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Effects of light deprivation on prolactin cells in golden hamsters: an immunoelectron microscopic study

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Summary. In the golden hamster light deprivation has been shown to induce gonadal regression and reduction of pituitary and plasma levels of prolactin (PRL). In the present study we examined changes in morphology and population ratios of three types of PRL cells 8 weeks after light deprivation, by means of blinding or exposure of hamsters to continuous darkness. In the pituitary of intact hamsters of either sex, which were entrained to a 14-h light: 10-h dark cycle, Type C cells with large secrtetory granules were the most numerous and Type A with smaller granules the least. After light deprivation the pituitary was found to contain remarkably atrophic PRL cells and showed a profound change in population ratio of PRL cell types, i.e., Type A cells prevailed over the other two types. Pituitary glands from light-deprived and concurrently pinealectomized hamsters exhibited structures and a population ratio of three types of PRL cells similar to those from intact animals. It is suggested that small-granule-containing PRL cells represent an inactive stage of PRL cells, whereas medium- and large-granule-containing cells are functionally active cells. The atrophy of PRL cells can account for the decreased pituitary level of PRL in light-deprived hamsters reported previously.

Key words: Golden hamsters, Prolactin cells, Pineal gland, Immunocytochemistry, Light deprivation

Introduction

Golden hamsters exposed to short photoperiod or subjected to blinding will exhibit gonadal regression and loss of reproductive function, and the events can be prevented if pinealectomy accompanied the light deprivation (Hoffman and Reiter, 1965; Reiter, 1968). In addition to LH and FSH, prolactin (PRL) has been proved to play an important role in maintaining the normal function of gonads in hamsters (Reiter and Johnson, 1974a,b ; Bartke et al., 1975, 1980; Bex et al., 1978; Matthews et al., 1978; Chen and Reiter, 1980). In both male and female hamsters that are either blinded or exposed to short photoperiod, pituitary levels of immunoassayable PRL are consistently reduced by as much as 80% (Reiter and Johnson, 1974a,b; Reiter, 1975). Our previous study by light microscopic immunohistochemical methods shows that PRL cells became atrophic and decreased in cell number in response to light deprivation, which were prevented by the simultaneous removal of the pineal gland (Wang et al., 1991). In the hamster pituitary, there are three types of PRL cells as revealed by immunoelectron microscopy (Wang et al., 1987). Thus, the purpose of this work was to examine the changes of relative proportion and the ultrastructure of the subtypes of PRL cells in the adenohypophysis of golden hamsters which were subjected to light deprivation, and to study the cellular mechanisms involved in the reduction of glandular size and secretory activity.

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Materials and methods

Animals

Golden hamsters at the age of 8 weeks were divided into 5 groups of males and females, respectively, each group consisting of 5 animals. Treatments in different groups were as follows: blinding by bilateral optic enucleation in group I, blinding and simultaneous pinealectomy (Hoffman and Reiter, 1965) in group II, exposure to continuous darkness in group III, group IV consisted of pinealectomized animals kept in continous darkness,

and group V were intact controls. The surgical procedures were performed while the animals were anesthetized with pentobarbital. Hamsters of groups I, II and V were entrained to a 14 h light : 10 h dark cycle (lights on at 6 a.m.), the same regime as they were before the experiment, whereas those of groups III and IV were kept under continuous darkness except for a short light period when foods and water were supplied or animal cages were changed in the morning twice a week. The room temperature was controlled at about 23° C.

Tissue preparation

After 8 weeks of experiments, the hamsters were sacrificed under anesthesia with intraperitoneal injection of pentobarbital. They were perfused via the aorta with 4% paraformaldehyde and 2% polyvinyl pyrrolidone in 0.1 M phosphate buffer for a few minutes. The pituitary glands were removed, cut into pieces and immersed in the same fixative for 3 h. After washing with phosphate buffer, they were postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer for 30 min, dehydrated, infiltrated and embedded in Polybed-Araldite (Polysciences, PA, USA). Thin sections were mounted on nickel grids and processed for immunoelectron microscopy.

