PINEAL GLAND OF THE KUWAITI DESERT GERBIL (*GERBILLUS CHEESMANI*): ALTERATIONS OF ITS STRUCTURE BY BROMOCRIPTINE TREATMENT

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In the present study, the pineal gland of the gerbil *Gerbillus cheesmani* was described for the first time. According to their electron density, two distinct cell types were observed (light and dark pinealocytes). The nuclei were either oval or irregular. Moderate amount of granular endoplasmic reticulum (GER) was observed. Free ribosomes were present throughout the cytoplasm. Mitochondria and lysosomes were among the most common organelles in the pinealocytes. Several dense core vesicles (DCV) were also noted. Blood capillaries with nonfenestrated endothelium were frequent. Bromocriptine treatment for two weeks influenced, to a degree, the physiology of the pinealocytes. It induced a loss of distinction between light and dark pinealocytes, a decrease in the amount of GER and a reduced frequency of lysosomes. On the contrary, lipid droplets and membrane bound vesicles became more frequent.

Key words: Pineal gland – Gerbil – Ultrastructure – Bromocriptine

The pineal gland exists, in one way or another, in all classes of vertebrates. In mammals, the major cellular component of the gland is the pinealocyte, which comprises about 90% of the total cell population. In some mammalian species, two different pinealocyte populations have been reported (Pevet et al. 1976, 1977; Miline 1979; Pevet and Racey 1981; Karasek and Hansen 1982) which are referred to as pinealocytes I and II (Karasek 1983). In animals such as rat (Arstila 1967), Syrian hamster (Sheridan and Reiter 1968), mole (*Talpa europaea* L) (Pevet 1974), kangaroo rat (*Dipodomys ordi*) (Karasek et al. 1982a) and gerbil (*Meriones unguiculatus*) (Welsh and Reiter 1978), a distinction was made between light and dark pinealocytes. In addition to the pinealocytes, glial cells are present in the pineal in varying numbers, while fibroblasts, mast cells, pigment-containing cells and nerve cells are occasionally present in some species (Karasek 1983).

The pineal gland and its hormones influence the adenohypophysis. Exposure of some mammalian species to short photoperiods induces marked alterations in the gonadotropic hormones (Reiter 1980). The most dramatic change is observed in prolactin level where it is diminished both in the plasma (Reiter 1980; Reiter et al. 1989) and in the anterior pituitary (Blask et al. 1986; Reiter et al. 1989). A reciprocal relationship between the pineal and the anterior pituitary gland was suggested (Karasek and Reiter 1982). Ultrastructural changes in the pineal gland have been observed following alterations in pituitary function (Karasek and Marek 1978; Karasek et al. 1982b; Karasek et al. 1983), castration (Karasek et al. 1976) and after thyroidectomy (Karasek and Stepien 1980).

In recent years, the morphology of the mammalian pineal gland has been extensively studied (see reviews by Bhatnagar 1992; Karasek 1992; Karasek and Reiter 1992). However, the number of species studied represents only about 2% or less of the over 4,200 mammalian species known (Bhatnagar 1992). Because of its structural diversity, the pineal gland requires more ultrastructural studies with emphasis on its functional aspects and hormonal regulation. Being a popular species in the Arabian Peninsula and its pineal gland has not been described yet; the gerbil *Ger-*

billus cheesmani was selected for the present study. The morphology of the pineal gland in the male and female gerbils was investigated. Also, to evaluate the existence of a reciprocal relationship between the pineal and adenohypophyseal hormones, the prolactin-inhibitory dopamine agonist bromocriptine was used.

Materials and Methods

Four male and eleven female gerbils *Gerbillus cheesmani*, collected from Kabd area, south west of Kuwait City, were used in the present study. This species is a medium-sized gerbil. It is widely distributed throughout the Arabian Peninsula and eastwards into southern Iran (HARRISON 1964). Animals (average 34g in body weight) were housed separately (at a temperature of 21+1˚C, under a 12L:12D light:dark cycle and a relative humidity of 50-60%) in clear plastic cages for two weeks, with food and water provided *ad libitum*. After acclimatization, animals were divided into three experimental groups:

Group I

Four male and four female, previously acclimatized, gerbils were kept under the previously mentioned conditions for 14 days. This group was used for the study of the normal pineal structure in male and female gerbils.

Group II

Five female gerbils received a daily sc injection (1mg/kg body weight) of bromocriptine mesylate (2-bromo-α-ergocriptine methane sulfonate, Sigma, St. Lewis, MO) dissolved in 100 µl physiological saline for 14 days.

