Immunocytochemical and Morphometric Studies on the Pars Distalis of the Golden Hamster from Perinatal to Senile Stage

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ABSTRACT. Six cell types of the pars distalis were studied from perinatal to senile stage in the golden hamster in combination with morphometric analysis and immunocytochemistry. GH cells occupied only a small area at 15 days of gestation and increased remarkably after birth to occupy a significantly larger area in the young adult than that in the other stages. PRL cells first appeared at 3 days after birth and increased rapidly thereafter to be distributed throughout the pars distalis. They always occupied a larger area in females than in males from 3 weeks after birth onward. ACTH cells were distributed mainly in the peripheral region of the pars distalis. They were dominant at 15 days of gestation, but relatively decreased after birth. The gonadotrophic cells were divided into LH cells and FSH cells. About 70% of LH cells were also immunoreactive to anti-FSH serum. LH cells occupied a larger area than that of FSH cells in every stage examined. TSH cells occupied the second largest area at 15 days of gestation, but rapidly decreased at 3 weeks after birth to occupy the smallest area among the immunoreactive cell types throughout the life-span. The chronological changes in the percentages of all cell types constituting the pars distalis seemed to reflect the activity of these cell types at a given stage.—**KEY WORDS:** hamster, immunocytochemistry, morphometric analysis, pars distalis, six cell types.

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Numerous immunocytochemical studies have been performed on the hormone-producing cells of the adenohypophysis in various vertebrate species. These studies have revealed the cell types of the adenohypophysis, their distribution, ultrastructure, development and differentiation, in many vertebrates. Morphometric studies are also performed on some of these cell types revealed by immunocytochemical method to demonstrate the developmental, reproductive or seasonal changes in the composition of the cell types of the adenohypophysis. However, there have been no morphometric reports on the age-related changes in each of these cell types in the hamster of both sexes. Morphometric analysis on such changes is expected to demonstrate the physiological or metabolic conditions throughout the life-span of animals in terms of the statistics. In the present study,

therefore, each of cell types of the adenohypophysis is studied from perinatal to senile stage in the golden hamster in combination with morphometric analysis and immunocytochemistry, because immunocytochemical reactions are also expected to reflect the activity of these cell types at a given stage.

MATERIALS AND METHODS

Golden hamsters maintained in our laboratory as a closed colony were used in the present study. Fetuses were obtained on 15 days of gestation, which was calculated from the end of witnessed copulation. Postnatal animals were obtained on 2 days, 3 days and 3, 7, 10, 14, 22 and 80 weeks after birth.

Fetuses were removed from the uterus and decapitated to obtain the whole brain tissue containing the hypophysis. The brains

Table 1. Antisera used for demonstration of cell types

Antiserum	Dilution
1. Anti-chicken GH serum (Dr. Scanes)	1:4000
2. Anti-ovine PRL serum (Miles)	1:2000
3. Anti-porcine ACTH ₁₋₃₉ serum (24H2T, Imm. Nucl. Co. Ltd.)	1:2000
4. Anti-rat LH serum (S-4, NIAMDD)	1:10000
5. Anti-rat FSH serum (S-9, NIAMDD)	1:2000
6. Anti-rat TSH serum (S-4, NIAMDD)	1:5000

were fixed in Bouin's solution without acetic acid for 6–24 hr, embedded in paraffin and sectioned serially at 3–4 μ m. Postnatal animals were anesthetized with ether, sacrificed by cardiac perfusion with the above fixative and decapitated. The hypophyses were obtained together with the brain (younger than 3 weeks after birth) or removed from the brain (older than 7 weeks after birth), fixed in the same fixative for an additional 5–24 hr and embedded and sectioned as in fetuses. Serial sections were placed on separate slides, 2 to 4 sections per slide, to make a series consisting of 6 slides to be stained with different antisera.