Immunostaining

The sections were immersed in 10% H_20_2 for 10 min, washed with PBS (phosphate-buffered saline), and treated with 50% normal goat-serum for 30 min. They were reacted with 1:200 diluted anti-prolactin antibodies at 4° C for 48 h. After several rinses in PBS, the sections were incubated with biotinylated horse anti-rabbit IgG (Vector Lab, CA, USA) for 1 h, washed in PBS, and reacted with avidin-biotin-peroxidase for 30 min. The sections were thoroughly washed in PBS and reacted in a substrate solution (5 mg 3, 3'-diaminobenzidine, 60 µl of 30% H_20_2 in 100 ml 0.05 M Tris-HCl, pH 7.6) for 5 min. Sections were either unstained or stained with uranyl acetate and lead citrate. Control staining was performed by omitting the incubation with the primary antibody. Some sections were also stained with irrelevant primary antibodies such as anti-growth hormone and anti-adrenocorticotropin antibodies. None of these procedures resulted in any specific staining on PRL cells.

Antibody

The rabbit anti-human prolactin antibody was purchased from Dako Co. (CA, USA). This antibody was proved to be specific to PRL in the hamster pituitary by immunoblotting as described in our previous paper (Wang et al., 1991).

Cell count of different types of PRL cells

Immunostained sections from nine blocks obtained from each group of 3 animals (3 blocks from each animals) were photographed at magnification of 2,000. Only PRL-immunoreactive cells with a nucleus were counted and classified into three types according to the size of secretory granules as defined in our previous study (Wang et al., 1987). Approximately 700 cells (about 200 cells per gland) were counted in each group. The relative proportion of different types of PRL cells to total PRL cells were represented by percentages. The data was expressed as the mean percentage with the standard error, and analyzed with Student's t-test for comparison of the means in different groups.

Results

At the autopsy of the animals it was macroscopically noticeable that the pituitary glands from the blinded or darkness-treated hamsters were extremely smaller than those from the intact hamsters. In the glands of light-deprived hamsters that were simultaneously pinealectomized displayed no visible difference in size and appearance from those of the intact controls.

Types of PRL cells

PRL cells were identified by the presence of PRL-immunoreactive secretory granules in the cytoplasm; classification of three types of PRL cells was based on the size of secretory granules as defined in our previous study (Wang et al., 1987). Type A cells contained small granules (100 - 230 nm) and a scanty cytoplasm. The secretory granules of Type B and C cells were medium-sized (230 - 280 nm) or large (280 - 570 nm), respectively; both types of cells displayed well developed organelles such as the Golgi apparatus and granular endoplasmic reticulum.

In the atrophic pituitary of the light-deprived hamsters all subtypes of PRL cells were reduced in cell size and usually contained a small number of secretory granules and scanty organelles as compared with those in the intact controls or the light-deprived and pinealectomized animals.

Examples of PRL immunoreactive cells in the pituitary from each group of female hamsters are shown in electron micrographs of sections not contrasted with the uranyl and lead stain (Figs. 1 - 5). Micrographs from male pituitaries are not presented, because the experiments with male hamsters gave similar ultrastructural features. Different population ratios of subtypes of PRL cells in each group of male and female hamsters are shown in Fig. 6 and Fig. 7, respectively.