Group III

Two female gerbils were sc injected daily for 14 days with 100 µl of the vehicle only and served as saline treated controls. All injections were carried out between 09:00h and 09:30h.

By the end of the two weeks of treatment, all experimental animals were decapitated between 09:00h and 10:00h.

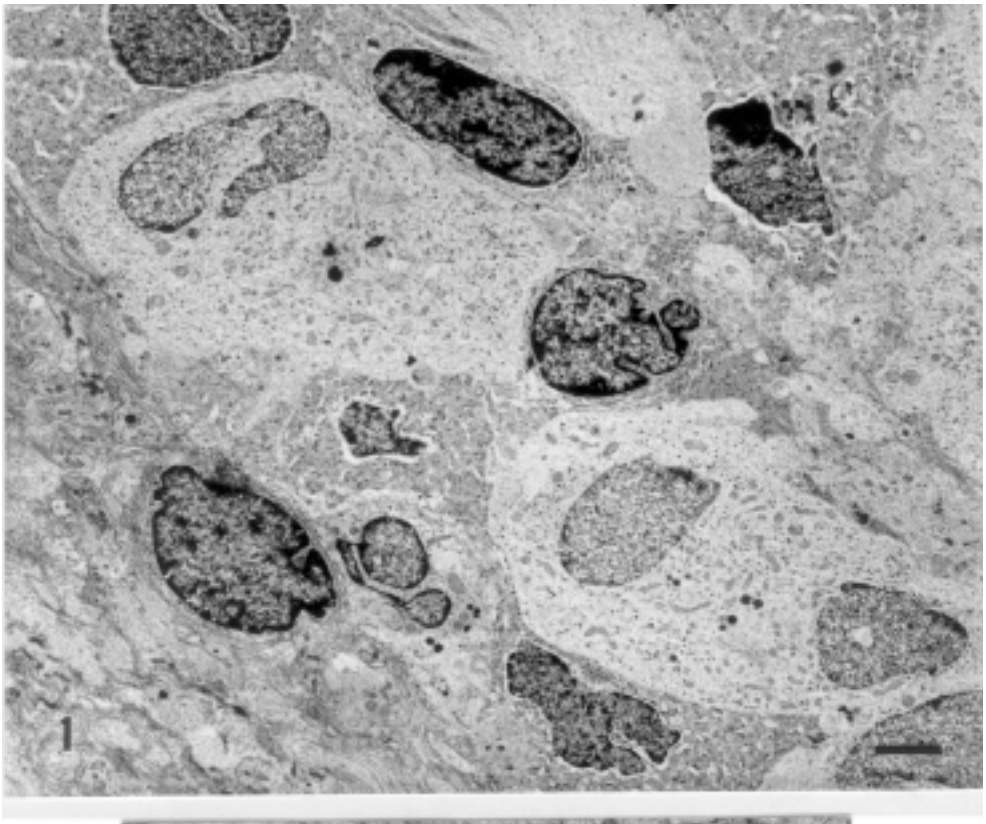
Electron microscopy

After decapitation, pineal gland was dissected, fixed by immersion in Karnovsky fluid (pH 7.4) for 24 h, washed in 0.2 M sodium cacodylate buffer (pH 7.2) and post fixed in 2% osmium tetroxide for 2 h. Pineal glands were then dehydrated, cleared in propylene oxide for 10 min and embedded in araldite. Ultra-thin sections (70-80 nm) were stained with uranyl acetate for 30 min, washed with double distilled water, counterstained with lead citrate and washed once more. Examination was performed in a Joel JEM-1200 EX II electron microscope.

Results

Pineals of male and female gerbils *Gerbillus cheesmani* were structurally identical. The pineal gland of *G. cheesmani* is mainly formed of two distinct cell types, dark and light pinealocytes, in which a recognizable difference in the electron density of their cytoplasm was observed (Fig. 1). The nuclei, especially of the dark pinealocytes, vary in shape being either oval or irregular with cytoplasmic invaginations (Fig. 1). Chromatin is marginally located (Fig. 1). In some cells, membranes with a lamellar form were observed (Fig. 2). The pinealocytes were found to contain granular endoplasmic reticulum (GER) in moderate amounts (Fig. 2) and they were rich in free ribosomes, mostly in the form of polyribosomes, either around the nucleus (Fig. 2) or throughout the cytoplasm of both dark and light pinealocytes (Fig. 3a). In most of the dark pinealocytes, but not in the light pinealocytes, several microtubular sheaves, ranging between 1-3 µm in length and about 0.1 µm in diameter, were observed (Fig. 3a). Mitochondria of various sizes and shapes and lysosomes were among the most common organelles of the pinealocytes (Fig. 3a). Several dense core vesicles (DCV) were also observed (Fig. 3b). The pineal gland of the gerbil was found to be highly vascularized. Blood capillaries with non-fenestrated endothelium were observed (Fig. 4). Treatment of animals with the vehicle only (saline) had no effect on the ultrastructure of the pineal gland.