Immunocytochemistry: All sections were deparaffinized in xylene, hydrated through a descending series of ethanol, and equilibrated in 0.02 M phosphate-buffered saline (PBS; pH 7.4). Immunocytochemical stainings were performed by avidin-biotin-peroxidase (ABC) method using antisera to each pituitary hormone. The details of antisera used for demonstration of pituitary cell types are shown in Table 1. The immunocytochemical stainings by ABC method and control immunocytochemical stainings were performed as described in our previous reports [23, 24].

Morphometric analysis: The percentages of the areas of the pars distalis occupied by each type of secretory cells were calculated using image-processing system (TOSPIX, Toshiba Co., Ltd.). Serial sections immunostained with each of the antisera were

photographed and enlarged to 12×16 cm on high-contrast paper. A photoimage was input into the image memory of the system as 512×512 pixels. The pixels were classified into 256 grades according to their light and shade, where 0 grade corresponded to black and 255 grade corresponded to white. A suitable threshold was then established among 256 grades of pixels to gain two-valued images, where reactive cells were black and non-reactive or non-specifically stained areas were white. Only the black areas were measured with the system to gain the percentages of areas occupied by reactive cells. Number of series used for morphometry were 2 to 3 for each animal; at least 6 sections reacted with different antisera were examined in each series. The cell types were identified by their immunocytochemical reactions using the respective antisera to pituitary hormones. The significance of the difference among obtained values was determined by Student's *t*-test.

RESULTS

General structure of the hypophysis: The hypophysis of golden hamster was dorsoventrally compressed and roughly trapezoidal in shape, with the broad wings of pars distalis directed laterally. It lay in the sella turcica and was enclosed in the meninges between the floor of the brain and the basisphenoid bone. The infundibulum was situated on the middorsal surface of the pars distalis and caudally directed to terminate in the pars nervosa. The pars tuberalis formed a thin cell layer covering the ventral surface of the median eminence and surrounding the infundibular stalk. The pars intermedia was situated between pars distalis and pars nervosa, enclosing the ventral and lateral surface of the pars nervosa. The pars distalis was flat and broad with lateral extension and well-developed in the caudal part to continue posteroventrally.

Fig. 1. a-f. Serial horizontal sections of the pars distalis of the female hamster aged 14 weeks, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH(a), ovine PRL(b), porcine ACTH(c), rat LH(d), rat FSH(e) and rat TSH(f), respectively. PD pars distalis; PN pars nervosa. $\times 50$.

Cell types of the pars distalis: Six types of endocrine cells, GH, PRL, ACTH, LH, FSH and TSH cells, were readily identified in the pars distalis of the hamster by immunocytochemical methods (Fig. 1). GH cells (Fig. 2a) were small and round or oval in shape with a small round nucleus. They

were distributed throughout the pars distalis, with prominent augmentation in the dorsolateral region, and observed singly or in the palisade-like arrangement along the capillaries. PRL cells (Fig. 2b) were generally small and pleomorphic and had large nuclei, round to oval and eccentrically

Fig. 2. a-f. Serial horizontal sections of the pars distalis of the female hamster aged 14 weeks, showing the distribution of pituitary cells, stained immunocytochemically with antisera against chicken GH(a), ovine PRL(b), porcine ACTH(c), rat LH(d), rat FSH(e) and rat TSH(f), respectively. $\times 350$.

located within the cytoplasm. They were almost evenly distributed throughout the pars distalis. ACTH cells (Fig. 2c) were large, round or polygonal in shape and usually observed as small groups in the rostroventral and ventrolateral regions of the pars distalis. Reaction products were coarse and localized in the periphery of these cells. Cells in the pars intermedia were also immunopositive to anti-ACTH serum. LH cells (Fig. 2d) and FSH cells (Fig. 2e)

were middle- to large-sized, oval in shape and scattered singly or in small groups throughout the pars distalis. About 70% of LH cells were also immunopositive to anti-FSH serum. An aggregation of LH cells and FSH cells was consistently observed in the zona tuberalis adjacent to the infundibular stalk to form the so-called sex zone. These cells were generally smaller and irregular in form than the scattered cells and contained more diffuse reaction product. TSH cells

Fig. 3. Diagrammatic frontal section of the hypophysis of adult hamster to show the distribution of cell types of the pars distalis.

