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An immunocytochemical study of effects of light deprivation on prolactin cells in the adenohypophysis of the golden hamster

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Summary. Population ratio and morphology of prolactin cells were studied by employing immunohistochemical methods in the adenohypophysis of normal and experimental golden hamsters of both sexes at 16 weeks of age. Prolactin cells occupied 29% of the total adenohypophyseal cells in the intact males exposed to 14/10 h light/dark schedule. After stimulation of the pineal activity by blinding or exposure of males to continuous darkness for eight weeks, prolactin cells became atrophic and were reduced in population to 17% and 13%, respectively. Pinealectomy prevented to some extent the effects of the above treatments; thus, prolactin cells constituted 27% in the pinealectomized and blinded hamsters, and 19% in the pinealectomized and darkness-treated group; and their morphology was comparable with that of the intact controls. Prolactin cells in the normal females were apparently larger in size and more numerous as compared with those of the normal males, comprising 47% of cell population in the anterior pituitary. In response to light deprivation, prolactin cells were atrophic with a diminished cytoplasm and decreased in cell number as reflected in the population ratio of 27% in the blinded and 21% in the darkness-treated groups. In pinealectomized females combined with blinding or darkness-treatment, prolactin cells contained an abundance of secretory granules in the cytoplasm and maintained the population ratio comparable to that in the intact females.

The present study revealed that light deprivation exerts an inhibitory effect on the secretory activity of prolactin cells and also causes hypoplasia of prolactin cells in the hamster adenohypophysis, the effects being mediated by the pineal gland.

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

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Introduction

It is now well established that the annual changes of the reproductive capacity in the golden hamster is dependent on the presence of an intact pineal gland (Reiter, 1972, 1973, 1975a; Steger et al., 1985). Light deprivation by means of exposure of hamsters to short photoperiod or bilateral optic enucleation, which activates the pineal activity, can induce the atrophy of the reproductive organs (Hoffman and Reiter, 1965; Reiter, 1967, 1968).

Light deprivation causes a significant reduction in the pituitary and plasma levels of LH and FSH in male hamsters (Reiter, 1973; Reiter and Johnson, 1974; Steger et al., 1983). The gonadotrophs are reported to be atrophic, displaying low synthetic and secretory activities in blinded males (Lin et al., 1986). In addition to the gonadotrophins (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, 1974; Bartke et al., 1975, 1980; Bex et al., 1978; Matthews et al., 1978). An inhibition of the synthesis, storage and release of PRL has been shown in pituitary tissues from light-deprived male and female hamsters (Reiter and Johnson, 1974; Reiter, 1975b, 1980a,b; Benson and Matthews, 1980; Chen and Reiter, 1980; Leadem and Blask, 1982; Steger et al., 1985; Blask et al., 1986). Although pinealectomy completely prevents the suppressive effects on PRL cells in males, it is only partially effective in females on the basis of the data from hormone assay (Reiter and Johnson, 1974; Blask et al., 1986; Orstead and Blask, 1987). This fact indicates a sexually dimorphic response of PRL cells to pineal extirpation in the golden hamster.

PRL cells of the golden hamster have been identified immunohistochemically in transplanted pituitaries (Campbell et al., 1979) and in pituitaries of hamsters treated with cadmium chloride (Girod and Dubois, 1976). They were further classified into three types according to the different size of the secretory granules

by immunoelectron microscopy in our laboratory (Wang et al., 1987). We have shown that numerical proportion of the different cell types exhibits a sexual dimorphism, and considerably fluctuates in response to the different physiological conditions. The immunocytochemical study on the morphological modification of hamster PRL cells by light deprivation has not yet been reported.

The present immunocytochemistry study reports some histological changes in the PRL cells in terms of the areas occupied by the cell and the cytoplasm, and the proportion of PRL cells in all of the adenohypophyseal cells with special reference to the effect of light deprivation in both sexes of the golden hamster.

Materials and methods

Animals

Golden hamsters at the age of 8 weeks were divided into 5 groups of males and females, each group consisting of 5 or more animals. Treatments in different groups were as follows: blinding by bilateral optic enucleation in group I; blinding and simultaneous pinealectomy in group II; exposure to continuous darkness without any surgery in group III; group IV consisted of pinealectomized animals kept in continuous 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 exposed to a 14 h light: 10 h dark schedule (lights on at 6 a.m.), whereas those of groups III and IV were kept under continuous darkness except for a short period when food 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 an 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 gland was removed, cut into pieces and immersed in the same fixative for an additional 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). The testes or ovaries with uteri of all experimental animals were removed and weighed.

