Effects of Photoperiod and Melatonin on the Development of Growth Hormone Cells and the Pituitary-Adrenal Axis in the Djungarian Hamster, *Phodopus sungorus*

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Summary. The development of GH cells and the pituitary-adrenal axis was morphologically examined in male Djungarian hamsters (*Phodopus sungorus*) exposed to short days and those kept under long days and receiving daily afternoon injections of melatonin, from the time of weaning (20 days) until 100 days of age. The postnatal increase in area of ACTH cells under long days was inhibited in short-day-exposed or melatonin-treated animals. It was suggested that a short photoperiod may suppress, via melatonin, the development of ACTH cells. GH cells were not affected by age, photoperiod or exogenous melatonin. Under long days, the zona fasciculata decreased in volume with age, while the zona reticularis increased. Such changes in the volumes of these adrenocortical zones were depressed under short days. In addition, the volumes of the zona fasciculata and zona reticularis in long-day-housed animals became respectively larger and smaller subsequent to orchidectomy and melatonin administration. These results suggest that fasciculata cells in deeper levels become progressively differentiated into reticularis cells, that short photoperiod inhibits development of both zonae, and that such an inhibition is caused mainly by the decreased secretion of androgens.

There is considerable evidence indicating that the pineal gland, via its hormone melatonin, exerts the photoperiodic regulation of pituitary function. Among a variety of photoperiodic rodents, the Syrian hamster and Djungarian hamster (*Phodopus sungorus*) have been used most extensively for study of the interactions of the pineal gland with pituitary hormones. However, previous studies have focussed mainly on adult animals and have primarily concerned the pituitary-gonadal axis and prolactin (PRL); exposure

to short photoperiod and properly timed injections or long duration infusions of melatonin, which respectively mimics short-day-induced responses in pineal-intact or pinealectomized animals, have been described as suppressing the secretion of gonadotropins and PRL in Syrian (TUREK and CAMPBELL, 1979; BARTKE et al., 1980; REITER, 1980; STEGER et al., 1985; BARTKE and STEGER, 1992) and Djungarian (DUNCAN et al., 1985; YELLON and GOLDMAN, 1987; BARTNESS and GOLDMAN, 1988; SCHLATT et al., 1993) hamsters. Pineal-thyroid interactions have also been studied in both species; it is generally believed that short photoperiod or administration of melatonin exerts an inhibitory action on the pituitary-thyroid axis (VRIEND, 1983; SEIDEL et al., 1987; MASUDA and OISHI, 1989; VAUGHAN and VAUGHAN, 1993). However, data on the pineal and adrenal relationships and the interactions of the pineal gland with growth hormone (GH) are scarce; in the Syrian hamster, plasma concentrations of glucocorticoids decrease under short photoperiods (OTTENWELLER et al., 1987), and exposure to short photoperiods and daily injections of melatonin have no effect (BORER et al., 1982; VRIEND et al., 1987) on serum or pituitary GH levels or cause an increase (VRIEND et al., 1990; LAARTZ et al., 1994) in circulating levels of this hormone. Nothing has been found in the literature concerning the influences of photoperiod or exogenous melatonin on the pituitary-adrenal axis or GH in the Djungarian hamster.

In the Djungarian hamster, somatic growth and gonadal development are greatly influenced by changes in photoperiod or exogenous melatonin (HOFFMANN, 1978, 1982; CARTER and GOLDMAN, 1983a, b; GOLDMAN et al., 1984; YELLON and GOLD-

MAN, 1984). In addition, these responses occur more rapidly in juveniles than in adults (CARTER and GOLDMAN, 1983 a, b). By contrast, the gonadal development of juvenile Syrian hamsters has been reported to be uninfluenced by photoperiod (GASTON and MENAKER, 1967; REITER et al., 1970; TUREK, 1979). Thus, the Djungarian hamster may be a good model for studying pineal and pituitary relationships during development. Regarding the effects of photoperiod or exogenous melatonin on pituitary hormones in juvenile Djungarian hamsters, only gonadotropins and PRL have been considered (GOLDMAN et al., 1984; YELLON and GOLDMAN, 1984), and interactions of the pineal gland with the other hormones have not been investigated.

