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Age-related changes of the ultrastructure in the cardiomyopathic hamster (UM-X7.1 Syrian hamster) parathyroid gland

M. Utsumi[1], K. Moriguchi[1], H. Takahashi[3], C. Kinoshita[3], A. Togari[2], M. Mizutani[1] and N. Ohno[1]

Departments of [1]Anatomy and [2]Pharmacology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan and [3]Education and Research Center of Animal Models for Human Diseases, Fujita Health University School of Medicine, Toyoake, Japan

Summary. We qualitatively and quantitatively investigated parathyroid glands of the UM-X7.1 cardiomyopathic hamster at 1, 2, 6 and 12 months of age to compare them with those of the normal hamster. We found that at 1 month of age in the UM-X7.1 hamster, the Golgi apparatus, lipid droplets and secretory granules decreased. There were no significant differences between the UM-X7.1 hamster and the control hamster at 2 months of age. At 6 months of age, the Golgi apparatus, rER and the secretory granules significantly increased in the UM-X7.1 hamster. At 12 months of age, the Golgi apparatus and lysosomes increased, while the secretory granules decreased. Ultrastructurally, we consider that in the UM-X7.1 hamster, the synthesis and release of the parathyroid at 6 months of age may be activated by an excessive amount of circulating catecholamine, and the functional activity of the parathyroid glands at 12 months of age may be depressed by the increased plasma calcium level. These findings suggest that the activities of the synthesis and release of the parathyroid hormone were the highest at 6 months of age in the UM-X7.1 hamster.

Key words: Cardiomyopathy, Morphometry, Parathyroid gland, Ultrastructure, UM-X7.1 Syrian hamster

Introduction

The UM-X7.1 Syrian hamster (the UM-X7.1 hamster) is a genetic strain established by crossbreeding diseased animals from the original BIO 14.6 strain with unrelated healthy animals (Jasmin and Eu, 1979) and is known as one of the animal models for idiopathic cardiomyopathy and congestive heart failure (Homburger, 1979; Makino et al., 1985). The disease of this hamster shows a progressive muscular dystrophy from about 40 days and dies of cardiac failure after about 1 year. The progress of cardiac damage in this hamster is caused by catecholamine effects on the sarcolemma, inducing overload of cellular calcium, hyper contraction of myofilaments due to calcium-dependent myofibrillar ATPase, deposition of calcium apatite granules within mitochondria and cardiac necrosis (Pangia et al., 1984; Finkel et al., 1987). Furthermore, Togari et al. (1989) showed a high level of plasma calcium and bone disorder associated with decreased bone formation in the UM-X7.1 hamster of different ages. These symptoms may indicate abnormality in its calcium regulating system in cardiomyopathic hamsters. On the other hand, the parathyroid gland (PTG) is considered to be one of the organs responsible for controlling calcium homeostasis, as it secretes the calcium-regulating hormones (PTH) thus controlling calcium influx and efflux to the blood at the level of the bone, the gut and the kidneys directly or indirectly. Ultrastructurally, we have reported that the functional activity of the PTG in the UM-X7.1 hamster at 6 months of age was higher than that in the normal hamster (Utsumi et al., 1999). The present study was undertaken with the object of obtaining quantitative data of the UM-X7.1 hamster of different ages on ultrastructural parameters related to parathyroid cell function and of comparing these data with those of the normal control.

Offprint requests to: Michiya Utsumi, Department of Anatomy, School of Dentistry, Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan. Fax: +81-52-752-5988. e-mail: kosmo@dpc.aichi-gakuin.ac.jp

Materials and methods

Male cardiomyopathic hamsters (UM-X7.1 strain) at 1, 2, 6, and 12 months of age were used as the experimental animals and normal male Syrian hamsters (GN strain) of comparable ages served as the control animals. They were divided into 8 groups of 5 animals each. The experimental protocol of this study followed

the Guideline of the Animal Experiment at the School of Dentistry, Aichi-Gakuin University. All animals were kept under automatically controlled conditions of temperature (23±1 °C), humidity (50±10%), and 12-h light/dark cycle, a standard laboratory diet (CMF, Oriental Yeast Co., Tokyo, Japan) and drinking water being taken ad libitum. The PTG of the animals in all groups was dissected under deep anaesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), fixed in a mixture of 2.5% glutaraldehyde and 2% OsO_4 in veronal acetate buffer, pH 7.4, for 1 hr at 4 ºC, and dehydrated through ascending concentrations of acetone and embedded in Epon 812. The body weight of all animals was measured after anaesthesia. Ultrathin sections were cut on a LKB ultramicrotome, stained with uranyl acetate and lead salts, and were examined by a JEM-1210 electron microscope (JEOL, Tokyo, Japan). In each hamster from all groups, 20 micrographs at a final magnification of 17,000 were taken at random from different regions of the PTG.