Immunoblotting

The rabbit anti-human prolactin antibody was purchased from Dako Co. (CA, USA). The specificity of this antibody was examined by immunoblotting. The homogenates of hamster anterior pituitaries, sheep prolactin (Sigma, MO, USA) and the molecular weight standard (Biorad, CA, USA) were electrophoresed on a 15% SDS-polyacrylamide gel and then electrotransferred to the nitrocellulose membrane. The membrane strips were either stained with amido black or processed for immunoblotting. Those for immunoblotting were blocked with 3% bovine serum albumin in PBS (phosphate buffered saline) for 1 h and incubated in 1:500 diluted anti-PRL overnight at 4° C. After washing with PBS-Tween (0.05% Tween in PBS), the strips were reacted with 1:200 diluted biotinylated horse anti-rabbit IgG (Vector Lab, CA, USA) and subsequently with the avidin-biotin-peroxidase mixture (Vector Lab). The strips were carefully washed with PBS-Tween and reacted in a substrate solution containing 4-chloro-1-naphthol as the chromogen.

Immunocytochemistry

Sections of 1.5 μm in thickness were prepared and picked up on albumin-coated slides. The sections were treated with saturated sodium ethoxide for 8 min and rehydrated in a series of ethanols and then in PBS. They were etched with 20% sodium metaperiodate for 20 min, washed with PBS, and treated with 10% normal goat serum for 1 h. The sections were incubated with rabbit anti-PRL (1:200 dilution) overnight at 4° C and then with 1:100 diluted biotinylated horse anti-rabbit IgG (Vector Lab, CA, USA) for 1 h. After washing with PBS, they were reacted with 1:100 diluted avidin-biotin-peroxidase mixture for 30 min. The sections were thoroughly washed in PBS and reacted in a substrate solution (50 mg 3,3'-diaminobenzidine, 60 μl of 30% H_2O_2 in 100 ml 0.05M Tris-HCl, pH 7.6) for 20 min. After washing with Tris-HCl buffer, the sections were mounted in Gel/mount (Biomeda, CA, USA) and examined in a Leitz orthoplan microscope.

Quantitative analysis

Our preliminary observations revealed that PRL cells were evenly distributed throughout the adenohypophysis in the golden hamster. Therefore, two blocks were chosen from each animal, and one thick section was obtained from each block. They were immunostained as described above and then briefly stained with toluidine blue for identification of the nuclei. Only cells with the nucleus were counted. For cell count, the sections were examined with a Nikon Microflex HFX 35 microscope on which the eyepiece was attached with a graticule. The proportion of the number of PRL cells to the total adenohypophyseal cells was represented by a percentage. The data thus obtained were analyzed by Student's t-test for comparison of the mean in different groups.

Morphometry

10 light micrographs were randomly chosen from each group and printed at a final magnification of 2,000.

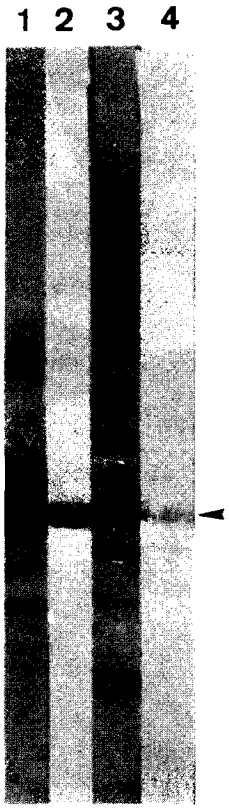


Fig. 1. The specificity of the rabbit anti-human prolactin antibody examined by immunoblot analysis. Lane 1 and 3, amido black stain. Lane 2 and 4, immunoblots. Lane 1 and 2, partially purified sheep prolactin. Lane 3 and 4, hamster pituitary homogenates. This antibody reacts to sheep PRL and hamster prolactin (arrowhead).

The area of immunoreactive cytoplasm was calculated by subtracting the nuclear from the total cell area, which were measured on an IBM personal computer provided with an AutoCAD program and a digitizing system.