The present study aims to examine whether or not the morphology of GH and ACTH cells and the adrenal cortex of developing Djungarian hamsters is affected by exposure to different photoperiods or daily afternoon injections of melatonin. The profile area of GH and ACTH cells and the volume of each adrenocortical zone were used as the morphological parameters for the development of GH cells and the pituitary-adrenal axis. In addition, data between intact and orchidectomized animals were compared to determine whether or not the effect of photoperiod or exogenous melatonin on GH cells and the pituitary-adrenal axis is dependent on changes in the reproductive status.

MATERIALS AND METHODS

Fifty-five male Djungarian hamsters (*Phodopus sungorus*) from our breeding colony were used for the present study. They were housed in clear plastic cages, each of which had four to five animals, and given a standard commercial diet and water *ad libitum*. The animal habitat was maintained at a temperature of $23 \pm 2^\circ\text{C}$. The animals were kept in LD 16:8 (lights on from 0500 to 2100 h) from birth until the time of weaning (20 days of age). Light was provided by ceiling-mounted, cool-white fluorescent lamps, with the intensity at the bottom of the cages being approximately 20 lux.

Five animals were killed at 20 days of age. Each group of 15 weaned animals was kept either under LD 16:8 or LD 8:16 (light on from 0900 to 1700 h); 5 animals kept under the long days and those under the short days were killed at the ages of 40, 60 and 100 days, respectively. These intact animals were killed between mid-September and mid-November. Orchidectomy and sham-orchidectomy were done on each group of 5 animals at 20 days. These animals

were kept under LD 16:8 and killed at 100 days between early September and early October. Each group of 5 animals was kept under LD 16:8 after weaning and received a daily subcutaneous injection of melatonin ($10 \mu\text{g}/\text{animal}$ in 0.1 ml of 5% ethanolic saline) or ethanolic saline vehicle at 1700 h (4 h before lights-off). The solutions were freshly prepared daily. The animals treated with melatonin or vehicle were killed at the age of 100 days between mid-August and mid-September. All the animals used in the present study were killed by decapitation at the middle of the light period (1300 h).

Intact animals were weighed at the time of sacrifice. Body weights of orchidectomized and sham-orchidectomized animals and those treated with vehicle and melatonin were recorded at 20, 40, 60 and 100 days. The pituitary glands, together with a portion of the base of the skull of all the animals—except for 40-day-old intact ones—and both adrenal glands of all the animals, were quickly removed and immersed in Bouin's fluid at room temperature. Both testes of the intact animals and those treated with the vehicle or melatonin were then removed and weighed. After fixation for 20 h, the pituitary glands, which were removed from the skull under the dissecting microscope, and adrenal glands were dehydrated in a graded series of ethanol and embedded in paraffin. Serial frontal sections of the pituitary and adrenal glands were prepared at a thickness of $6 \mu\text{m}$ and $8 \mu\text{m}$, respectively. The sections of the pituitary gland were processed for immunohistochemical staining, and those of the adrenal gland were stained with hematoxylin-eosin.

Immunohistochemical identification of GH and ACTH cells was performed with the antisera raised in rabbits against GH (polyclonal; Chemicon International; AB940) or ACTH (1–24) (polyclonal; Chemicon International; AB902) using Vectastain Universal Elite ABC kit (Vector, Burlingame, CA, USA). Sections were pretreated in 3% H_2O_2 , preincubated in 1.5% normal goat serum for 30 min at room temperature, and then incubated at room temperature overnight in the antisera against GH (diluted 1:1,000) or ACTH (diluted 1:1,000). Sections were incubated with biotinylated anti-rabbit IgG at a dilution of 1:200 for 1 h at 32°C and then with the avidin-biotin-peroxidase complex (diluted 1:25) for 1 h at 32°C . Finally, sections were reacted with 0.01% diaminobenzidine tetrahydrochloride (DAB) and H_2O_2 in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) for 4 min at 37°C . Sections were counterstained with veronal acetate-buffered 1% methyl green (pH 4.0). The staining specificity of the antisera against GH or ACTH was tested by omitting the antisera or replacing them

with normal rabbit serum or PBS. No specific immunostaining was detected under these conditions.