PRL cells in males

In the pituitary of the intact males approximately

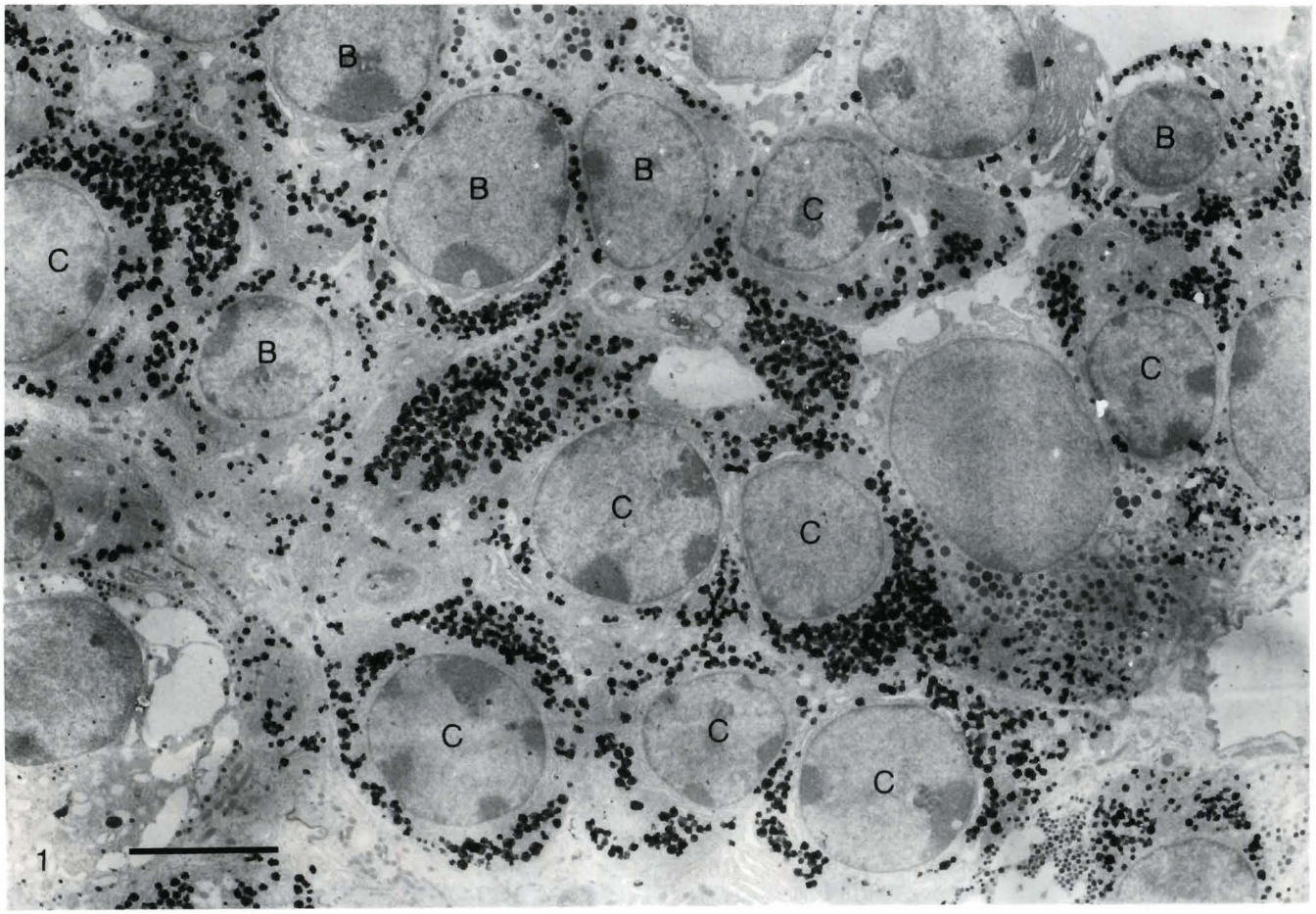

Fig. 1. PRL cells containing immunoreactive secretory granules of the pituitary of an intact female hamster. Different types of PRL cells are labelled on the nucleus. Bar = 5 μm.

half the number of PRL cells were Type C cells (48%) and the proportion of Type B (33%) was slightly higher than that of Type A cells (19%).

After optic enucleation each type of PRL cells drastically changed in proportion. Thus, the majority of PRL cells were of Type A, which amounted to 87%, whereas Type B and C cells became insignificant. The difference of the percentage of each type was highly significant between the blinded and the control hamsters. When blinding was combined with pinealectomy, the proportion of PRL cell types was similar to that observed in the intact controls.

The population ratio of PRL subtypes in hamsters kept in continuous darkness was very close to that shown in the blinded animals. Hamsters which were pinealectomized and exposed to continuous darkness displayed proportions of subtypes similar to those exhibited in the intact controls.