Results

Gonadal change

The weights of reproductive organs were markedly decreased in light-deprived hamsters. The combination of pinealectomy with light deprivation abolished the atrophic response of the reproductive systems.

Characterization of the antibody specificity

Immunoblot analysis of the rabbit anti-human PRL showed that it specifically reacted with PRL (MW. 22,500) in the hamster pituitary gland (Fig. 1). Although the antibody was raised against the human PRL, it crossreacted with the hamster PRL with a high specificity.

PRL cells in male hamsters

In the intact control, PRL cells were angular in shape and consisted of 29% of the total adenohypophyseal cells (Figs. 2, 4). After blinding, the population ratio of PRL cells was significantly reduced as compared with that in the control males (Fig. 2). PRL cells in blinded-pinealectomized males occupied a population percentage of 28% which was comparable to 29% in the intact control, but was significantly higher when compared with 16% in the blinded males (Fig. 2). Moreover, the cytoplasmic area of PRL cells was smaller in blinded males and was considerably larger in blinded-pinealectomized males as compared with that in control males (Figs. 5, 6, Table 1).

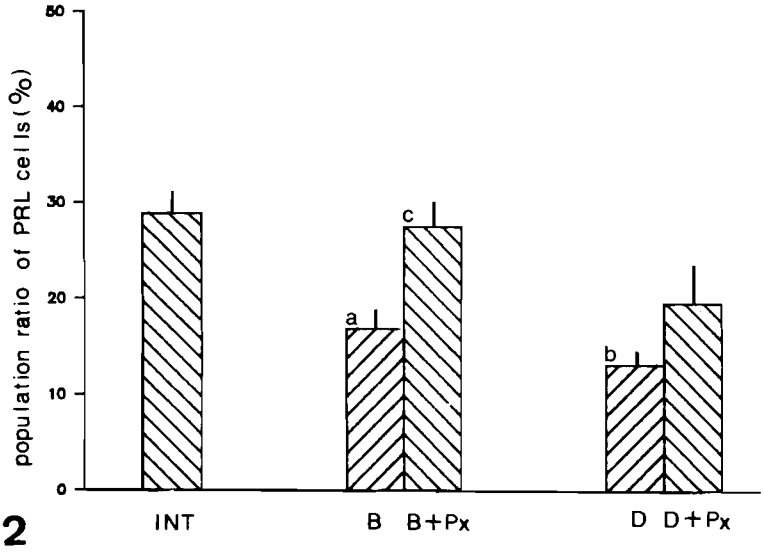


Fig. 2. Mean population ratios of PRL cells in anterior pituitaries of 5 groups of male hamsters: intact control (INT); rendered blind for 8 weeks (B); pinealectomized and rendered blind for 8 weeks (B + Px); exposed to continuous darkness for 8 weeks (D); pinealectomized and exposed to continuous darkness for 8 weeks (D + Px). Each bar represents the mean ± SE. a: $p < 0.01$ vs. INT. b: $p < 0.001$ vs. INT. c: $p < 0.05$ vs. B.

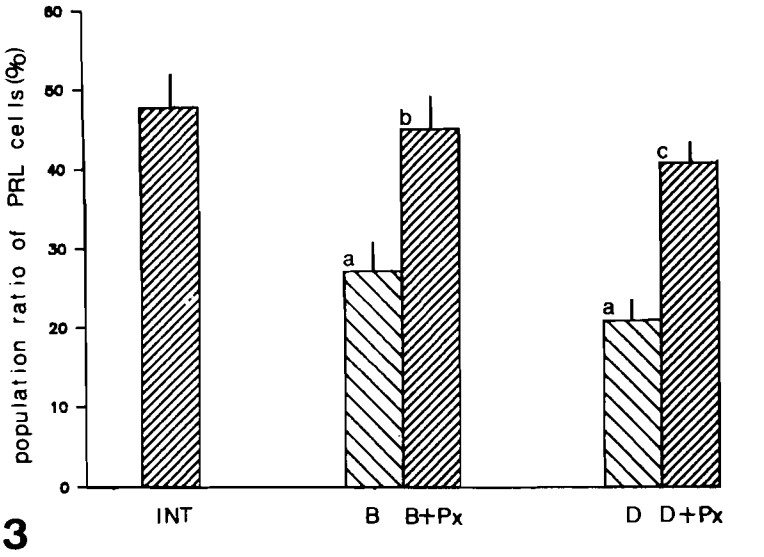
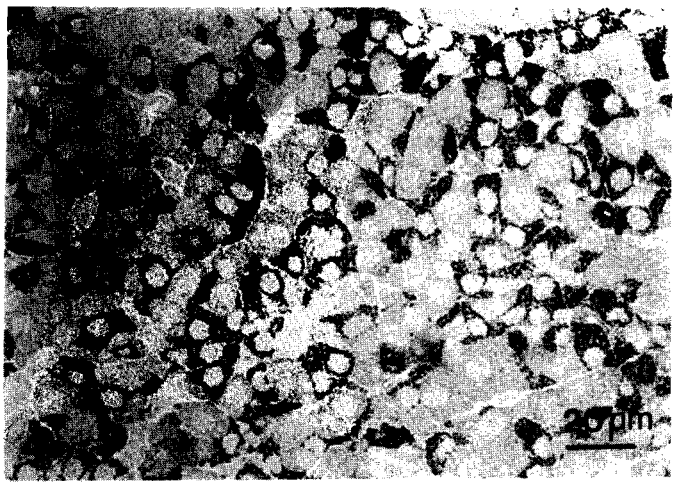