Tracing paper was placed over the micrographs and the outline of the areas of cytoplasm, the Golgi apparatus (Golgi vesicles, Golgi vacuoles and Golgi lamellae), the cisternae of the rough endoplasmic reticulum (rER), lysosomes, large vacuolar bodies and lipid droplets were drawn on the tracing paper. Quantitation of the areas of cytoplasm, cytoplasmic organelles and cytoplasmic inclusions was performed with a GT-6000 scanner (EPSON, Tokyo, Japan) interfaced to a Power Macintosh 7600/132 computer (Apple, Cupertino, USA). Scans were served as 8-bit TIFF files and imported into Adobe Photoshop (Adobe Systems, Mountain View, USA) and NIH Image program (version 1.58, Wayne Rasband at the U.S National Institutes of Health, Bethesda, USA) for analysis. The regions of the Golgi apparatus, the rER, lysosomes, lipid droplets and large vacuolar bodies were expressed as a percentage of the cytoplasmic area. Additionally, the number of secretory granule profiles per 100 μm^2 in the cytoplasm was measured.

All data are presented as means±SEM. After F-test, Student's t-test was used to calculate significant differences between the control and the UM-X groups at the same age. Among the UM-X7.1 groups, mean values were compared by one-factor analysis of variance followed by Scheffe's method. Significance was accepted at $p<0.05$.

Table 1. Comparison of body weight between control and UM-X7.1 groups.

AGE	GROUPS	BODY WEIGHT (g)
1 m	Control	89.2±1.4
	UM-X	58.1±3.0*
2 m	Control	120.8±1.3
	UM-X	70.2±4.4*
6 m	Control	173.2±9.2
	UM-X	115.8±4.4*
12 m	Control	165.0±7.4
	UM-X	125.0±7.1*

Values are means±SEM. m: month, *: $p<0.05$ vs. control group.

Results

Body weight

Changes of body weight in the control and the UM-X7.1 hamsters are shown in Table 1.

The mean body weight in the UM-X7.1 hamster at all ages was significantly less than that in the control hamster.

Ultrastructure of the PTG

In the control and the UM-X7.1 hamsters at all ages, the parenchyma of the PTG consisted of the chief cells, which were oval or polygonal in shape and contained abundant free ribosomes and mitochondria (Figs. 1-4). The morphology of the PTG of the control hamster in each group (Figs. 1a-4a) resembled, respectively, that of the normal golden hamster, as reported earlier (Emura et al., 1984; Shoumura et al., 1991).

In the UM-X7.1 hamster, the chief cells of each experimental group, except the 1-month-old group, contained a well-developed Golgi apparatus consisting of small vesicles and large, dilated vacuoles when compared with those of each control group (Figs. 2b-4b). In the chief cells of the UM-X7.1 hamster at all ages (Figs. 1b-4b), the rER was well-developed, and, especially at 6 months of age, was arranged as curvilinear whirls rather than as parallel arrays and was randomly distributed in the cytoplasm (Fig. 3b,c). The appearance of lysosomes increased gradually in an age-related fashion in the UM-X7.1 hamster as well as in the control hamster (Figs. 1b-4b). Large vacuolar bodies contained floccular material or vesicles were sometimes observed in the chief cells of the UM-X7.1 hamster at all ages (Fig. 3b). In the UM-X7.1 hamster, many lipid droplets were noted at 2 months of age (Fig. 2b). The secretory granules at all ages were scattered in the cytoplasm, and at 6 and 12 months of age relatively numerous secretory granules were situated close to the plasma membrane (Figs. 3c, 4b). The tortuous course of the plasma membranes of adjacent chief cells was occasionally observed with increasing age as well as that in the control hamsters. In the UM-X7.1 hamster at 12 months of age few secretory granules were present as compared with those in the control animals of the same age (Fig. 4).

Morphometry of the PTG

The results of morphometric measurements are shown in Table 2 and 3. Table 2 shows a comparison of

organellar profiles on the PTG among UM-X7.1 groups and Table 3 shows those on the PTG between control and UM-X7.1 groups. The mean value of the Golgi apparatus in the UM-X7.1 hamster showed a slight increase from 2 to 12 months but there were no significant differences among the 4 groups. The mean value of the Golgi apparatus in the UM-X7.1 hamster was significantly lower than that in the control hamster at 1 month of age and significantly higher than that in the control hamster at 6 and 12 months of age. The mean value of rER in the UM-X7.1 hamster was significantly higher at 6 months of age than at 2 and 12 months of age and significantly lower at 12 months of age than at 1 month of age. The mean value of rER in the UM-X7.1

Table 2. Comparison of organellar profiles on the PTG among UM-X7.1 groups.

