Histomorphometric Study of the Parathyroid Glands of the Spontaneously Hypercholesterolemic (SHC) Rats with Chronic Renal Failure

Ryoichi TSUKUDA, Akio SHINO, and Shoji YAMAUCHI¹⁾

Biology Laboratories, Central Research Division, Takeda Chem. Ind., Ltd., 17-85, Jusohon-machi 2-chome, Yodogawa-ku, Osaka 532, and ¹⁾Department of Veterinary Anatomy, College of Agriculture, University of Osaka Prefecture, 4-804, Mozuume-machi, Sakai City, Osaka 591, Japan

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ABSTRACT. Light and electron microscopic morphometry was performed on the parathyroid (PT) glands of the SHC rats with naturally occurring chronic renal failure. Macroscopically, the PT glands were distinctively hypertrophied in 24-week-old male SHC rats when compared with the corresponding controls, Sprague Dawley (SD) rats. Light microscopic morphometry on consecutive sections of the PT glands showed that the volume was about 3 times greater in the SHC rats, whereas there was no difference in the size of the chief cells. Mitoses were often found in the PT glands of the SHC rats. The total number of mitoses was about 8.5 times greater in the SHC rats, and was closely related to the volume of the PT glands. Ultrastructural morphometry of the chief cells revealed an increase in the cell surface area by the interdigitation of the plasma membrane and increases in the volume of mitochondria and Golgi complex. Secretory granules sometimes existed close to the cell surface in the SHC rats, but not in the SD rats. These results suggest that the PT glands of the SHC rats are hyperplastic mainly because of the proliferation of the chief cells. Concurrently, an increase in volume of the cell organelles suggests enhanced secretion activity in the chief cells.—**KEY WORDS:** histomorphometry, hyperplasia, parathyroid gland, spontaneously hypercholesterolemic (SHC) rat.

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The spontaneously hypercholesterolemic (SHC)/Ta rat was developed by Imai *et al.* [10]. The SHC rats spontaneously show chronic renal failure [10], hypercholesterolemia [10], uremia [10], hyperphosphatemia [18], enlargement of the kidney [10] and PT glands [19], metastatic calcification in soft tissues [19] and renal osteodystrophy [18]. These changes are more severe in males than in females and the mean life span of males is as short as about 30 weeks. The renal osteodystrophy and metastatic calcification in soft tissues are considered to be caused by secondary hyperparathyroidism following metabolic disturbance of blood calcium, which arises from hyperphosphatemia resulting from insufficient excretion of phosphate in the blood by chronic renal failure [7]. The morphology of the PT glands hypertrophied secondarily by chronic

renal failure has been well documented [9, 13–15, 17], but no studies seem to have been done on the morphometry of the PT glands at tissue and cell levels. Recently, ultrastructural morphometry of the PT glands stimulated by a transient reduction of serum calcium concentration has demonstrated a rapid shift of membranes from the secretory granules and Golgi complex to the plasma membrane with subsequent retrieval of plasma membrane components [20–22].

The purposes of the present investigation were to examine the PT glands of the SHC rats at 24 weeks of age using light and electron microscopic morphometry, and to determine the morphometric features of the glands and cells stimulated chronically by renal insufficiency in the rats.

MATERIALS AND METHODS

Animals: Male SHC rats (mean $\pm$ SEM: 427 $\pm$ 44 g, BW) reared in our laboratories and the corresponding controls, SD rats (581 $\pm$ 16 g) purchased from CLEA Japan, were used at 24 weeks of age. The rats were fed a commercial diet (CE-2, CLEA Japan) and water *ad libitum* in an air-conditioned environment (23 $\pm$ 2 °C, 55 $\pm$ 5% humidity).

Preparations for light microscopy: The rats were killed by exsanguination through the abdominal aorta under ether anesthesia. The PT glands were removed with the thyroid gland and fixed in a 10% buffered formalin solution. The glands were embedded in paraffin, consecutively sectioned at 5 μ m, and stained with hematoxylin-eosin (HE).

Light microscopic morphometry: An image analyzer system (IBAS-2000, Carl Zeiss, West Germany) and a light microscope equipped with a drawing tube (Carl Zeiss) were used for histomorphometry. The areas of the PT glands were measured at 81 $\times$ magnification in the consecutive sections to calculate the volume of the PT glands. One of the largest sections of the left gland was used to determine nuclear number, area, perimeter, and maximal diameter of the chief cells in 2 square fields (89 $\times$ 89 μ m) of the center and periphery in the PT gland at 1130 $\times$ magnification. The blood vessels and connective tissue were excluded from the calculation. The nuclear number per unit area was calculated by dividing the total number by the measured area. Three sections were selected at an interval of about 60 μ m from consecutive sections and analyzed at 900 $\times$ magnification to determine the number of mitoses of the chief cells. The mitotic cells were counted to calculate the mitotic number per unit area.