PRL cells in females

In the pituitary of intact female hamsters Type C cells amounted to 68% of all PRL cells, and Type B constituted most of the remaining PRL cells (30%). After blinding Type A cells, which composed only 2% of the total PRL cells in the intact females rose to 91%, and accordingly Type B and C cells scarcely appeared. Similar situation in the changes of population ratio of different PRL subtypes was found in the hamsters exposed to continous darkness. There were slight differences in the population ratio of subtypes between blinded and darkness-treated groups, demonstrating different degrees in the effect of optic enucleation and exposure to continuous darkness on the PRL cells. When either means of light deprivation was carried out in combination with pinealectomy, the subtypes of PRL cells display a proportion approximating that shown in the intact females.

Discussion

We have studied the changes in population ratio and the morphology of three types of PRL cells in light-deprived hamsters. The evidence indicates that light deprivation inhibits the secretory function of PRL cells in both male and female hamsters.

The present results in the males showed no

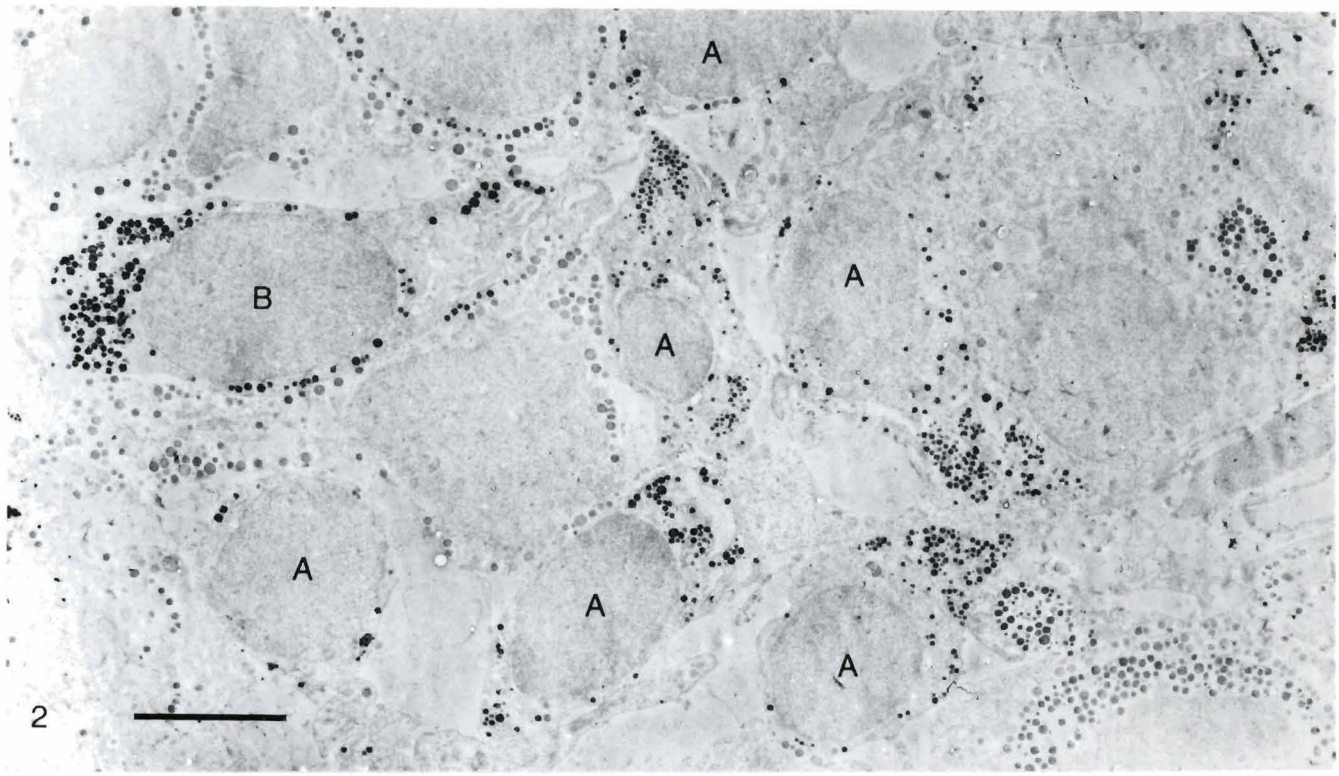

Fig. 2. PRL cells in the pituitary of a blinded female. Bar = 5 μm.