Sections of the pituitary gland stained with the antisera against GH or ACTH were used for the determination of profile areas of GH and ACTH cells. The profiles of these cells were drawn at a magnification of $\times 1,000$, and their areas were determined using a digitizer (KD4610A, Graphtec, Yokohama, Japan) and computer (NIH, Image 1.59). Only solitary, nucleated cells were measured. For one animal kept under LD 16:8 and killed at the age of 100 days, it was found that the differences for each cell type between the mean profile areas obtained from each of the five sets of 50 cells and from 250 cells were less than 5%. Thus, in this study, the mean profile areas of 50 cells were determined for each cell type.

The volumes of the three adrenocortical zones were determined. Sections of the adrenal gland were examined at a magnification of $\times 100$, camera lucida drawings of each cortical zone were made, and their areas were measured using a digitizer and computer. In one 40-day-old animal under LD 8:16, the sum of the areas of the profiles on all the sections was found to be the same as 5 times the areas on every fifth section. No differences were observed between the data obtained from the right and left glands. Accordingly every fifth section of the right gland was used for the measurement. Due to difficulty in distinguishing between the zona glomerulosa and zona fasciculata, and the absence of the zona reticularis, only the total area of the adrenal cortex was measured in 20-day-old animals.

Data were expressed as the mean $\pm$ the standard error of the mean, and were analyzed for statistical significance by non-parametric tests (Mann-Whitney U-test or Kruskal-Wallis H-test). Possible differences in testes and body weights as well as adrenocortical volumes were evaluated by a two-way analysis of variance (ANOVA), with subsequent comparisons between individual means made by the least significant difference (L. S. D.) test.

RESULTS

Distribution of GH and ACTH cells

GH and ACTH cells were distributed abundantly and evenly in the pars distalis. The distribution and density of these cells showed no marked changes in relation to age, photoperiod, orchidectomy, and melatonin administration.

Fig. 1. Profile area of GH and ACTH cells in intact animals under long (white bar) and short (black bar) photoperiods. Each group consists of five animals ($n=5$). *Significantly smaller than intact animals under long photoperiod at the respective ages ($p<0.008$, U test).

Fig. 2. Profile area of GH and ACTH cells in sham-operated (S), orchidectomized (O), and vehicle (V)- and melatonin (M)-treated animals. Each group consists of five animals ($n=5$). *Significantly smaller than vehicle-treated animals ($p<0.008$, U test).

Fig 3. Adrenal cortex of 40-, 60- and 100-day-old intact animals under long photoperiod (LP), and 100-day-old, short-day-exposed (SP) and melatonin-treated animals. Hematoxylin-eosin. *G* zona glomerulosa, *F* zona fasciculata, *R* zona reticularis, *M* medulla. *Arrowheads* and *arrows* indicate the boundaries between zonae glomerulosa and fasciculata, and between zonae fasciculata and reticularis, respectively. Bar = 50 μ m

Profile area of GH and ACTH cells

In long-day-housed, intact animals, ACTH cells increased in area with advancing age (Fig. 1; $p < 0.01$, H test), whereas the area of GH cells showed no significant differences from 20 to 100 days. The areas of GH and ACTH cells in short-day-housed animals at 60 and 100 days were similar to those at 20 days (Fig. 1). At 60 and 100 days the areas of ACTH cells, but not those of GH cells, showed significant differences between animals under both photoperiods (Fig. 1). GH and ACTH cells were similar in size between orchidectomized and sham-operated animals (Fig. 2). Daily afternoon injections of melatonin induced a marked atrophy of ACTH cells, whereas the

size of GH cells was unaffected by this indolamine (Fig. 2). The area of ACTH cells in animals treated with melatonin (Fig. 2) was the same as that in 100-day-old, intact animals under the short photoperiod (Fig. 1).