(Fig. 2f) were large and polygonal in shape and distributed singly in the ventral and central regions of the pars distalis. They were rather minor in cell population. The distribution of each cell type is summarized in Fig. 3.

Morphometric analysis: The mean percentage of an area occupied by each type of secretory cells was estimated by the image-processing system in several sections of the pars distalis at each stage from 15 days of gestation to 80 weeks after birth. The results were summarized in Tables 2 and 3 and Figs. 4 and 5.

Fifteen days of gestation. The pars distalis was immature at this stage and immunoreactive cells totally occupied only about a half of the area of the pars distalis. ACTH cells occupied the largest area (20.34%) among immunoreactive cell types and were distributed throughout the pars distalis. Cells in the pars intermedia were also immunoreactive to anti-ACTH serum. TSH cells occupied the second largest area (11.11%) and were localized in the ventrocentral region of the pars distalis. LH cells and FSH cells were next to TSH cells in the area and were distributed in the ventral region. GH cells occupied the smallest area (6.32%) among the cell types and were distributed mainly in the central to dorsocaudal region of the pars distalis. PRL cells were not observed at this stage.

Three days after birth. The pars distalis was still immature and a relatively smaller area was totally occupied by immunoreactive cells than in the adult. PRL cells first appeared at this stage and were observed singly in the central region. They were very small in number and occupied only the smallest area (0.39%) among the immunoreactive cell types. GH cells increased remarkably and were distributed throughout the pars distalis. They occupied the second largest area. ACTH cells still occupied the largest area (18.94%). LH cells and FSH cells decreased relatively to the other cell types.

Three weeks after birth. The pars distalis grew up to be similar in features to those of the adult. PRL cells increased conspicuously to occupy 16.62% and 20.58% of the area in males and females, respectively, and were distributed throughout the pars distalis. LH cells also increased conspicuously and occupied the largest area (24.90% in males, 26.78% in females) among the immunoreactive cell types. FSH cells increased especially in females (21.57%). In contrast, TSH cells decreased in percentage remarkably to occupy the smallest area (2.52% in males, 2.15% in females) among the cell types.

Seven weeks after birth. Hamsters were regarded as young adult from this stage. The percentage and distribution of immunoreactive cell types were almost similar to those in the previous stage except that PRL cells tended to show a sex difference in percentage; the percentage of an area occupied by PRL cells was relatively larger in females than in males.

Fourteen weeks after birth. Although the percentage and distribution of immunoreactive cell types changed little from those in the previous stage, PRL cells, LH cells and FSH cells showed the obvious sex difference in percentages. The percentage of PRL cells was significantly larger in females (26.36%) than in males (12.45%). The percentages of

Table 2. Percentage of an area occupied by each type of secretory cells in the pars distalis of various ages of golden hamsters