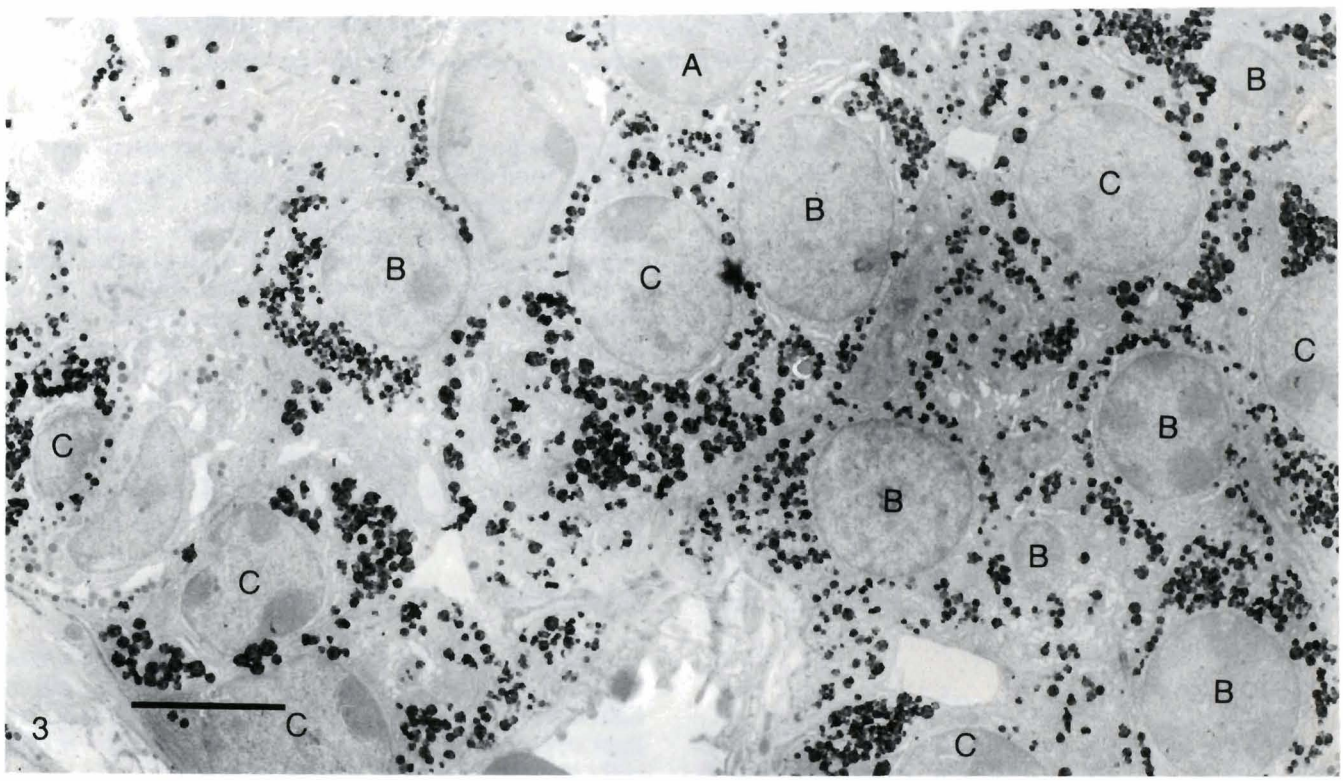

Fig. 3. PRL cells in the pituitary of a blind-pinealectomized female. Bar = 5 μm.