Morphology of adrenal cortex

The adrenal cortex was unable to be distinguished into three zones until 40 days, when it was constituted by the thin, superficial zona glomerulosa and deep zona reticularis, with the thick zona fasciculata intervening between them (Fig. 3). The zona glomerulosa consisted of small cells endowed with a weakly acidophilic cytoplasm and deeply stained, rounded

Fig. 4. Superficial (upper figure) and deep (lower figure) portions of adrenal cortex of 40-day-old, intact animals under long photoperiod. *G* zona glomerulosa, *F* zona fasciculata, *R* zona reticularis, *M* medulla. Hematoxylin-eosin. Bar = 20 μ m

nuclei (Fig. 4 upper figure). In the zona fasciculata, the cells were larger in size, and their nuclei, which were also round in shape, were more lightly stained than those in the zona glomerulosa (Fig. 4 upper figure). The cells in the zona fasciculata, especially in its outer region, contained small amounts of tiny vacuoles corresponding to lipid droplets (Fig. 4 upper figure). The cells of the zona reticularis were small and had densely stained, elliptical nuclei (Fig. 4 lower figure). The cytoplasm of the fasciculata and reticularis cells was more strongly acidophilic than that of the glomerulosa cells. The microscopic appearance of the zona glomerulosa and zona fas-

ciculata was almost similar while that of the zona reticularis differed between 40-day-old animals under either the long or short photoperiods; the zona reticularis was less developed under LD 8 : 16 and did not form a continuous band around the medulla in many short-day-exposed animals.

At 20 days, the boundary between the zona glomerulosa and zona fasciculata was poorly marked. The cells in a narrow, superficial region underneath the capsule contained a weakly acidophilic cytoplasm, and the remaining adrenocortical region consisted of more intensely acidophilic cells. Nuclei of adrenocortical cells increased in size, and their nuclear density

Fig. 5. Zona glomerulosa (*G*) and a superficial portion of zona fasciculata (*F*) (upper figure) and the border zone between zonae fasciculata and reticularis (*R*) (lower figure) of 100-day-old, intact animals under long photoperiod. Hematoxylin-eosin. Bar = 20 μ m

decreased gradually in deeper regions. Lipid droplets were not found in adrenocortical cells. The zona reticularis was absent.

In 40- to 100-day-old animals under LD 16:8, the thickness of the zona glomerulosa remained constant, whereas that of the zona fasciculata and zona reticularis respectively decreased and increased with age (Fig. 3). The amount of lipid droplets in fasciculata cells, and the number of these cells containing abundant lipid droplets increased toward deeper levels of the zona fasciculata with advancing age

(Fig. 3, Fig. 4 upper figure, Fig. 5 upper figure). Fasciculata cells appeared to become larger as lipid droplets accumulated. At 100 days, a very narrow layer consisting of smaller fasciculata cells devoid of large lipid droplets and showing a much lower cell density than the zona reticularis overlay the zona reticularis (Fig. 5 lower figure). Exposure to short photoperiods, orchidectomy, and the administration of melatonin induced morphological changes in all adrenocortical zones except the zona glomerulosa in 100-day-old animals. After orchidectomy, lipid

droplets in the zona fasciculata decreased to the level in 60-day-old animals under LD 16:8. A more prominent decrease in the amount of lipid droplets in this zone occurred through exposure to short photoperiod (Fig. 3) or treatment with melatonin (Fig. 3). The zona fasciculata and zona reticularis respectively increased and decreased in thickness in animals under LD 8:16 (Fig. 3) and in those treated with melatonin (Fig. 3). Thus, the two zones were very similar to one another in these animals, and the zona reticularis was extremely thin as in 40-day-old animals under LD 16:8 (Fig. 3).

