Electron Microscopic Study of Impuberal and Adult Rats Pineal Body

The neurosecretory activity of mammalian pineal body (PELLEGRINO DE IRALDI and DE ROBERTIS[1]; CLEMENTI, FRASCHINI, MULLER, ORNESI, and ZANOBONI[2]; DE ROBERTIS[3]) seems to be controlled by the hypophysis, the diencephalon (CASSANO, TORSOLI, PERUZY, and DE MARTINO[4]) and the adrenal gland (FARRELL[5]).

The N-acetyl-5-methoxytryptamine, which was isolated from the ox pineal body (LERNER, Case, TAKAHASHI, LEE, and MORI[6]), seems to induce, in rats, hypothyroidism (BASCHIERI, DE LUCA, CRAMAROSSA, DE MARTINO, OLIVERIO, and NEGRI[7]), a decrease in the incidence of oestrus and a reduction of ovarian weight (WURTMAN, AXELROD, and CHU[8]). Furthermore, an acetone soluble fraction extracted from ox pineal body was found to promote aldosterone secretion (FARRELL[5], JOUAN[9]) by direct stimulation of the glomerular adrenal gland (GIACOMELLI[10]). This substance, called 'glomerulotropin' (FARRELL[5]), has been chemically individualized as 1-methyl-6-methoxy-1, 2, 3, 4-tetrahydro-2-carboline (FARRELL and MCISAAC[11]).

Nevertheless, while there is general agreement on the endocrine activity of the epiphysis, its function in late stages of life is still under discussion.

This preliminary report deals with the ultrastructural features of the pineal body in young and adult rats. The study was made in order to detect morphological signs of different secretory activity in the two ages.

Material and methods. Twelve Wistar male rats were divided in two groups. The first was formed of animals 20–30 days old and the second one of rats 180–240 days old. The pineal bodies were removed after decapitation.

The glands from the animals of each group were fixed in osmium tetroxide solution buffered at pH 7.2 according to MILLONIG[12] and embedded in Durcupan-ACM Fluka. Sections 200–300 Å thick were cut on a Porter Blum or Leitz type ultramicrotome equipped with glass knives. The sections were examined in a Siemens Elmiskop I° electron microscope.

Results. The pinealocytes in impuberal rats are small in size. The cell limits are clearly outlined by straight contours and show a few cytoplasmic processes which contain rare 'plurivesicular material' (DE ROBERTIS[3]). Only a few granules of irregular shape and low electron density are visible.

In the adult rats, the shape and size of the pinealocytes are not uniform. Numerous cytoplasmic processes extend towards the perivascular spaces and interdigitate with each other so that the single cell limits are difficult to determine. 'Plurivesicular material' is localized in the cytoplasmic processes which are visible in the perivascular area. The nuclei are prominent and indented at their periphery. Eterochromatin is frequently visible, mostly close to the nucleoli.

Two types of granules are present in the adult rat pinealocytes. The granules of the first type have an irregular shape, contain an homogeneous material of low electron density and are regularly distributed throughout the cell. The limiting membrane of dense granules is almost constantly visible. The granules of the second type show a more even shape, contain an heterogeneous

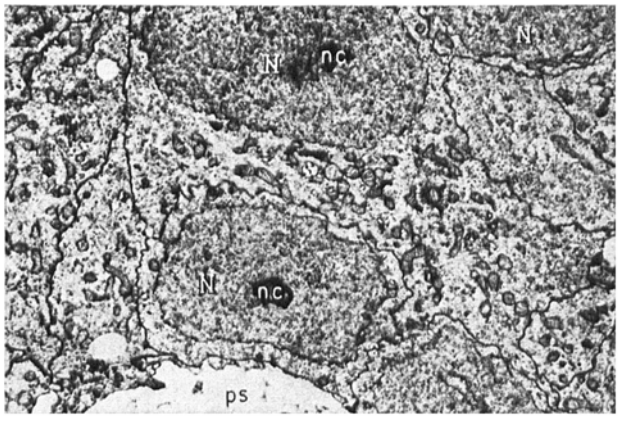

Fig. 1. Immature rat pineal body. The cell limits are clear cut. Neither cytoplasmic processes around the perivascular space (ps) nor granules within the cytoplasm are visible. The nuclei (N) show few indentations and contain small nucleoli (nc). Magnification × 3470.

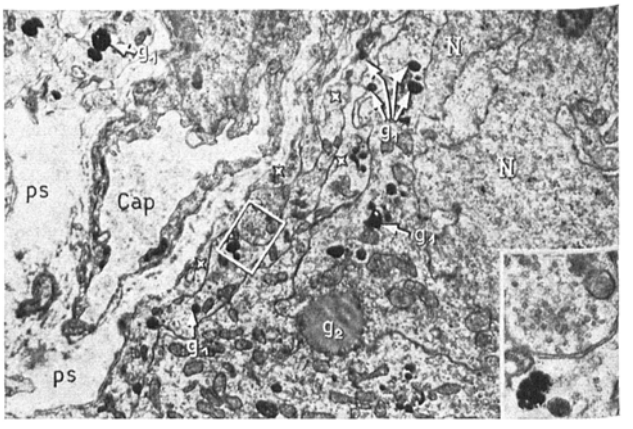

Fig. 2. Adult rat pineal body. The presence of numerous cytoplasmic processes (★) which contain plurivesicular material (boxed area) makes it difficult to individualize the limits of the single pinealocytes. In the cytoplasm around the perivascular space (ps) many osmiophilic granules (g_1) of small size are present. A less dense granule (g_2) of major size is localized in the centre of the picture. In the right upper part a nucleus (N) is seen, whose periphery appears notched. The inset shows a higher magnification of the boxed area. The cytoplasmic process contains numerous small vesicles (plurivesicular material). In the lower part a granule appears to be formed by several osmiophilic droplets separated by a less dense material. Magnification × 7500. Inset × 12,000.