AGE	G	ER	Ly	LD	V	SG
1 m	5.45±0.47	5.08±0.46[d]	0.18±0.02[c,d]	0.06±0.02[b]	0.15±0.02	1.55±0.06[c,d]
2 m	5.26±0.27	3.36±0.22[c]	0.21±0.02[c,d]	0.25±0.05[a,c]	0.21±0.03[d]	2.85±0.35[c,d]
6 m	5.64±0.43	5.61±0.41[b,d]	0.32±0.04[a,b,d]	0.07±0.01[b]	0.13±0.03	7.35±0.98[a,b]
12 m	6.28±0.32	3.12±0.54[a,c]	0.52±0.01[a,b,c]	0.17±0.04	0.09±0.02[b]	6.64±0.37[a,b]

Values are means±SEM. m: month; G: the Golgi apparatus; ER: rough endoplasmic reticulum; Ly: lysosome; V: large vacuolar body; LD: lipid droplet. Values are presented as percentage of cytoplasmic areas. Value of secretory granule (SG) is presented as number of profiles per 100 μm^2 in the cytoplasm. [a]: $p<0.05$ vs. 1-month group; [b]: $p<0.05$ vs. 2-month group; [c]: $p<0.05$ vs. 6-month group; [d]: $p<0.05$ vs. 12-month group.