Volume of adrenocortical zones

Under LD 16:8, the total volume of the adrenal cortex increased from 20 to 40 days, when it attained the same value as at 100 days (Table 1). Photoperiod, orchidectomy and the administration of melatonin exerted no apparent influence on the total volume of all the adrenocortical zones (Table 1). The volume of the zona fasciculata and zona reticularis, but not that of the zona glomerulosa, was much influenced by photoperiod, age, orchidectomy, and the administration of melatonin (Table 1). ANOVA revealed a significant main effect of photoperiod on the volume

of the zona fasciculata ($F=7.50$, $df=1/24$, $p<0.05$). Although there was no significant difference in the volume of the zona fasciculata in 60-day-old animals under short and long photoperiods, this zone was significantly larger in volume under LD 8:16 than under LD 16:8 at 100 days (Table 1). There was no significant main effect of age on its volume ($F=0.59$, $df=2/24$, $p>0.05$). ANOVA showed significant main effects of age ($F=76.53$, $df=2/24$, $p<0.01$) and photoperiod ($F=106.78$, $df=1/24$, $p<0.01$) on the volume of the zona reticularis (Table 1). Significant differences were noted in the volume of this zone in short- and long-day-exposed animals at the respective ages (Table 1). A significant correlation existed between the volumes of the zona fasciculata and zona reticularis in animals under LD 16:8 ($r=-0.55$, $p<0.05$). The volumes of the zona fasciculata and zona reticularis were respectively larger and smaller in orchidectomized animals than in sham-operated ones (Table 1). However, these values in orchidectomized animals did not reach the level seen in 100-day-old, intact animals under LD 8:16 (Table 1). Likewise, the volumes of the zona fasciculata and zona reticularis respectively increased and decreased, by the administration of melatonin (Table 1). The volumes of these zones in melatonin-treated animals corre-

Table 1. Total volume (mm^3) of the adrenal cortex (A), and volume (mm^3) of the zonae glomerulosa (G), fasciculata (F) and reticularis (R) in intact animals under long (LP) and short (SP) photoperiods, sham-operated and orchidectomized animals, and vehicle- and melatonin-treated animals. Each group consists of five animals ($n=5$).

	Age(days)	Intact (LP)	Intact (SP)	Sham-operated	Orchidectomized	Vehicle	Melatonin
(A)	20	0.592 $\pm$ 0.022					
	40	0.915 $\pm$ 0.038	0.835 $\pm$ 0.033				
	60	0.874 $\pm$ 0.100	0.981 $\pm$ 0.119				
	100	0.834 $\pm$ 0.043	1.023 $\pm$ 0.059	0.818 $\pm$ 0.026	0.871 $\pm$ 0.043	0.820 $\pm$ 0.024	0.881 $\pm$ 0.033
(G)	40	0.154 $\pm$ 0.005	0.134 $\pm$ 0.006				
	60	0.147 $\pm$ 0.010	0.146 $\pm$ 0.015				
	100	0.154 $\pm$ 0.005	0.158 $\pm$ 0.008	0.147 $\pm$ 0.004	0.149 $\pm$ 0.004	0.155 $\pm$ 0.005	0.164 $\pm$ 0.009
(F)	40	0.718 $\pm$ 0.039	0.691 $\pm$ 0.033				
	60	0.606 $\pm$ 0.087	0.778 $\pm$ 0.092				
	100	0.475 $\pm$ 0.036	0.793 $\pm$ 0.061*	0.462 $\pm$ 0.019	0.623 $\pm$ 0.039**	0.445 $\pm$ 0.015	0.635 $\pm$ 0.029****
(R)	40	0.043 $\pm$ 0.003	0.010 $\pm$ 0.001*				
	60	0.121 $\pm$ 0.004	0.057 $\pm$ 0.015*				
	100	0.205 $\pm$ 0.006	0.072 $\pm$ 0.010*	0.209 $\pm$ 0.005	0.099 $\pm$ 0.014***	0.220 $\pm$ 0.016	0.064 $\pm$ 0.007****

* $p<0.05$ when compared with intact (LP) animals at the respective ages (L.S.D. test).