Preparations for electron microscopic morphometry: Under ether anesthesia, each

rat was perfused with physiological saline for 3 to 5 min and subsequent Karnovsky's fixative [11] at 4°C for 10 to 15 min through left ventricle at a flow speed of 20 ml/min using a roller pump (RP-N3, Furue Science Co., Ltd.). The PT glands were then removed and minced under a stereomicroscope, and immersed in Karnovsky's fixative. The glands were washed, postfixed in OsO₄, dehydrated and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and photographed using a JEM-1200 EX electron microscope at 4000 $\times$ magnification.

Ultrastructural morphometry: Morphometric analysis was performed on negative films of 30 chief cells having nuclei from 1 section of each gland, using IBAS-2000. The negative images of the chief cells were input from a TV camera at 3 different magnifications in correspondence with morphometric variables, converted into positive images, and analyzed. The cellular area, the nuclear area and perimeter, and cellular and nuclear maximum diameter were determined at 2.93 $\times$ magnification of the negative figure (final mag.: $\times$ 11700). The length of the plasma membrane and rough endoplasmic reticulum (RER), and areas of the mitochondria and lysosomes were measured at 6.47 $\times$ magnification of the negative (final mag.: $\times$ 25900). The areas of the Golgi complex and secretory granules were estimated at 16 $\times$ magnification (final mag.: $\times$ 64000). The area of the Golgi complex was defined as a field comprising Golgi lamellae, and vesicles and vacuoles adjacent to the lamellae, excluding vesicles and vacuoles detached from the lamellae.

Statistical analysis: The statistical significance of differences between group means was determined by the Student's *t*-test. Correlation analysis was conducted when needed.

RESULTS

Light microscopic observation and morphometry: The PT glands of SHC rats were markedly hypertrophied in comparison with those of SD rats, and the parenchyma was loosely divided into lobules by thin septa consisting of blood vessels and connective tissue (Figs. 1A, B). No nodular foci of PT tissue were seen.

The total volume of right and left glands was significantly increased in the SHC rats and reached about 3 times greater in the SHC rats (Table 1). There was no significant difference in the volume between the right and left PT glands in either SD or SHC rats.

There were no significant differences in the area, perimeter, and maximal diameter of the chief cells, or in the nuclear number per unit area between the 2 strains of rats (Table 2).

The total number of mitoses per unit area

in both glands of the SHC rats was 6.65 ± 1.71 N/mm² (mean $\pm$ SEM, n=4) and was about 8.5 times ($P < 0.05$) as much as that of SD rats (0.78 ± 0.19 N/mm², n=3). In both strains, the right and left glands did not differ significantly in any mitotic number. The total number of mitoses exhibited strong positive correlations with the volume of both glands ($r = 0.99$, $P < 0.01$; n=4) or each ($r = 0.72$, $P < 0.05$; n=8) in the SHC rats, but did not show such a correlation in the SD rats.

Electron microscopic observation: The parenchyma of the glands of the rats of both strains comprised chief cells of polygonal shape. The nuclei of the chief cells were round or oval, and were accompanied by frequent shallow indentations and a few invaginations. The plasma membrane showed numerous infoldings and complex interdigitations between contiguous chief cells. They were more distinct in the SHC

Fig. 1. Light micrographs of the PT glands from a control SD rat and an SHC rat at 24 weeks of age. A: Normal PT gland of the SD rat. B: Hypertrophied PT gland of the SHC rat. (HE stain. $\times 44$).

Table 1. Light microscopic morphometry: volume of the PT glands

	SD	SHC
Volume (mm ³)		
Right PT	0.323±0.053 (3)	0.954±0.071 (4)**
Left PT	0.337±0.017 (5)	1.147±0.081 (4)**
Total PTs	0.658±0.083 (3)	2.101±0.136 (4)***

Mean±SEM.

Number of rats in parentheses.

** P<0.01, *** P<0.001.

Table 2. Light microscopic morphometry: nuclei of the PT cells

	SD (n=5)	SHC (n=4)	t-test
Area (μm ²)	25.7±1.2	25.4±1.0	NS
Perimeter (μm)	19.1±0.4	18.9±0.4	NS
Diameter, maximum (μm)	7.27±0.11	7.08±0.14	NS
Number / 100μm ²	1.25±0.03	1.35±0.06	NS

Mean±SEM.