Groups	No. of hamsters	GH	PRL	ACTH	LH	FSH	TSH
15-day fetuses	2	6.32±0.22	0.00	20.34±0.21	8.68±3.16	8.20±4.89	11.11±4.48
2 days	3	10.49±1.46	0.00	19.36±4.83	6.60±1.74	2.85±0.40	10.25±3.72
3 days	3	12.28±3.89	0.39±0.22	18.94±3.06	6.23±0.84	3.28±1.85	10.65±7.00
3 weeks male	2	16.00±1.53	16.62±0.06	13.36±5.19	24.90±3.53	11.99±2.47	2.52±0.33
female	2	11.82±1.37	20.58±4.79	13.88±5.32	26.78±0.40	21.57±2.62	2.15±1.20
7 weeks male	4	17.83±2.49 ^{d)}	16.20±5.58	21.76±4.48 ^{d)}	27.38±6.44	20.20±5.61	2.21±1.13
female	4	12.53±1.92 ^{a)}	23.34±5.67	13.98±1.22 ^{a)}	18.96±2.83	14.55±2.17	1.64±0.57
10 weeks male	4	15.42±2.49 ^{d)}	17.46±3.48	19.44±1.84 ^{d)}	24.13±1.06 ^{c)}	18.12±4.51	1.87±0.51
female	3	16.02±1.40	22.53±1.05	13.81±2.65 ^{a)}	15.02±2.01 ^{b)}	10.34±0.53 ^{a)}	1.47±0.41
14 weeks male	4	17.77±1.74 ^{d)}	12.45±2.27	20.07±1.87 ^{d)}	21.18±1.99	14.60±0.89	2.27±0.62
female	6	14.98±1.82 ^{a)}	26.36±5.86 ^{b)}	12.67±2.80 ^{b)}	13.60±2.13 ^{b)}	10.18±2.72 ^{a)}	1.69±0.72
22 weeks male	2	15.18±3.83	16.25±1.22	17.19±2.28	14.79±3.03	14.11±0.03	2.30±0.39
female	2	14.32±1.07	25.99±3.80	12.25±1.61	15.60±6.00	13.31±4.76	1.36±0.22
80 weeks male	4	8.98±1.12	10.59±5.06	9.68±2.27	18.73±4.30	15.06±0.99	2.10±1.20
female	2	14.21±3.37	14.55±6.30	11.23±3.82	16.74±3.61	12.81±5.46	1.55±0.52

Mean±S.D. a) p<0.05 vs. male b) p<0.01 vs. male c) p<0.05 vs. 80 weeks male d) p<0.01 vs. 80 weeks male

Table 3. Percentage of an area occupied by total of secretory cells in the pars distalis of various ages of golden hamsters

Age	Male	Female	Total
15-day fetuses			54.63± 5.78
2 days			48.98±12.54
3 days			51.78±12.36
3 weeks	85.37±12.33	96.78± 5.32	91.08±10.17 ^{b)}
7 weeks	105.59±11.86 ^{a)}	84.85± 9.35	95.28±14.79 ^{d)}
10 weeks	96.44± 5.91 ^{c)}	79.19± 4.07	89.05±10.39 ^{d)}
14 weeks	88.33± 3.89 ^{e)}	79.48± 7.26	82.46± 7.27 ^{d)}
22 weeks	79.81± 2.33	82.82±17.46	81.31±10.32
80 weeks	65.13± 7.66	71.08±22.03	67.11±11.90

Mean±S.D. a) p<0.05 vs. female b) p<0.05 vs. 80weeks c) p<0.01 vs. female d) p<0.01 vs. 80weeks e) p<0.05 vs. 7weeks male f) p<0.05 vs. 14weeks

Fig. 4. Changes in percentage of each type of cells in the growing pars distalis of male hamsters.

Fig. 5. Changes in percentage of each type of cells in the growing pars distalis of female hamsters.

LH cells and FSH cells were significantly smaller in females (LH cells 13.60%, FSH cells 10.18%) than in males (LH cells 21.18%, FSH cells 14.60%).

Twenty-two weeks after birth. Hamsters were regarded to be in full maturity around this stage. The percentage and distribution of the immunoreactive cell types were almost similar to those in the young adult except that the percentage of LH cells decreased a little in males. However, none of the cell types showed significant sex differences in percentage by statistical analysis.

Eighty weeks after birth. Hamsters were regarded as senile from this stage. The pars distalis became a little atrophied to be thin in lateral and caudal regions. According to the statistical analysis between hamsters 14 and 80 weeks after birth, the total area occupied by immunoreactive cell types decreased in seniles. The percentage of GH cells was significantly smaller in male seniles (8.98%) than that in younger ones (17.83% at 7 weeks, 17.77% at 14 weeks). The percentage of PRL cells decreased to be 14.55% in female seniles. On the other hand, the percentages of LH and FSH cells did not so much decrease or increased in seniles (LH cells 18.73% in males, FSH cells 15.06% in males).