** $p<0.016$ and *** $p<0.008$ when compared with sham-operated animals (U test).

**** $p<0.008$ when compared with vehicle-treated animals (U test).

sponded to those found in 100-day-old, intact animals under LD 8 : 16. There was no significant difference in the volume of the zona glomerulosa between orchidectomized and sham-operated animals, or between melatonin- and vehicle-treated animals (Table 1).

The volume of the adrenal medulla (not shown) increased from 20 to 60 days, when it attained the level found at 100 days. The adrenal medulla was similar in volume in short- and long-day-exposed animals aged 20 to 100 days.

Testes weight

ANOVA revealed a significant main effect of photoperiod on testicular weight at the age of 40 to 100 days ($F=517.22$, $df=1/24$, $p<0.01$), when the testes showed a marked atrophy under LD 8 : 16 (Table 2). Daily injections of melatonin induced a similar reduction of the testicular weight (Table 2).

Body weight

ANOVA showed significant main effects of age ($F=$

12.07 , $df=2/24$, $p<0.01$) and photoperiod ($F=19.63$, $df=1/24$, $p<0.01$) on the body weight of intact animals aged 40 to 100 days. Significant differences existed in the body weight of short- and long-day-exposed animals at the age of 40 to 100 days (Table 3). The increase in body weight from the time of weaning until 100 days in short-day-exposed animals was found to be about half that in long-day-exposed animals (Table 3). A similar inhibition in somatic growth occurred by orchidectomy (Table 3) and the administration of melatonin (Table 3). ANOVA demonstrated significant main effects of age ($F=9.45$, $df=3/32$, $p<0.01$, orchidectomized and sham-orchidectomized animals; $F=79.81$, $df=3/32$, $p<0.01$, melatonin- and vehicle-treated animals) and treatment ($F=72.04$, $df=1/32$, $p<0.01$, orchidectomized and sham-orchidectomized animals; $F=27.31$, $df=1/32$, $p<0.01$, melatonin- and vehicle-treated animals). The postnatal weight gain was almost identical among short-day-exposed, orchidectomized, and melatonin-treated animals, and among long-day-exposed, sham-orchidectomized and vehicle-treated animals (Table 3).

Table 2. Testicular weights (mg/g body weight) in intact animals under long (LP) and short (SP) photoperiods, and vehicle- and melatonin-treated animals. Each group consists of five animals ($n=5$).

Age (days)	Intact (LP)	Intact (SP)	Vehicle	Melatonin
20	9.54 ± 1.21			
40	37.06 ± 2.45	$1.51 \pm 0.17^*$		
60	38.64 ± 2.64	$1.34 \pm 0.14^*$		
100	32.51 ± 1.95	$0.86 \pm 0.04^*$	39.28 ± 1.71	$0.75 \pm 0.03^{**}$

*Significantly smaller than intact (LP) animals at the respective days ($p<0.001$, L.S.D. test).

**Significantly smaller than vehicle-treated animals ($p<0.008$, U test).

Table 3. Body weights (g) of intact animals under long (LP) and short (SP) photoperiods, sham-operated and orchidectomized animals, and vehicle- and melatonin-treated animals from 20 to 100 days of age. Each group consists of five animals ($n=5$).

Age (days)	Intact (LP)	Intact (SP)	Sham-operated	Orchidectomized	Vehicle	Melatonin
20	13.3 ± 0.9		13.2 ± 0.6	13.1 ± 0.6	12.7 ± 0.6	12.5 ± 0.6
40	25.3 ± 0.6	$19.5 \pm 1.5^*$	24.9 ± 0.6	20.8 ± 0.4	21.4 ± 1.1	18.3 ± 0.5
60	28.7 ± 0.7	$21.1 \pm 0.7^*$	31.5 ± 1.0	23.4 ± 1.0	28.7 ± 0.8	23.5 ± 0.9
100	34.2 ± 1.3	$23.1 \pm 1.3^*$	35.9 ± 1.3	23.5 ± 1.1	34.2 ± 0.7	24.5 ± 1.3

*Significantly smaller than intact (LP) animals at the respective ages ($p<0.05$, L.S.D. test).