Fig. 3. Mean population ratios of PRL cells in the adenohypophyses of 5 groups of female hamsters. The abbreviations are the same as in Fig. 2. Each bar represents the mean ±SE. a: $p < 0.001$ vs. INT. b: $p < 0.05$ vs. B. c: $p < 0.01$ vs. D.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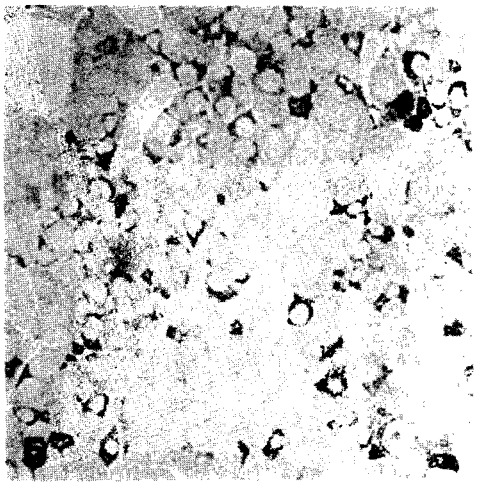

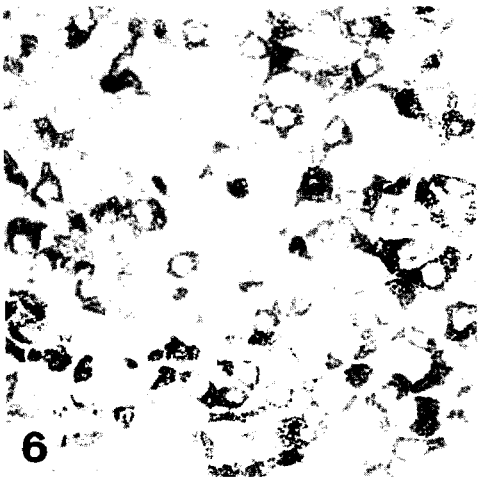
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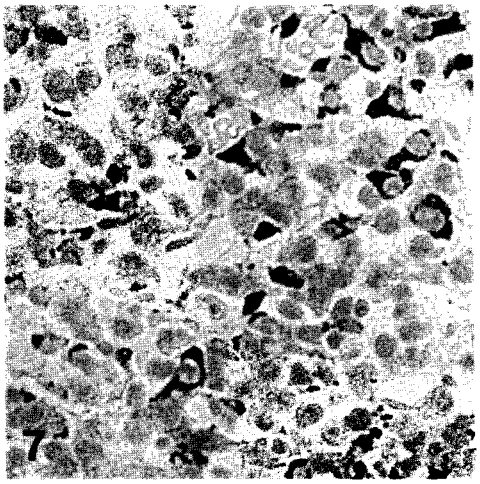
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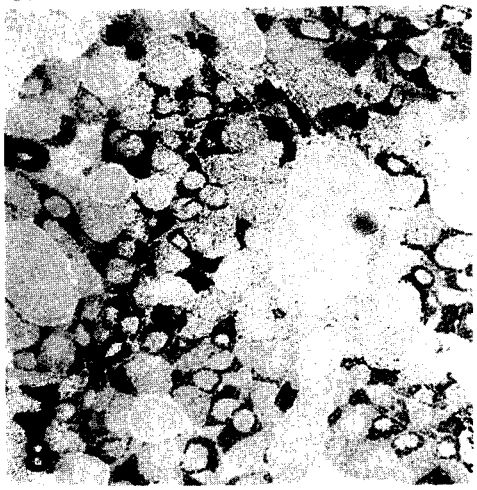
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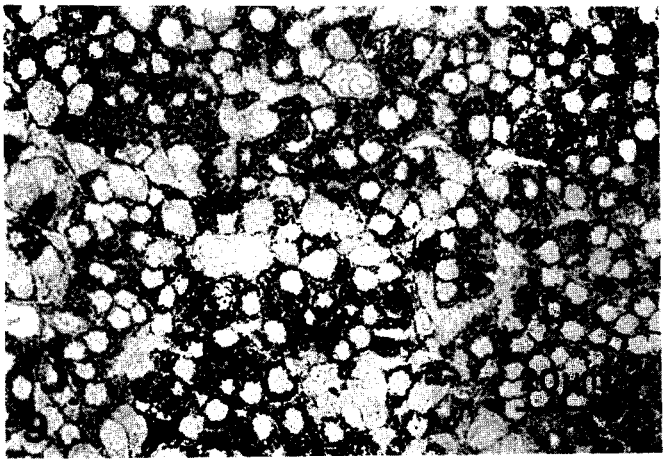