Treatment of female gerbils with a daily dose of bromocriptine for two weeks produced changes in the ultrastructure of the pineal gland. The overall appearance of the pinealocytes was preserved; however, there was no distinction between dark and light pinealocytes (Fig. 5a). An increase in the numbers of lipid droplets as well as membrane bound vesicles of various sizes and internal structures was observed in the perikarya (Figs. 5a & b). Also, numerous vesicles were found in the terminal buds of pinealocyte processes (Figs. 6a, b & c). The vesicles showed extremely wide variations in their internal structure. Some vesicles contained flocculent ma-



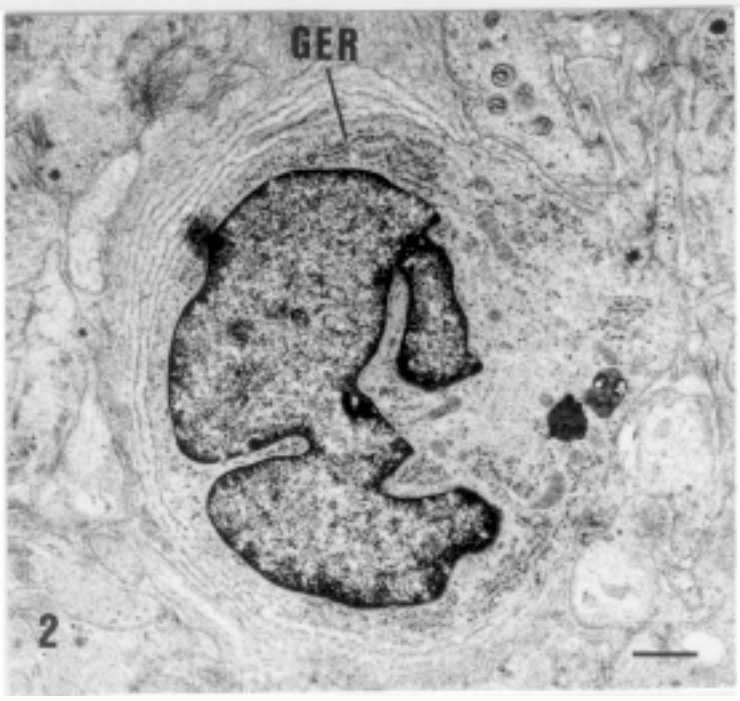


Fig.1. The general structure of the gerbil's pinealocytes of a control gerbil. Light and dark pinealocytes, oval and irregular nuclei with marginally located chromatin are evident (bar, 2 μm).

Fig.2. A concentric lamellar structure in a pinealocyte of a control gerbil. Granular endoplasmic reticulum (GER) and free ribosomes are seen (bar, 2 μm).