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material of higher electron density and have different sizes. The smaller ones are mostly seen close to perivascular and intercellular spaces, while those of larger size have a more peripheral localization.

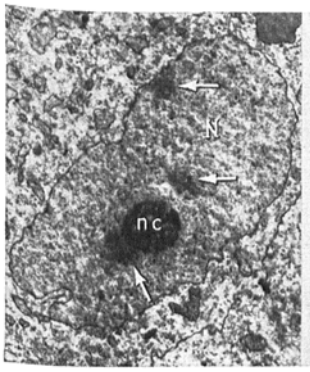

Fig. 3

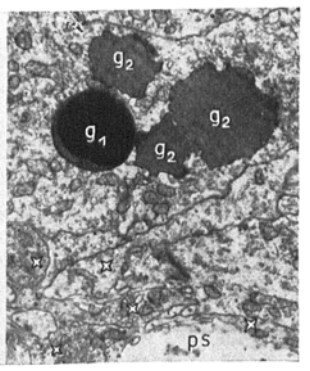

Fig. 4

Fig. 3. Adult rat pineal body. The picture illustrates that the nucleus of a pinealocyte (N) is greater than those observed in Figure 1. The nucleolus (nc) is larger and eterochromatin is well visible. Magnification × 3300.

Fig. 4. Adult rat pineal body. In the cytoplasm of a pinealocyte four granules are present. One of them (g_1), of regular round shape, appears to contain an electron dense droplet. Other granules (g_2) have a more irregular shape and lower electron density. Intersecting cytoplasmic processes (★) are visible around the perivascular space (ps). Magnification × 4300.

The granules of pinealocytes are supposed to contain lipids, serotonin, melatonin and other various biogenic amines (Csillik[13], De Martino, De Luca, Minio Paluello, Tonietti, Orci[14]).

Conclusions. In previous investigations, carried out during different ages, the ^{32}P uptake, the nuclear and cytoplasmic volumes of pinealocytes (De Martino, Peruzy, Pavoni, Capone, and Lintas[15]) and the number of osmiophilic granules (De Martino, De Luca, Minio Paluello, Tonietti, Orci[14]) appeared to increase in the adult rats.

Our present ultrastructural findings give further support to the opinion that neurosecretory activity of the pineal body increases during the adult life[16].

Riassunto. Sezioni di pineale di ratto impubere e adulto sono state studiate col microscopio elettronico. I reperti ottenuti depongono per una maggiore attività neurosecretoria della ghiandola nell'animale adulto.

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Action of Plastid Phospholipase D on Free and Lipoprotein-Bound Phospholipids

We have previously reported that phospholipase A (phosphatide-acyl hydrolase EC 3.1.1.4) from snake venom[1] and human or ox pancreas[2] splits lipoprotein-bound phospholipids in egg yolk and serum at a higher rate than in their total lipid extracts or than purified ovolecithin. In the present study we investigated whether phospholipase D (phosphatidylcholine phosphatidohydrolase EC 3.1.4.4) from spinach chloroplasts shows a similar preference for lipoprotein-bound phospholipids.

Spinach chloroplasts, prepared according to Kates[3], were suspended in distilled water to the amount of 26–33 mg dry weight per ml, and stored for a maximum of 4 days. The chloroplast suspension was acted on egg yolk and human serum, on their total lipid extracts and on purified ovolecithin, prepared as described previously[1]. Both the egg yolk and egg yolk lipid extracts were diluted with half volume of distilled water. The purified lecithin was suspended in distilled water at a concentration of 50 mg per ml, corresponding to the concentration of lecithin in the diluted egg yolk[4]. Phospholipase D activity was measured in the presence of acetate buffer of pH 4.7[3]. The incubation temperature was 37°C in the absence of ether, and 25°C whenever ether was added to the system. Hydrolysis of the substrates was followed by both the release of free choline, determined by precipitation with ammonium reineckate[3], and by qualitative chromatography on silicic acid impregnated paper[5].

As shown in Figure 1, an amount of enzyme, effecting a rapid release of choline from egg yolk, produced only slight hydrolysis of egg yolk lipid extract and of ovolecithin. The discrepancy between the hydrolysis of egg yolk and that of purified ovolecithin is further illustrated in Figure 2 by the decrease in the lecithin spots. Essentially similar results were obtained when comparing the rates of hydrolysis of lecithin in human serum and in serum lipid extract.

These results suggested that, similarly to what had been found for phospholipase A[1], the integrity of the lipoprotein structure is essential for optimal activity of phospholipase D. Indeed, plastid phospholipase D showed a

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