DISCUSSION

The present data suggest that the size of GH cells is not influenced by photoperiod and melatonin, and that GH cells are already well developed at 20 days. The frequency or volume density of GH cells in rats (SMETS et al., 1989) and Syrian hamsters (TANIGUCHI et al., 1989) was reported to increase from late embryonic stages to 15-20 days after birth, and to remain constant thereafter until adulthood. Electron microscopic observations on secretory granules in GH cells in rats indicated that there was no difference in their size among neonatal, immature and adult animals, suggesting that GH cells have already become morphologically mature at birth (SMETS et al., 1987). However, GH cells in rats were classified, according to the size of secretory granules, into three subtypes, the type I, II and III cells (KUROSUMI et al., 1986), which, based on the age changes in their frequency, were supposed to represent mature, intermittent and immature types, respectively (TAKAHASHI, 1991, 1992). Whether or not GH cells in the Djungarian hamster are divided into subtypes remains a topic for further study.

There are considerable species variations in the frequency of ACTH cells. The number of ACTH cells in rats is generally less than that of GH and PRL cells, which constitute a large population of pars distalis cells (OSAMURA and NAKANE, 1982; DADA et al., 1984); however, in the Syrian hamster, the volume density of ACTH cells, which is correlated with their number and size, is large enough to correspond to that of the other pars distalis cells excluding TSH cells (TANIGUCHI et al., 1989). As in the Syrian hamster, the pars distalis of the Djungarian hamster contains abundant ACTH cells. Previous morphometric studies on ACTH cells reported that their number and volume density showed no apparent postnatal changes in rats (SMETS et al., 1989) and Syrian hamsters (TANIGUCHI et al., 1989). We have here demonstrated for the first time that, in the Djungarian hamster, the size of ACTH cells from 20 to 100 days increased under LD 16:8, but remained constant under LD 8:16 and that administration of melatonin suppressed the increase in the size of these cells seen under the long photoperiod. These findings suggest that short photoperiod inhibits, via melatonin, the postnatal growth of ACTH cells in this animal. The present data obtained from orchidectomized animals deny the possibility that such inhibitory influences of melatonin are secondary to changes in gonadal hormones.

In is not until 40 days that the zona glomerulosa

can be identified in the Djungarian hamster. This zone attained the volume of 100-day-old animals at 40 days, whereas the volumes of the zona fasciculata and zona reticularis showed marked changes after 40 days of age. Thus, the zona glomerulosa, unlike the other adrenocortical zones, may reach its full development relatively early after birth in this animal. In the Djungarian hamster, the nuclei of the reticularis cells predominantly assume an elliptical shape, in contrast to those of the fasciculata cells showing rounded contours. The available data on the nuclear morphology of the fasciculata and reticularis cells in mammals are scanty, but in rats and some other species these cells are found to be similar in having round nuclei (NUSSDORFER, 1986). The apparent morphological difference in the cells of the zona fasciculata and zona reticularis enabled us to discriminate these zones and to examine their developmental changes in the Djungarian hamster.

Quantitative morphological studies of the postnatal development of the adrenal cortex have previously been made on the rat and Syrian hamster; in these animals, the volumes of all the adrenocortical zones increase with age between 21 and 77 days (MAJCHRZAK and MALENDOWICZ, 1983; NIKICICIZ et al., 1984). In long-day-housed Djungarian hamsters, by contrast, the adrenocortical volume increases from 20 to 40 days, when it attains the same value as at 100 days. The volume of the zona fasciculata decreases but that of the zona reticularis increases between 40 and 100 days. In addition, larger fasciculata cells containing abundant lipid droplets increase in number with age toward the deeper levels of the zona fasciculata and, as a result, the inner layer of the zona fasciculata, consisting of smaller cells with a lesser amount of lipid droplets, decreases progressively in width. As in the rat (VAN DORP and DEANE, 1950; MAJCHRZAK and MALENDOWICZ, 1983) and Syrian hamster (ITO, 1956; NIKICICIZ et al., 1984), the development of the zona reticularis begins late in comparison with that of the remaining cortical zones.