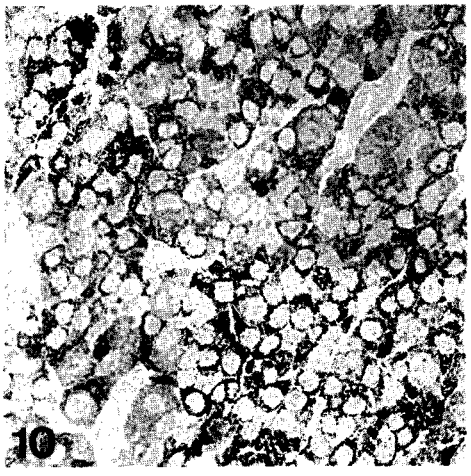
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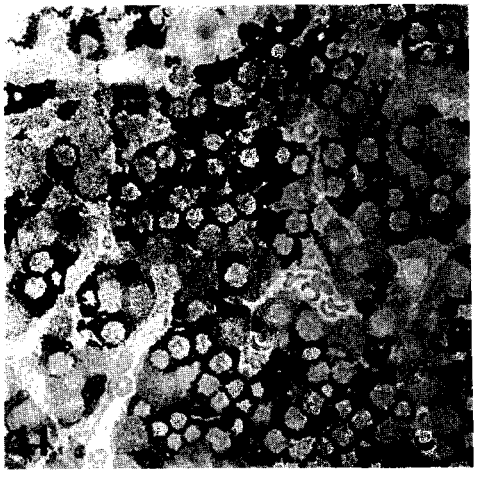

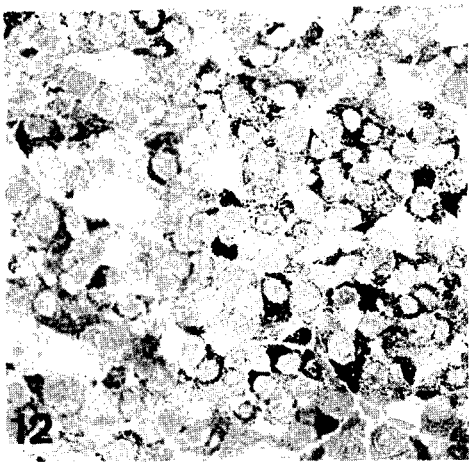
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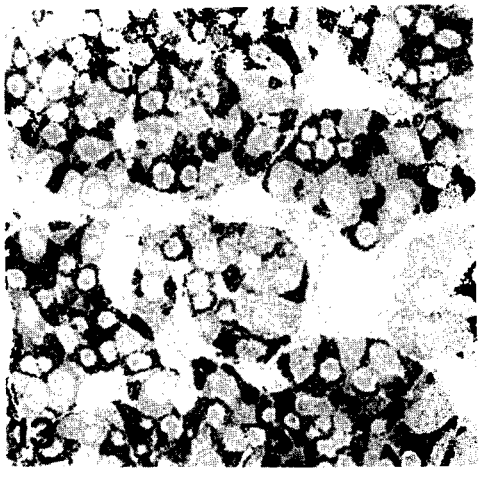
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Fig. 4. Immunoperoxidase-labelled PRL cells in a control male. They are angular cells with numerous immunoreactive secretory granules. × 440. Bar, 20 μm.