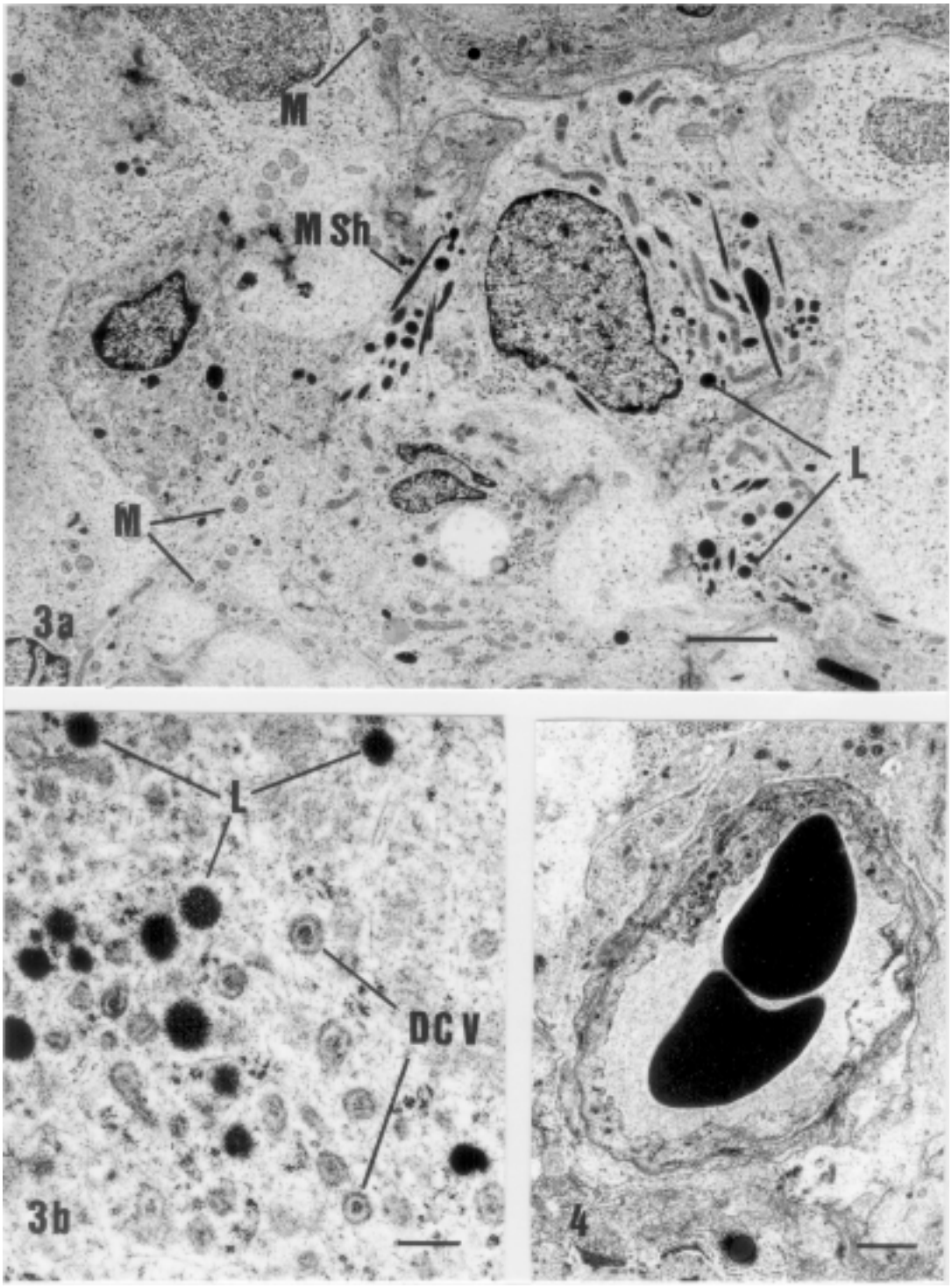


Fig.3. (a) Microtubular sheaves (M Sh), mitochondria (M) and lysosomes (L) in the pinealocytes of a normal untreated female gerbil. Notice the abundance of the free ribosomes throughout the cells (bar, 2 μm). (b) A higher magnification showing several dense core vesicles (DCV), lysosomes (L) and free ribosomes (bar, 500 nm).
Fig. 4. Electron micrograph of a blood capillary with nonfenestrated endothelium (bar, 1 μm).

terials, whereas others had dense, fine or coarse, granular materials (Fig. 5b). Mitochondria were either unaltered (Fig. 7) or they had a dense matrix and cristae with wide, clear space between their membrane (tigroid-like) (Figs. 6a & 7). The granular endoplasmic reticulum (GER) almost disappeared, while free ribosomes were observed throughout the pinealocyte (Fig. 5b). Lysosomes were observed in the perikarya (Fig. 7) and in the terminal buds of pinealocyte processes (Figs. 6a & b) but they were less frequent than in the pinealocytes of the control group. The Golgi apparatus was well developed, with vesicles pinching off from the Golgi sacs (Fig. 8). Blood capillaries with nonfenestrated endothelium (Fig. 6b) surrounded by a wide perivascular space containing numerous endings of pinealocyte processes were observed (Fig. 6b).