Taking all the above together, it is suggested that, in the Djungarian hamster, the hypertrophy of fasciculata cells due to an accumulation of lipid droplets is associated with their differentiation, that the differentiation proceeds centripetally in the zona fasciculata, and that undifferentiated cells constituting the deeper layer of the zona fasciculata of developing animals become progressively transformed into reticularis cells. However, the age-related changes in the volumes of the zona fasciculata and zona reticularis are not exactly the same; the decrease in the volume of the former exceeds the increase in that of the latter. This difference may occur if fasciculata

cells become smaller when transformed into reticularis cells. As far as we know, the present study is the first to demonstrate the effects of photoperiod and chronic administration of melatonin on the volume of the adrenal cortex of mammals. Our observations that age changes in the volumes of the zona fasciculata and zona reticularis seen under long days were depressed under short days suggest that the adrenocortical development in the Djungarian hamster is suppressed by short photoperiod, and that such an inhibition is mediated by melatonin.

The present data which an increase in volume of the zona fasciculata occurs simultaneously with a decrease in volume of the zona reticularis following orchidectomy strongly suggest that gonadal androgens directly exert a facilitating influence on the adrenocortical development in the Djungarian hamster. A stimulatory action of androgens on the adrenal cortex has been demonstrated in the Syrian hamster; the volumes of the zona fasciculata and zona reticularis of adult Syrian hamsters decrease following orchidectomy, and the volume of the zona fasciculata, but not that of the zona reticularis, in orchidectomized hamsters is restored to the control value after treatment with testosterone (MALENDOWICZ et al., 1982). Examination of our data reveals that the volumes of the zona fasciculata and zona reticularis in orchidectomized animals are not identical with those in short-day-housed animals. This suggests that orchidectomy does not so greatly suppress the development of the zona fasciculata and zona reticularis as does short photoperiod. As mentioned, the development of ACTH cells is supposed to be depressed under short photoperiod. It is possible that ACTH also participates in the photoperiodic regulation of the adrenocortical development in the Djungarian hamster.

Relations between serum concentrations of glucocorticoids and photoperiod have been examined in a few species. Conflicting results have been obtained from adult Syrian hamsters; some investigators have observed similar circadian rhythms in plasma cortisol levels irrespective of daylength (DE SOUZA and MEIER, 1987), while others have found that the rhythms in plasma concentrations of cortisol and corticosterone seen under long days (LD 14:10) are suppressed under short days (LD 10:14) (OTTENWELLER et al., 1987). Serum corticosterone levels in juvenile (GOWER et al., 1992) and adult (GOWER et al., 1996) collared lemmings, which were determined at either one time or several different times during a 24-h period, were found to be higher under LD 20:4 than under LD 16:8 and LD 8:16; the corticosterone values were similar under the latter two conditions.

The present study indicates that the growth curve of orchidectomized or melatonin-treated animals is almost identical with that of short-day-housed animals. These observations clearly differ from those obtained from adult Djungarian hamsters; the body weight of adult male hamsters housed under LD 16:8 shows no significant differences after gonadectomy (WADE and BARTNESS, 1984). In developing Djungarian hamsters, unlike in adult hamsters, the inhibitory effect of short photoperiod or exogenous melatonin on body weight may be caused mainly by decreased secretion of androgens. As mentioned, body weight increases even in animals exposed to short photoperiod after weaning, as in those housed in the same condition after birth (HOFFMANN, 1978). In addition, a similar somatic growth is observed in orchidectomized or melatonin-treated animals. Although nothing definite can be stated concerning factors involved in the somatic growth of these animals, GH may be one candidate. We have found that, under the same experimental conditions employed in the present study, PRL and TSH cells and gonadotrophs decrease in size in Djungarian hamsters kept under short days or receiving melatonin (unpublished observations). Thus, among the anterior pituitary cells, only GH cells are found to be uninfluenced by short photoperiod and melatonin administration.

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