NS: not significant.

rats (Figs. 2, 3). At sites where chief cells face capillaries, the cell surface was smooth. The lamellae of the RER formed narrow cisternae and were distributed diffusely or collectively in the cytoplasm. The mitochondria were round, oval or tubular in shape, and observed diffusely in the cytoplasm. The number of mitochondria increased in the SHC rats (Figs. 2, 3). Lipid droplets occurred diffusely or collectively as large dense granules. The lysosomes were round or irregular in shape and contained a heterogeneous dense substance. The Golgi complex consisted of 3 to 4 stacks of lamellae, vesicles on the forming face, and vacuoles on the maturing face. The Golgi complex was observed more frequently in the SHC rats. Limiting membrane-bounded secretory granules, 0.1~0.3 μm and occasionally around 0.5 μm in diameter, contained a dense core surrounded by a translucent space inside the membrane (Figs. 4A~D). A few secretory granules were found in both

SD and SHC rats, but were sometimes seen close to the cell surface in the SHC rats (Figs. 4C, D).

Ultrastructural morphometry: The surface area of the chief cells in the SHC rats was about 1.2 times (P<0.01) as much as that in the SD rats (Table 3). However, there were no significant differences in the volume of chief cells, cytoplasm and nuclei, and in the volume ratio of nuclei per cells between the 2 strains. Nor did they differ in either nuclear surface area or cellular and nuclear diameter.

The density of cell organelles per cytoplasm in the chief cells is shown in Table 4. The volume densities of mitochondria and Golgi complex were about 1.3 (P<0.01) and 1.6 times (P<0.05) higher in the SHC rats. The numerical density of the Golgi complex was about 1.6 times (P<0.01) greater. No significant differences were noted in the surface density of RER, and the volume density of lipid, lysosomes, and secretory

Fig. 2. An electron micrograph of chief cells showing normal structure in a PT gland of an SD rat. $\times 11900$.

Fig. 3. An electron micrograph of chief cells showing increases in the interdigitation of the plasma membrane between the contiguous cells, and in number of the mitochondria in a PT gland of an SHC rat. $\times 11900$.

Fig. 4. Electron micrographs of chief cells in the PT glands of SD (A, B) and SHC (C, D) rats. $\times 49500$. A, B: Limiting membrane-bounded secretory granules (arrows) containing a dense core surrounded by a translucent space inside the membrane. C, D: Secretory granules (arrows) seen close to the plasma membrane.

granules between SHC and SD rats.

DISCUSSION

The light microscopic morphometry presented here revealed an increase in the volume of the PT glands in 24-week-old male SHC rats when compared with control SD rats. However, neither nodular foci nor neoplastic lesions were found in the glands of the SHC rats. No such lesions have been observed in the glands of male SHC rats from 6 to 27 weeks of age [19]. Therefore, it is conceivable that the hypertrophic change

of the glands in the SHC rats does not belong to any category of either primary hyperparathyroidism caused by a primary tumor and hyperplasia [2, 13, 16], or tertiary hyperparathyroidism caused by neoplastic lesions following secondary hyperplasia of the PT glands in the human [8, 9]. Instead, the hypertrophic change in the SHC rats is considered to belong to a category of secondary hyperparathyroidism in the human [9, 13, 15], inasmuch as the hypertrophy of the glands seen in the rats, at least from 18 weeks of age, is preceded by chronic renal failure and hyperphos-

Table 3. Ultrastructural morphometry: cells, cytoplasm and nuclei in the PT glands

	SD	SHC
Volume (μm^3)		
Cell	50.8 $\pm$ 3.8	50.1 $\pm$ 1.1
Cytoplasm	31.4 $\pm$ 2.3	31.6 $\pm$ 0.9
Nucleus	19.1 $\pm$ 1.7	18.4 $\pm$ 0.6
Surface (μm^2)		
Cell	55.9 $\pm$ 2.3	69.8 $\pm$ 3.0**
Nucleus	19.7 $\pm$ 0.5	19.4 $\pm$ 0.4
Diameter, maximum (μm)		
Cell	12.1 $\pm$ 0.1	11.9 $\pm$ 0.2
Nucleus	7.18 $\pm$ 0.22	6.97 $\pm$ 0.18
Volume density per cell (%)		
Nucleus	38.0 $\pm$ 1.2	37.0 $\pm$ 1.1

Mean $\pm$ SEM; n=4.

** P<0.01.

Table 4. Ultrastructural morphometry: density of organelles in the PT cells

Density per cytoplasm	SD	SHC
Surface ($\mu\text{m}^2/\mu\text{m}^3$)		
RER	0.91 $\pm$ 0.05	1.71 $\pm$ 0.12
Volume (%)		
Mitochondria	7.56 $\pm$ 0.32	10.12 $\pm$ 0.45**
Lipid	2.89 $\pm$ 0.55	3.93 $\pm$ 1.01
Lysosome	0.83 $\pm$ 0.13	0.66 $\pm$ 0.05
Golgi complex	1.04 $\pm$ 0.11	1.68 $\pm$ 0.13**
Secretory granules	0.14 $\pm$ 0.04	0.16 $\pm$ 0.02
Number (N/ μm^3)		
Golgi complex	0.062 $\pm$ 0.006	0.097 $\pm$ 0.011*

Mean $\pm$ SEM; n=4.