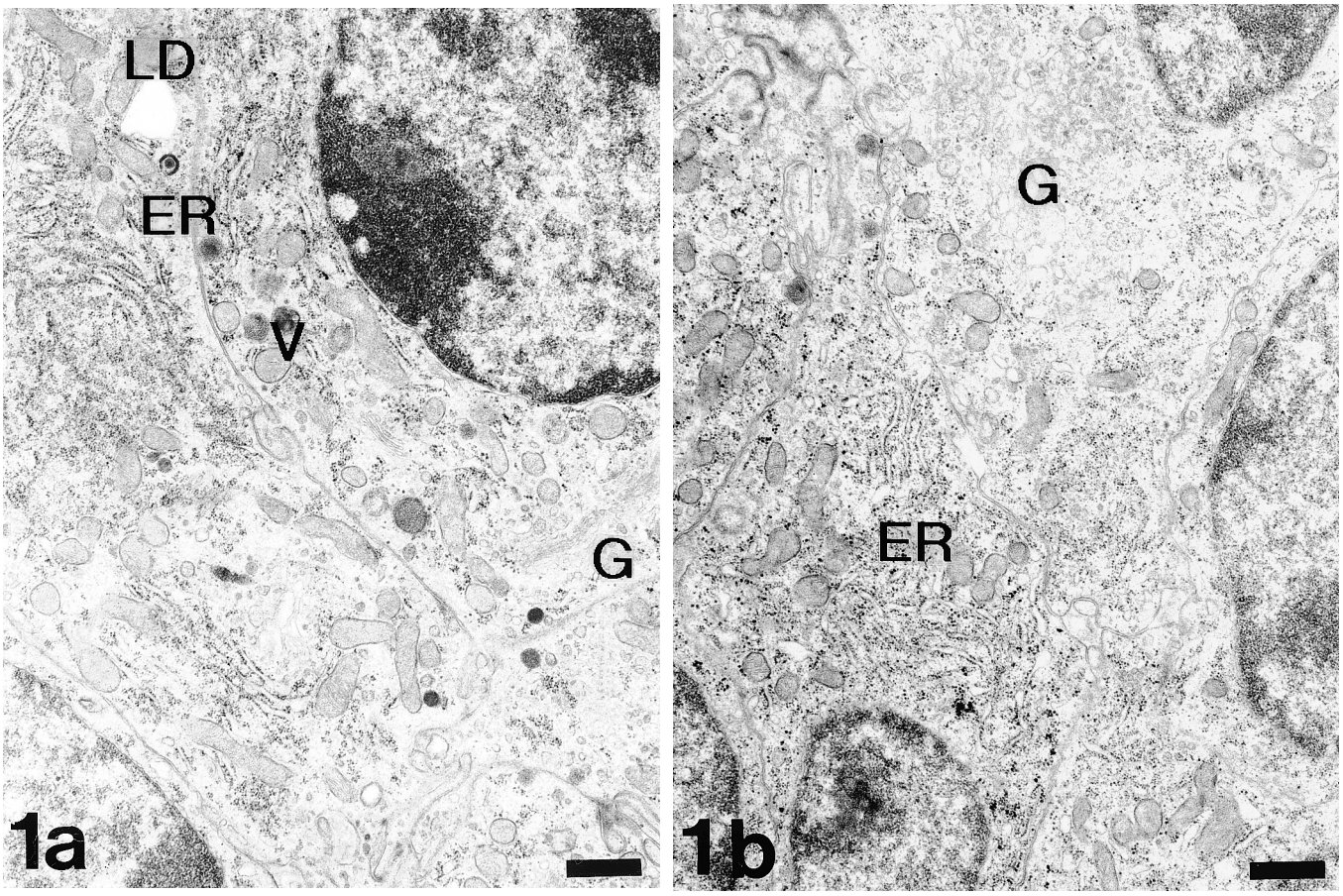


Fig. 1. a. Parathyroid chief cells of a control hamster at 1 month of age. The chief cells contain a abundant free ribosomes, mitochondria, a moderately developed Golgi apparatus (G), cisternae of the rough endoplasmic reticulum (ER), a large vacuolar body (V), lipid droplets (LD) and secretory granules. Scale bar: 1 μm. **b.** Parathyroid chief cells of a UM-X7.1 hamster at 1 month of age. The Golgi apparatus (G) and cisternae of the rough endoplasmic reticulum (ER) are seen. Scale bar: 1 μm.

hamster at 6 months of age was significantly higher than that in the control hamster. The mean value of lysosomes in the UM-X7.1 hamster increased markedly with age and was significantly higher at 6 months of age than at 1 and 2 months of age and significantly higher at 12 months of age than at 2 months of age. The mean value

Table 3. Comparison of organellar profiles on the PTG between control and UM-X7.1 groups.

AGE	GROUPS	G	ER	Ly	LD	V	SG
1 m	Control	7.48±0.46	6.08±0.41	0.20±0.02	0.13±0.02	0.16±0.01	3.06±0.39
	UM-X	5.45±0.47*	5.08±0.46	0.18±0.02	0.06±0.02*	0.15±0.02	1.55±0.06*
2 m	Control	4.52±0.33	3.50±0.17	0.25±0.03	0.30±0.07	0.06±0.02	4.46±1.05
	UM-X	5.26±0.27	3.36±0.22	0.21±0.02	0.25±0.05	0.21±0.03	2.85±0.35
6 m	Control	3.77±0.33	3.53±0.32	0.31±0.02	0.33±0.10	0.06±0.02	3.50±0.81
	UM-X	5.64±0.43*	5.61±0.41*	0.32±0.04	0.07±0.01*	0.13±0.03	7.35±0.98*
12 m	Control	4.83±0.31	2.59±0.53	0.47±0.02	0.18±0.05	0.06±0.01	19.11±0.36
	UM-X	6.28±0.32*	3.12±0.54	0.52±0.01*	0.17±0.04	0.09±0.02	6.64±0.37*

Values are means±SEM. m: month; G: the Golgi apparatus; ER: rough endoplasmic reticulum; Ly: lysosome; V: large vacuolar body; LD: lipid droplet. Values are presented as percentage of cytoplasmic areas. Value of secretory granule (SG) is presented as number of profiles per 100 μm^2 in the cytoplasm. *: $p<0.05$ vs. control group.

