 2 % uranyl acetate diluted in 50 % ethanol, followed by lead citrate according to Reynolds (1963). After staining a thin layer of carbon was evaporated over the sections. For reasons of

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topographic localization, large semi-thin sections were occasionally cut and stained with silver methenamine following Cardno (1965). They were examined by light microscopy. Pineal tissue of 6 animals, processed in this way, was investigated under a Philips EM 200 electron microscope.

Results

Alternating semi-thin and ultra-thin sections of rabbit pineal tissue were screened under the light and electron microscope, respectively.

On the ultrastructural level, the presence of a cortex, a medulla and a transition area already mentioned in a preceding light microscopic study (Romijn, 1973) could be confirmed. This distinction can be based on the distribution pattern of two different types of pineal cells, light and dark pinealocytes, as originally termed by Wartenberg and Gusek (1965). It appeared that both cell types are present in the medulla and the transition area, whereas in the cortex only light pinealocytes occur.

Light Pinealocytes. The light pinealocytes are characterized by a frequently lobulated nucleus, the longest diameter of which measures about 6–9 μ. (Fig. 1). The nucleus shows a single dense nucleolus and scattered dense granular areas of chromatin situated close to the nuclear membrane. Many mitochondria of the crista type, present in the cytoplasm, are often conically arranged around a centriole. Numerous microtubules radiate from these centrioles to the mitochondria (see also Leonhardt, 1966). The cytoplasm also contains zones of regularly shaped, closely spaced rough endoplasmic reticulum, tubular smooth endoplasmic reticulum, clusters of free ribosomes, and a well-developed Golgi complex. Numerous microtubules, measuring about 200–250 Å across, are scattered throughout the cytoplasm. In a single case giant mitochondria were observed (cp. Wartenberg and Gusek, 1965).

Three types of vesicles seem to be pinched off from the Golgi sacculi, smooth-surfaced grey vesicles, rough-surfaced grey vesicles, and dense-core vesicles (Figs. 2 and 3). The rough-surfaced vesicles, also named “coated” vesicles or acanthosomes, owe their name to small spines oriented perpendicularly to the outer surface of the limiting membrane. The diameter of the smooth-surfaced grey vesicles and of the acanthosomes, the latter excluding their spiny coat, is about 350–1200 Å, the majority being between 400 and 1000 Å. The length of the spines amounts to about 180 Å (see also Wolfe, 1965). The dense-core vesicles show a diameter varying between 550 and 2500 Å, most of them measuring from 1000–2000 Å. The width of the lucent rim between the dense core and the limiting membrane of the vesicle varies between 125 and 175 Å. Although dense-core vesicles are usually seen to be directly pinched off from Golgi sacculi, not rarely pictures have been observed suggesting the formation of dense-core vesicles from acanthosomes (Fig. 2). During this transformation the contents of the acanthosome becomes more dense and the spines get lost.

Finally, electron-dense and -lucent droplets, probably lipid, and lysosome-like bodies, can be found.

The light pinealocytes have several offshoots which are mostly rather long. In the medulla these offshoots terminate as follows:

(a) in close relationship with the cell body or the processes of dark pinealocytes, which are almost exclusively situated around blood vessels. Frequently the