Fig. 5. PRL cells in the adenohypophysis of a male blinded for 8 weeks. A marked reduction in the cell number and size is noted. × 440

Fig. 6. PRL-cells in the adenohypophysis of a male that was pinealectomized and blinded for 8 weeks. Many PRL cells are much larger in size and contain more secretory granules than those in blinded males. × 440

Fig. 7. PRL cells in the adenohypophysis of a male exposed to continuous darkness for 8 weeks. The cells are decreased in number and appear atrophic as do those in blinded males. × 440

Fig. 8. PRL cells in a male that was pinealectomized and exposed to continuous darkness for 8 weeks. PRL cells are larger and more numerous than those in Fig. 7. × 400

Fig. 9. PRL cells in a control female. The cells are large and oval or angular in shape. The cytoplasm is filled with immunoreactive secretory granules. × 440. Bar 20 μm.

Fig. 10. Atrophic PRL cells in a female animal blinded for 8 weeks. × 440.

Fig. 11. PRL cells in the adenohypophysis of a female animal pinealectomized and blinded for 8 weeks. They are larger than those in Fig. 10. × 440

Fig. 12. Atrophic PRL cells in a female hamster exposed to continuous darkness for 8 weeks. The cytoplasm of PRL cells contains less numerous secretory granules as compared with those in Fig. 9. × 440

Fig. 13. PRL cells in the adenohypophysis of a female animal that was pinealectomized and exposed to continuous darkness for 8 weeks. The immunoreactive secretory granules are significantly increased in number when compared with those in Fig. 12. × 440

Table 1. The cellular and cytoplasmic areas (in μm^2) of prolactin cells in different groups of experimental animals. the abbreviations are the same as in Fig. 2.

	MALE		FEMALE	
Group	**Cell**	**Cytoplasm**	**Cell**	**Cytoplasm**
INT	81.3 ± 7.3	50.2 ± 7.1	109.4 ± 7.6	75.8 ± 8.6
B	65.9 ± 6.0[a]	42.6 ± 6.6	70.6 ± 5.8[a]	45.3 ± 8.4[a]
B + Px	80.8 ± 6.8[b]	57.2 ± 6.2[a,b]	75.9 ± 6.0[a,b]	49.3 ± 9.0[a,c]
D	67.0 ± 6.3[a]	45.6 ± 6.7	58.8 ± 6.0[a]	33.2 ± 6.8[a]
D + Px	71.1 ± 5.6[a]	47.4 ± 6.9[e]	88.7 ± 7.9[a,d]	57.4 ± 8.0[a]

a : $p < 0.001$ vs. INT - **b** : $p < 0.001$ vs. B - **c** : $p < 0.05$ vs. B - **d** : $p < 0.001$ vs. D - **e** : $p < 0.01$ vs. D

PRL cells in male hamsters exposed to continuous darkness significantly decreased in cell number (Fig. 2), and exhibited a regression in the cytoplasm (Fig. 7, Table 1). The population ratio of PRL cells was slightly increased in the hamsters that were pinealectomized and exposed to continuous darkness but failed to reach a significant difference as compared with that in continuous darkness-treated hamsters (Fig. 2). However, most of the PRL cells from hamsters exposed to continuous darkness in combination with pinealectomy were smaller in cell size but displayed a similar staining intensity as in the intact control (Fig. 8 and Table 1).

PRL cells in female hamsters

PRL cells comprised 47% of cell population in the pituitary of the control females (Fig. 3). They were oval or angular in shape and their nucleo-cytoplasmic ratio was usually smaller than that of PRL cells from intact males (Fig. 9). A drastic decrease in cell population and cytoplasmic area of PRL cells was noted after optic enucleation (Fig. 10, Table 1). The population ratio of PRL cells was 45% in the blinded and pinealectomized females. The value was significantly greater than that in blinded females and comparable to that in the intact animals (Fig. 3). In the blinded and pinealectomized females, PRL cells possessed a larger cytoplasmic area when compared with that in blinded females (Fig. 11, Table 1). Pinealectomy partially prevented the effect of blin ness on the cell and cytoplasmic areas; the PRL cells appeared smaller in blinded-pinealectomized females than those in control females (Table 1).