DISCUSSION

There have been so far made several morphometric studies on the pars distalis of mammals such as men [29], bats [23], rats [7, 9, 10, 11, 12, 13, 15, 20, 22, 28, 34, 35, 36, 37] and hamsters [1, 14, 32]. These studies were, however, performed by tinctorial methods, or focused mainly on a part of the cell types of the pars distalis or on the relatively short period of the life-span. Therefore, the present study examined the changes in the percentages of all cell types constituting the pars distalis throughout the life-span of the golden hamster.

GH cells. GH cells were reported to appear relatively late in the gestational period [33, 40]. In the golden hamster, they were reported to appear at 15 days of gestation [24]. According to the morphometric analysis of the present study, they occupied only a small area at 15 days of gestation and increased remarkably after birth to occupy a significantly larger area in the young adult than that in the other stages. Dekker [14] also reported the increase of GH cells in the developing hamster and their decrease thereafter to occupy a significantly smaller area in the seniles than in the young adult.

On the other hand, Birge [5] reported that the content of GH in the hypophysis increased rapidly along with the postnatal development and decreased in the seniles. This seems to correspond to the results of the morphometric analysis of GH cells in the present study.

PRL cells. The time of initial appearance of PRL cells varies according to authors. Some reported that they appear during the gestational period [2, 4, 33, 38]; while others reported their absence during this period [8, 16, 24, 40]. In the present study, they first appeared at 3 days after birth and increased rapidly thereafter to be distributed throughout the pars distalis.

According to the morphometric analysis of the present study, the percentage of an area occupied by PRL cells was always larger in females than in males from 3 weeks after birth onward. On the other hand, Takahashi *et al.* [36, 37] reported that PRL cells and their mitotic figures were larger in number in females than in males of sexually matured rats. Dada *et al.* [13] also reported the similar results. These reports suggested the dominance of PRL cells in females and were consistent with the present results.

In seniles, the percentage of the area occupied by PRL cells decreased in both sexes, especially in females, where it drew

near to that of males. This seems to suggest the regression of PRL cells in seniles. The present results would support Baker's report [4] describing the regression of PRL cells in senile men.

ACTH cells: In the present study, ACTH-immunoreactive cells were distributed mainly in the peripheral region of the pars distalis. Most of cells in the pars intermedia were also immunoreactive to anti-ACTH serum. Since MSH secreted from the pars intermedia was composed of N-terminal 13 amino residues of ACTH, the immunoreaction in the pars intermedia was thought to be caused by MSH. However, since Moriarty and Halmi [26] and Naik [27] reported the presence of ACTH in the pars intermedia of the rat, the present immunoreaction in the pars intermedia may partly be originated from ACTH.

According to the morphometric analysis, ACTH cells were dominant at 15 days of gestation in the present study. This may partly be attributed to the early appearance of ACTH cells reported by Mikami *et al.* [24]. Thereafter, the percentage of an area occupied by ACTH cells remained above 12% in both sexes except in seniles. On the other hand, Dada *et al.* [13] and Baker *et al.* [3] reported the paucity of ACTH cells in the rat. This discrepancy seems to be attributable to the difference in the property of antisera used in their and present studies.

The percentage of ACTH cells showed sex difference in the young adult and significantly larger in males than in females. On the other hand, it decreased significantly in seniles in comparison with the young adult. These results seem to reflect the difference in the metabolic activity between males and females in the young adult and between young adults and seniles.

LH cells and FSH cells: The gonadotrophic (GTH) cells have been divided into 2 functional groups, LH cells and FSH cells, on the basis of cyclic alterations in number

and tinctorial properties as correlated with the reproductive cycles. Recently, several immunocytochemical studies were performed on LH cells and FSH cells to reveal that selective immunoreaction to both LH and FSH antisera was primarily localized in the same cell type, with the exception of some cells that reacted solely with antisera to either LH or FSH [6, 17, 18, 19, 30, 31, 39]. In the present study, the percentage of an area occupied by LH cells was larger than that of FSH cells in every stage examined. This suggests that all of GTH cells do not always contain both LH and FSH. According to the present comparison of adjacent sections stained with antisera to LH or FSH, about 70% of GTH cells were immunoreactive to both antisera, the other being immunoreactive to either of them.