Discussion

The present study describes for the first time the ultrastructure of the pineal gland of the gerbil *G. cheesmani*. The pineal gland of *G. cheesmani* was found to be formed mainly of two distinct cell types (dark and light pinealocytes) according to the difference in their electron density. Similar observations were reported in other mammalian species (Arstila 1967; Sheridan and Reiter 1968; Pevet 1974; Karasek et al. 1982a; Welsh and Reiter 1978). This difference in electron density may be a reflection of the physiological status of the pinealocytes as evidenced by their differential susceptibility to the fixative (Sheridan and Reiter 1973; Welsh and Reiter 1978; Karasek 1983). In general, the pinealocyte organelles in *G. cheesmani* are in accordance with previous reports in different animal species. The nuclei were either oval or irregular with cytoplasmic invaginations. Similar observations have been reported by Karasek and Reiter (1992). Chromatin is marginally located, which is a characteristic feature of pinealocytes of other animal species such as the kangaroo rat (Karasek et al. 1982a). A moderate amount of GER was observed in the gerbil's pinealocytes; this is in accordance with the report of Karasek and Reiter (1992). In addition, free ribosomes, mostly in the form of polyribosomes, were present throughout the cytoplasm of both light and dark pinealocytes. The existence of the GER as well as the high amount of ribosomes is suggestive for active protein synthesis in the pinealocytes of *G. cheesmani*. Microtubular sheaves were also observed in some dark pinealocytes of *G. cheesmani*. While the final proof of the role of this structure is still lacking, it is suggested that it might play a role in microtubule formation (Welsh and Reiter 1978) or in transport mechanisms of pineal active compounds (Karasek 1981). The present study also shows that mitochondria and lysosomes were among the most common structures in the pinealocytes. It is known that large numbers of mitochondria of different shapes exist in the mammalian pinealocytes (Karasek 1983). Lysosomes, as well, are another type of cellular organelles typically present in mammalian pinealocytes and their number varies greatly among species (Karasek and Reiter 1992). The present study also shows the presence of several DCV in the gerbils pinealocytes. Dense core vesicles are components of the mammalian pinealocytes though their number varies among species (Karasek and Reiter 1992). It was suggested that DCV are formed by the Golgi apparatus (Karasek 1983).

The pineal gland of the gerbil is highly vascularized as revealed by the existence of numerous blood capillaries with nonfenestrated endothelium. It has been reported that the minimum rate of rat pineal blood flow per gram tissue exceeds that of most endocrine organs, equal to that of the neurohypophysis and is surpassed only by that of the kidney (Goldman and Wurtman 1964). Blood capillaries with nonfenestrated endothelium has been reported in several mammalian species (for review see Karasek and Reiter 1992).

A reciprocal relationship between the pineal gland and the adenohypophysis has been suggested (Karasek and Reiter 1982). Several reports have shown an effect of the pineal gland and/or its products on the adenohypophysis (Reiter and Johnson 1974; Seegal and Goldman 1975; Reiter 1980; Reiter et al. 1989; Benson and Machen 1994; Esquifino et al. 1994; Gower et al. 1994; Nelson et al. 1994). Additionally, some reports suggest an effect of the anterior pituitary secretory products on pineal ultrastructure as well (Karasek et al. 1982, 1990; Przybylska et al. 1992).

The results of the present study clearly show that treatment of female gerbils with a daily dose of bromocriptine for two weeks induced ultrastructural changes at the level of the pinealocytes. However, bromocriptine in previous studies was found to have no marked effect either on pineal melatonin production or on the activity of its rate-limiting enzyme N-acetyltransferase