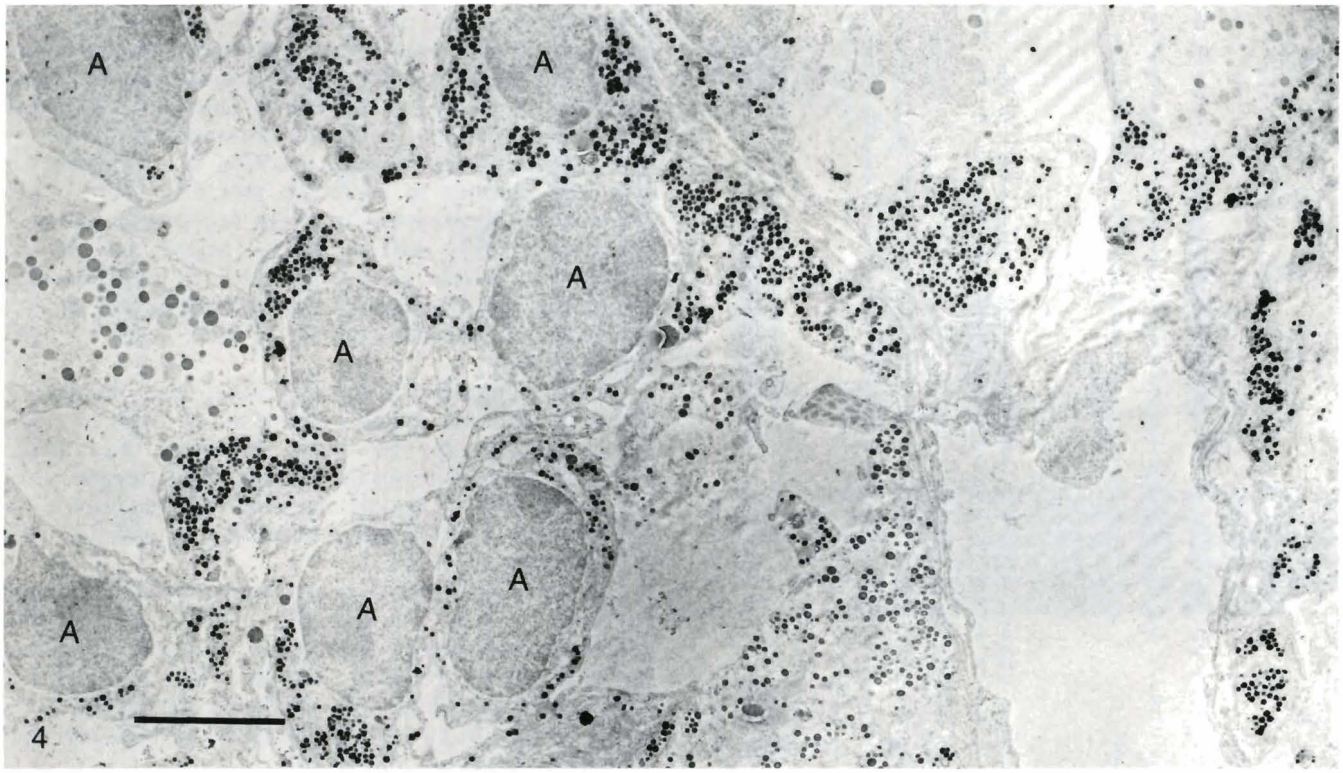

Fig. 4. PRL cells in the pituitary of a female hamster exposed to continuous darkness. Bar = 5 μm.