There have been several morphometric reports on the presence of sex difference in LH cells or FSH cells between both sexes [11, 13, 15, 20, 22]. These reports differ in opinion with each other. In the present study on the percentages of areas occupied by LH cells and FSH cells, the percentages of both cell types were significantly larger in males than in females in the young adult, especially at 10 and 14 weeks after birth. On the other hand, Lisk [21] reported that the LH content of adenohypophysis of the rat was larger in the female until the sexual maturation and reversed thereafter. Moore [25] also reported the decrease in the LH content of adenohypophysis after the first ovulation in the female rats. These and present results seem to suggest that a larger quantity of GTH hormone was released into blood stream in company with the sexual maturation in females than in males to cause the decrease in the immunoreactivity of GTH cells in females.

TSH cells: TSH cells were reported to appear at 12.5 days of gestation in the hamster [24]. In the present study, the percentage of an area occupied by TSH cells

was the second largest at 15 days of gestation. In spite of the early appearance of TSH cells, however, it rapidly decreased at 3 weeks after birth to maintain the smallest value among the immunoreactive cell types throughout the life-span. This seems to indicate that TSH cells did not so much increase as the other cell types in company with the development of the pars distalis to result in the decrease in the relative ratio of the area to the pars distalis. Childs [10] reported that the percentage of TSH cells reached the peak at 9 days after birth in the male rat and decreased thereafter to maintain the smallest value among the cell types in the adult. Dada *et al.* [13] also reported that TSH cells were the smallest in number in the rat. These reports agree well with the present results. The significance of the early appearance and subsequent low frequency of TSH cells is to be investigated in the future.

The total percentage of the area occupied by immunoreactive cell types of the pars distalis seems also to reflect the degree of the activity of the pars distalis. Until 3 days after birth, it amounted only about 50%. This seems to reflect the immaturity of the pars distalis in perinatal hamsters. From 3 weeks after birth, it exceeded 85% and maintained around or over this value in the young adult and fully matured hamsters. Although it exceeded 100% in the males aged 7 weeks, this may be attributed to the fact that about 70% of LH cells also reacted to anti-FSH serum. The high percentage of the total area occupied by the cell types seems to reflect the high activity of the pars distalis in the young adult and fully matured hamsters. In the hamsters aged 80 weeks, the percentage decreased significantly in comparison with that in the young adult. This seems to reflect the low activity of the pars distalis in seniles.

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要 約

周生期から老齢期までのゴールデン・ハムスターの下垂体前葉細胞の免疫細胞化学的ならびに形態計測学的研究：谷口和之・金崎彩織・見上晋一(岩手大学農学部家畜解剖学教室)——ゴールデン・ハムスターの下垂体前葉の6種類の細胞型について、周生期から老齢期まで免疫細胞化学と形態計測学の手法を併用して検討した。GH細胞は胎齢15日では前葉全体のうちごく僅かの面積を占めるに過ぎなかったが、出生後急激に増加して若い成体で最大となり、以後やや減少した。PRL細胞は生後3日に初めて出現し、その後急激に増加して前葉全体に分布し、生後3週齢以降では常に雌で雄より大きな面積比を示した。ACTH細胞は前葉辺縁部に分布し、胎齢15日で各細胞型中最大の面積比を示したが、それ以後相対的に減少して細胞型として2番目以下となった。LH細胞とFSH細胞はその約70%が両方の抗血清に反応したが、その面積比は常にLH細胞の方が大きかった。TSH細胞は胎齢15日には各細胞型中2番目の面積比を示したが、それ以後相対的に減少し、3週齢以降では常に各細胞型中最小の面積比を示した。下垂体前葉を構成する各細胞型における面積比の経時的变化は各段階におけるこれらの細胞型の活性を反映するものと思われた。