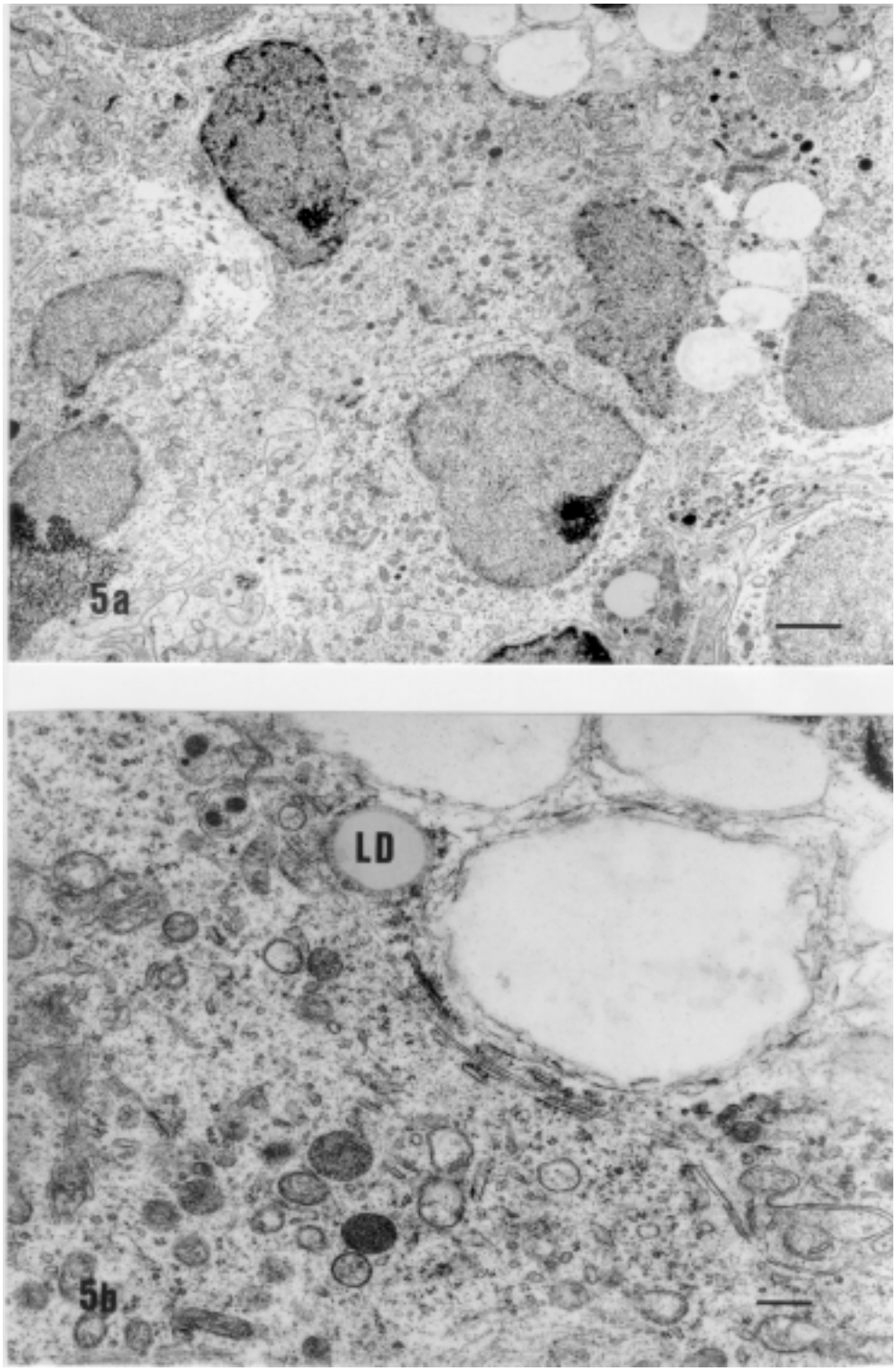


Fig.5. (a) Pinealocytes from a bromocriptine treated female gerbil. Notice the disappearance of distinction between light and dark pinealocytes (bar, 2 μm). (b) A higher magnification showing lipid droplets (LD) and vesicles of various sizes and internal structures (bar, 500 nm).