PRL cells of the female hamsters exposed to continuous darkness showed a marked reduction in cell population and cytoplasmic area (Figs. 3, 12, Table 1); the results were partially reversed in pinealectomized

females exposed to continuous darkness. Thus, the PRL cells in the latter group were still smaller in size than those in the intact females (Fig. 13).

Discussion

We used immunostaining in identification of PRL cells in the hamster adenohypophysis and investigated the changes in the cell morphology and population ratio of this cell type with special reference to the pituitary of the hamsters exhibiting pineal-induced gonadal atrophy. PRL cells in the hamster showed a sexual dimorphism, especially in the cell morphology and population ratio. PRL cells were apparently larger and more numerous in the pituitary of the female than those in the male gland. Light deprivation in the hamsters of both sexes resulted in a significant decrease in the cell number of PRL cells. In addition, it induced an atrophy of PRL cells as characterized by the diminished area of cytoplasm with the depletion of secretory granules. It appears that PRL cells in the darkness-treated hamsters were more severely affected than those in the blinded hamsters on the basis of the changes in the population ratio. The inhibitory effect on PRL cells was apparently mediated by the pineal gland since pinealectomy combined with light deprivation largely prevented the above-mentioned changes.

Our results were in agreement with the studies of immunoassay of PRL by previous investigators which demonstrate that the synthesis of PRL is greatly inhibited in blinded male and female hamsters (Blask et al., 1986; Orstead and Blask, 1987) or in hamsters exposed to a short photoperiod (Reiter and Johnson, 1974; Reiter, 1975b, 1980b; Goldman et al., 1981; Borer et al., 1982; Steger et al., 1983). Our data also correlated well with the reduced amount of pituitary PRL reported in light-deprived hamsters (Reiter and Johnson, 1974; Bartke, 1980; Reiter, 1980a,b; Blask et al., 1986).

Hypoplasia and atrophy of mammotroph cells are noticed in blind-anosmic female rats (Leadem and Blask, 1982) and a marked reduction of mature secretory granules in the mammotrophs is shown in female rats exposed to constant darkness (Relkin et al., 1972). The present observations in the light-deprived female hamsters were consistent with those morphological changes in female rats. Leadeam and Blask (1982) used immunohistochemical staining to identify PRL cells at the light microscopic level. However, they did not provide a statistical analysis on the changes of cell population in their experimental specimens. Relkin et al. (1972) and Shiino et al. (1974) demonstrated that the secretory granules of PRL cells were decreased in number in light-deprived and anosmic rats by conventional electron microscopy. Recent studies using immunocytochemistry have classified three types of PRL cells according to the different size of secretory granules in the rat adenohypophysis (Nogami and Yoshimura, 1980, 1982). PRL cells described by Relkin et al. (1972) and Shiino et al. (1974) apparently belong to the large granule-containing PRL cells. This indicates that the changes of the other two types of PRL cells could not be found in the earlier nonimmunocytochemical studies. Therefore, the immunocytochemical methods are justified in being employed to study the morphological alteration of PRL cells after light deprivation.

The present observation of low PRL cell activity in light-deprived hamsters is in agreement with the gonadal atrophy. Since prolactin stimulates the production of LH receptors in Leydig cells which then increases testosterone production (Bartke, 1980), a low level of pituitary PRL might indirectly result in a low level of circulating testosterone.

The precise mechanism by which the pineal gland accomplished its inhibition of PRL cell function is unclear. A possible involvement of the hypothalamic dopaminergic system in this aspect was proposed by Leadem et al. (1988); an increase in dopaminergic neuron activity was found to precede the inhibition of prolactin and the reproductive system in blind-anosmic female rats. Dopamine, a physiological prolactin-inhibiting factor, might exert an inhibition on PRL cell activity which in turn retards the growth of reproductive organs. Further study on the activity of the hypothalamic dopaminergic neurons under different pineal activities will provide an insight into the mechanism of pineal-mediated prolactin secretion.

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