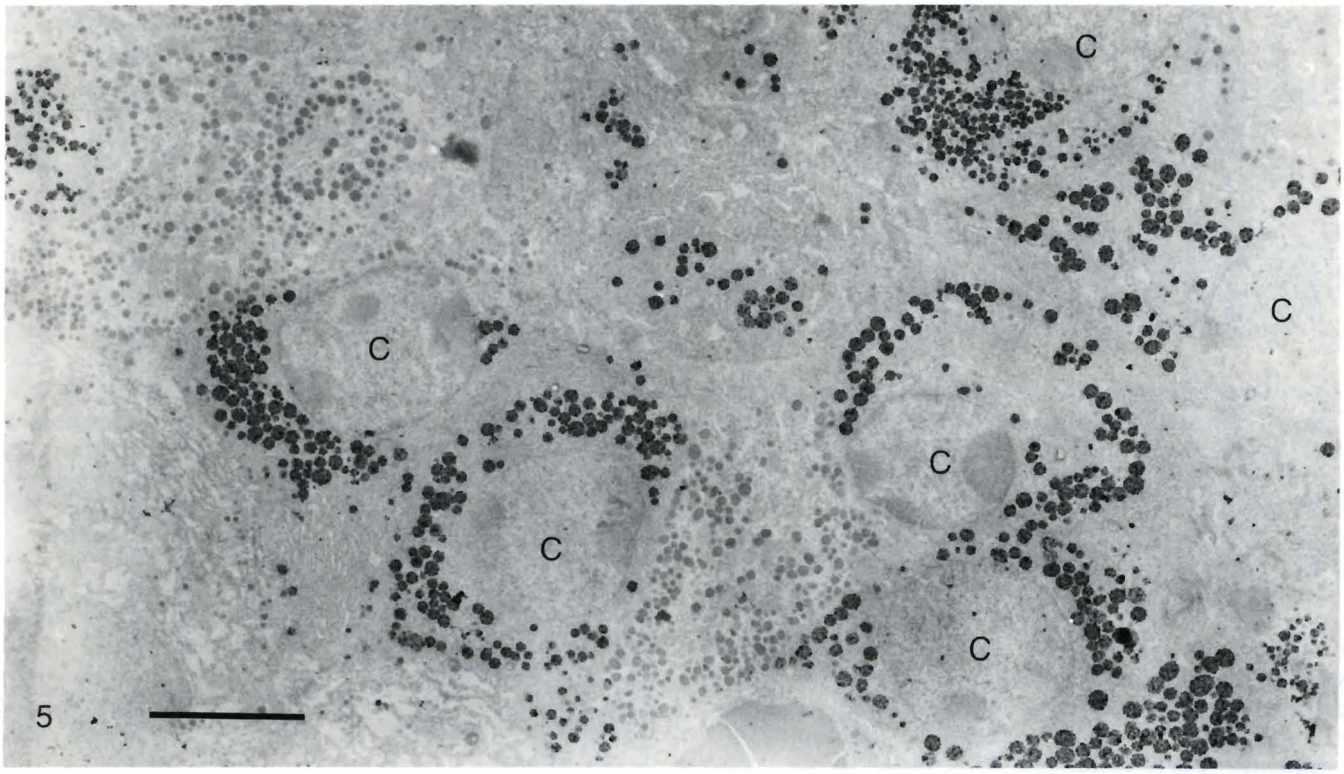

Fig. 5. PRL cells in the pituitary of a pinealectomized female kept in continuous darkness. Bar = 5 μm.

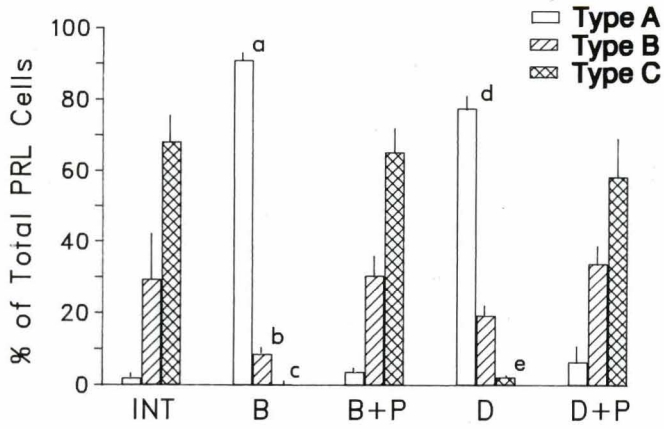

Fig. 6. Population ratios of 3 types of PRL cells in intact (INT), blind (B), blind and pinealectomized (B + P), darkness-treated (D) and darkness-treated and pinealectomized (D + P) male hamsters. Values are mean ± SE. a: $p < 0.001$ vs INT, B + P. b: $p < 0.01$ vs INT. c: $p < 0.001$ vs INT, B + P. d: $p < 0.001$ vs INT, D + P. e: $p < 0.001$ vs INT, $p < 0.01$ vs D + P. f: $p < 0.001$ vs INT, D + P