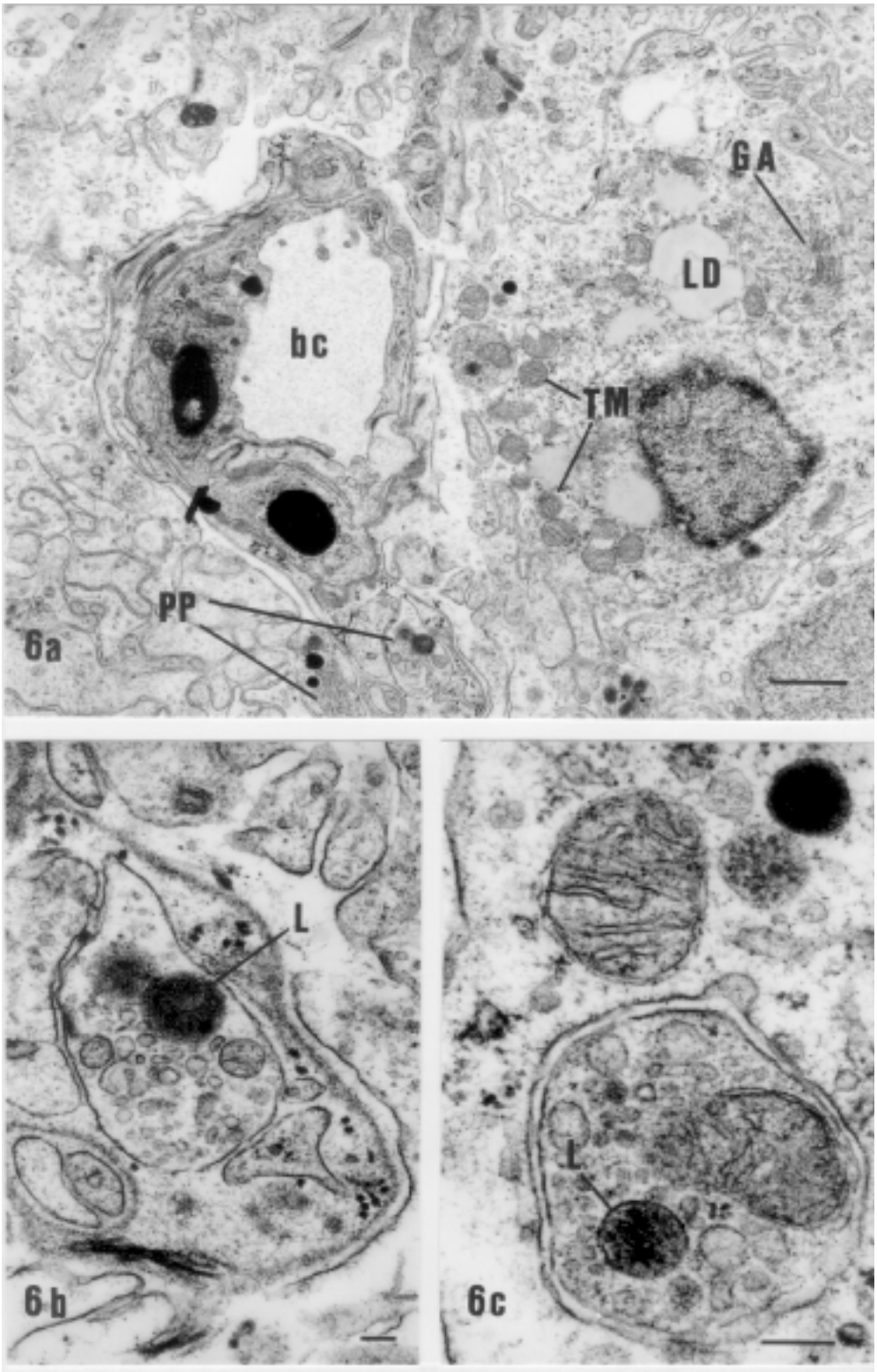


Fig.6. (a) The pineal gland of a bromocriptine treated female gerbil, blood capillary (bc) with nonfenestrated endothelium surrounded by a wide perivascular space containing numerous pinealocyte processes (PP) and several tigroid-like mitochondria (TM). Notice the well developed Golgi apparatus (GA) and lipid droplets (LD) (bar, 1 μm). (b) and (c) terminal buds of pinealocyte processes rich in membrane bound vesicles of different internal structures and lysosomes (L) (bars, 200 nm).