* P<0.05, ** P<0.01.

phatemia [18, 19].

Mitoses were often found light microscopically in the glands of the SHC rats. The morphometric analysis demonstrated the increase in the total mitotic number per unit area in the glands of the rats, and a strong correlation between the total number of mitoses and the volume of the glands. These results suggest that the hypertrophy of the glands is due mainly to proliferation of the chief cells by mitosis.

The ultrastructural morphometry of the PT cells in the SHC rats quantitatively

confirmed the increase in the interdigitation of plasma membrane between contiguous cells and the significant increase in the cell surface area. Moreover, the morphometry demonstrated increases in the volume density of mitochondria and Golgi complex in the SHC rats, the latter being resulted mainly from an increase in number. Secretory granules, although few, were sometimes observed close to the cell surface in the SHC rats. These findings and the morphometric evidence are in good accord with those in chronically stimulated chief cells from pa-

tients with secondary hyperparathyroidism due to renal disease [9, 13, 15]. No significant differences were observed between SD and SHC rats in the surface density of RER, or in the volume density of lipid and lysosomes in the chief cells. However, there is a difference in chronically stimulated chief cells between the SHC rats and human in that increased RER was observed in the human [9, 13, 15], but not in the SHC rats.

Wild *et al.* [20–22] have recently shown that a transient depression of the serum calcium concentration results in an initial centrifugal membrane shift from the Golgi complex and secretory granules to the plasma membrane and a subsequent centripetal membrane shift indicating enhanced endocytotic retrieval of the plasma membrane in stimulated PT cells [21]. In the present study, the PT chief cells in the SHC rats showed increases in the volume of Golgi complex and mitochondria, and the surface area of the cells. According to the hypothesis by Wild *et al.* [20–21], it is conceivable that in the PT cells in the SHC rat, prolonged stimulation by deranged calcium metabolism may result in the increased exocytosis of secretory granules, subsequently the persistent centrifugal membrane shift from the Golgi complex and secretory granules to the plasma membrane, and the retarded centripetal membrane shift. The increase in mitochondria may suggest increased production of energy sustaining hyperfunction of the chief cells.

Ultrastructural changes of the PT cells in secondary hyperparathyroidism have been reported in animals under hypocalcemia [4] subsequent to nutritional causes such as calcium or phosphate deficiency [1, 3, 5], parturition [6], and vitamin D deficiency [15]. The ultrastructural features of chief cells stimulated chronically in such a nutritional secondary hyperparathyroidism appear to be similar to those in renal secondary hyperparathyroidism [17]. The

light and electron microscopic morphometry presented here exhibited no hypertrophy of the chief cells in the hyperplastic PT glands in the SHC rats. However, chronically stimulated chief cells have been reported to display hypertrophy in rats with renal disease [17], rabbits injected with ferric glycerophosphate [12], and cats fed a low calcium diet [5]. Although the significance of this difference is unclear, it might arise from some differences in the severity and the period of the morbid state in secondary hyperparathyroidism, and the presence of concomitant lesions.

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要 約

慢性腎不全を伴う自然発症高コレステロール血症 (SHC) ラットにおける上皮小体の組織形態計測学的研究：佃 良一・志野晟生・山内昭二¹⁾ (武田薬品, 中央研究所, ¹⁾大阪府立大学, 家畜解剖学教室) ——SHC ラットの上皮小体を光顕及び電顕的に形態計測した。24週齢の雄性 SHC ラットでは対照の同週齢の SD ラットに比べ, 上皮小体の肥大が肉眼的に明瞭であった。上皮小体の連続切片の光顕的形態計測の結果, SHC ラット上皮小体の体積は対照ラットの約3倍に増大していたが, 上皮小体主細胞の核の平均面積及び単位面積当りの核数はSD ラットと変わらなかった。SHC ラット上皮小体の有糸分裂細胞数は, SD ラットの約8.5倍に増加すると共に, 上皮小体の体積と高い相関を示した。電顕的形態計測の結果, SHC ラットの上皮小体主細胞ではSD ラットに比べ, 細胞膜の皺壁形成が著明で, その表面長が増加すると共に, ミトコンドリア及びゴルジ装置の面積も有意に増加していた。分泌顆粒は時折, 細胞膜に密接して観察された。これらの結果, SHC ラット上皮小体の顕著な肥大は, 主に主細胞の分裂増殖による細胞数の増加に起因することを示し, 同時に主細胞での上皮小体ホルモンの合成と分泌に関連した細胞小器官の面積の増加は, 主細胞の分泌機能亢進を示唆していた。