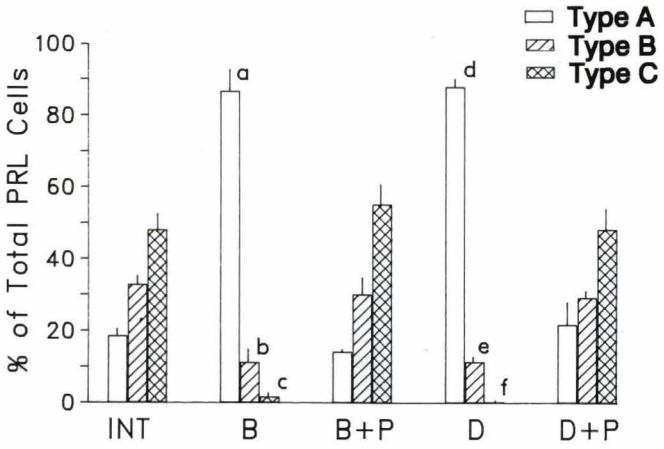

Fig. 7. Population ratios of 3 types of PRL cells in different groups of female hamsters. Abbreviations are the same as in Fig. 6. a: $p < 0.01$ vs INT, B + P; $p < 0.05$ vs D. b: $p < 0.05$ vs INT, D; $p < 0.01$ vs B + P. c: $p < 0.001$ vs INT, B + P; $p < 0.01$ vs D; d: $p < 0.001$ vs INT, D + P. e: $p < 0.001$ vs INT; $p < 0.01$ vs D + P.

significant differences in the change of the population ratio of PRL cell types between blinded hamsters and darkness-treated ones. In the following discussion, therefore, both groups of male hamsters will be collectively called light deprived ones.

In the intact males of the present study Type C cells comprised about half the total number of PRL cells, whereas Type A cells were scarcely recognized. Conversely, Type A cells sharply increased in proportion at the expense of the other two types 8 weeks after light deprivation. In predominant Type A cells secretory granules were found reduced in number in addition to the considerable diminution of organelles when compared with those in the intact animals. The similar atrophic organelles with degranulation were also noticed in Type B and C cells in light-deprived males, both types in normal hamsters being considered to represent functionally active stages of PRL cells (Wang et al., 1987). Thus, it is suggested that the treatments induced a sustained inhibition of PRL synthesis and storage, leading to a decrease in size and number of secretory granules. Our results support the observation that light deprivation causes a decline in PRL mRNA levels (Massa and Blask, 1990). The effects were prevented by pinealectomy, indicating involvement of the pineal gland in the inhibition of cellular activity of PRL cells by light deprivation.

In the females light deprivation also induced a dramatic increase in the proportion of Type A cells. The increase in the blind hamsters was more remarkable than in the darkness-treated animals. It is suggested that in female hamsters the magnitude of the response of PRL cells to blinding was greater than to continous darkness.

The present result is in accordance with that of our previous study employing light microscopic immunohistochemical techniques and morphometric analysis, which revealed atrophic PRL cells with a diminished cytoplasm and a reduced population ratio of PRL cells in all pituitary cells from light-deprived hamsters (Wang et al., 1991).

Heterogeneous populations of PRL cells have been reported in different species of mammals, the classification of subtypes being made on the basis of different size of secretory granules: two types in the guinea pig (Beauvillain et al., 1977) and swine (Dacheux, 1980), three types in the rat (Nogami and Yoshimura, 1982) and golden hamster (Wang et al., 1987). It is generally believed that subtypes might represent different stages of secretory activity of PRL cells. In rats large-granule-containing PRL cells predominate in pituitaries of high PRL secretion, whereas small-granule-containing cells become increased in number in pituitaries of low PRL secretion (Maurer, 1982; Osamura et al., 1982; Nogami, 1984; Kurosumi et al., 1987; Smets et al., 1987; Shull and Gorski, 1989; Tong et al., 1989).

The enhanced pineal activity caused by light deprivation exerts an inhibitory action on the PRL secretion (Reiter and Johnson, 1974a,b; Reiter, 1975), and the present study shows that light deprivation brings about changes in morphology and population ratio of different PRL subtypes. In intact hamsters Type A cells constituted a small fraction of total population of PRL cells, whereas they became a predominant cell type after light deprivation. Such an extraordinary change in proportion of the subtypes and a dramatic reduction in population ratio of PRL cells among the total pituitary cells (Wang et al., 1991) suggest that most of Type B and C cells were converted to Type A cells after light deprivation. Present results support the view that PRL cells with small secretory granules are less active than those with medium and large granules.

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