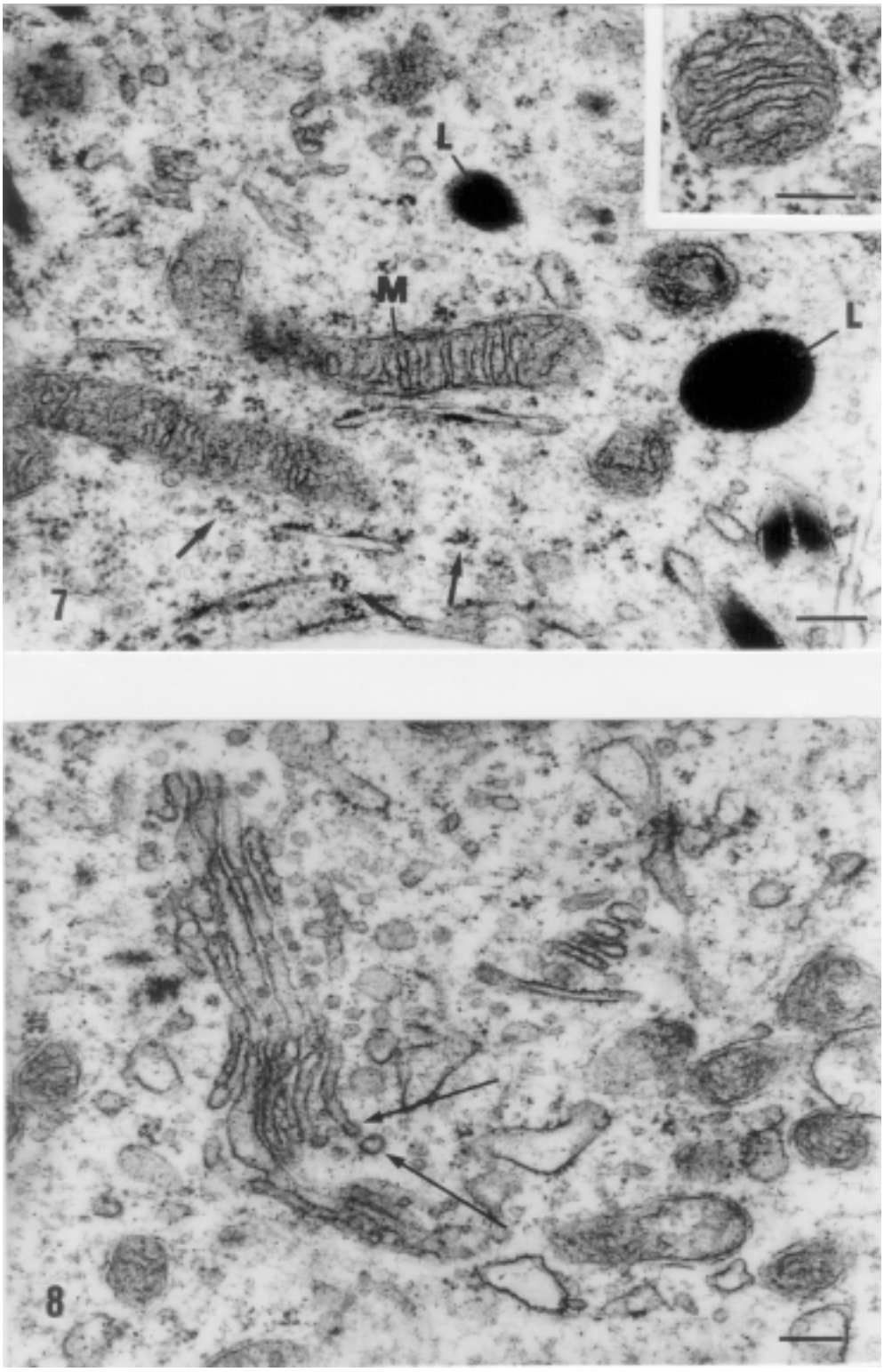


Fig.7. A pinealocyte of a bromocriptine treated female gerbil with normal mitochondria (M), lysosomes (L) and polyribosomes (arrows) (bar, 200 nm). The in-set shows a tigroid-like mitochondrion (bar, 200 nm).
Fig. 8. A well developed Golgi apparatus in the pinealocyte of a bromocriptine treated female gerbil, vesicles (arrows) are pinching off from the Golgi cisterns (bar, 200 nm).

(SABRY and REITER 1989). On the other hand, hypophysectomy, a process known to cause a marked reduction in a number of pituitary hormones, induced a reduction in both pineal melatonin content and N-acetyltransferase activity (SABRY and REITER 1989).

In the present study, the distinction between light and dark pinealocytes was lost after bromocriptine treatment for two weeks. A reduction in the GER was observed and lysosomes were less frequent than in the controls. On the other hand, lipid vacuoles and membrane bound vesicles were found to be more frequent. The Golgi apparatus was well developed and vesicles were observed pinching off the Golgi sacs. The present results agree, in part, with previous reports. In hypophysectomized rats, a significant decrease in the areas of GER, lysosomes and lipid droplets was observed and prolactin administration reversed some of these effects (KARASEK et al. 1982b). While fluctuation in the number of lysosomes under natural and experimental conditions (KARASEK 1986) suggests that they may play a role in the secretory mechanisms of the pinealocyte, their exact role in the pinealocytes has not been established (KARASEK and REITER 1992). It has been proposed that lipids, together with smooth endoplasmic reticulum, are involved in pinealocyte secretion (COLLIN 1981) and also as a possible storage site of indolamines (QUAY 1974). Fluctuations in the number of lipid droplets under various experimental conditions (KARASEK 1992) support the idea that they are of importance in pineal metabolism, probably in synthetic/secretory processes of the pinealocyte (KARASEK 1981; PEVET 1981). However, KARASEK and REITER (1992) favor the idea that fluctuation in pineal lipids is an indicator of pinealocytic general metabolic activity rather than secretory activity.

It is concluded from the present study that bromocriptine treatment influences to a degree the physiology of the pinealocytes as evidenced by the loss of distinction between light and dark pinealocytes, a decrease in the amount of GER and in the frequency of lysosomes and by the increase in frequency of lipid droplets and membrane bound vesicles.

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