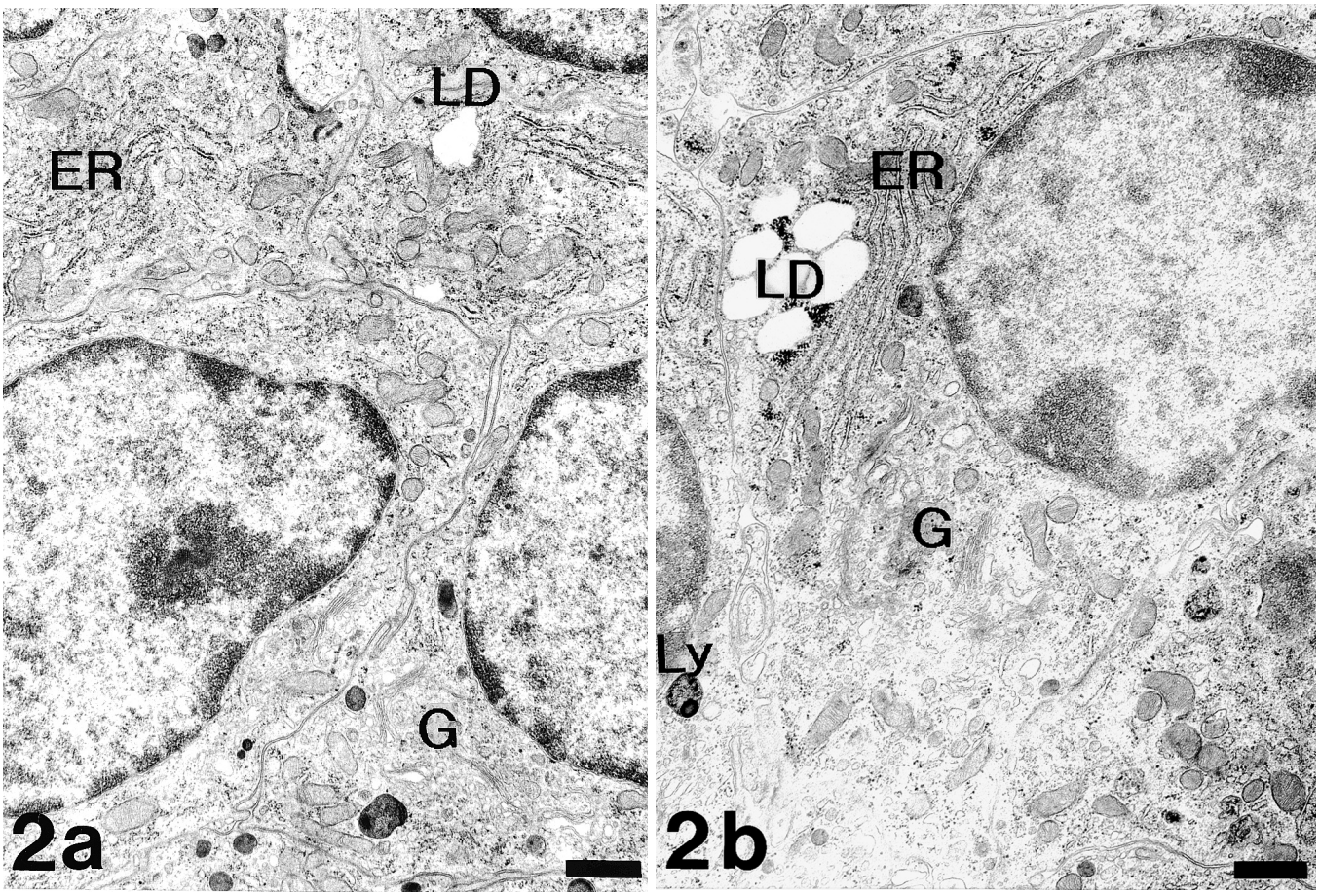
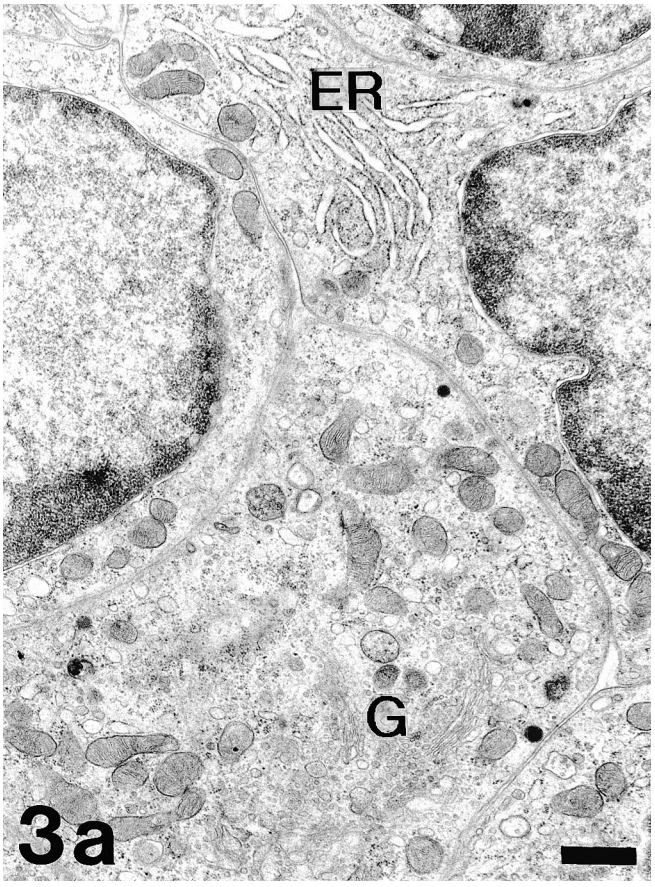
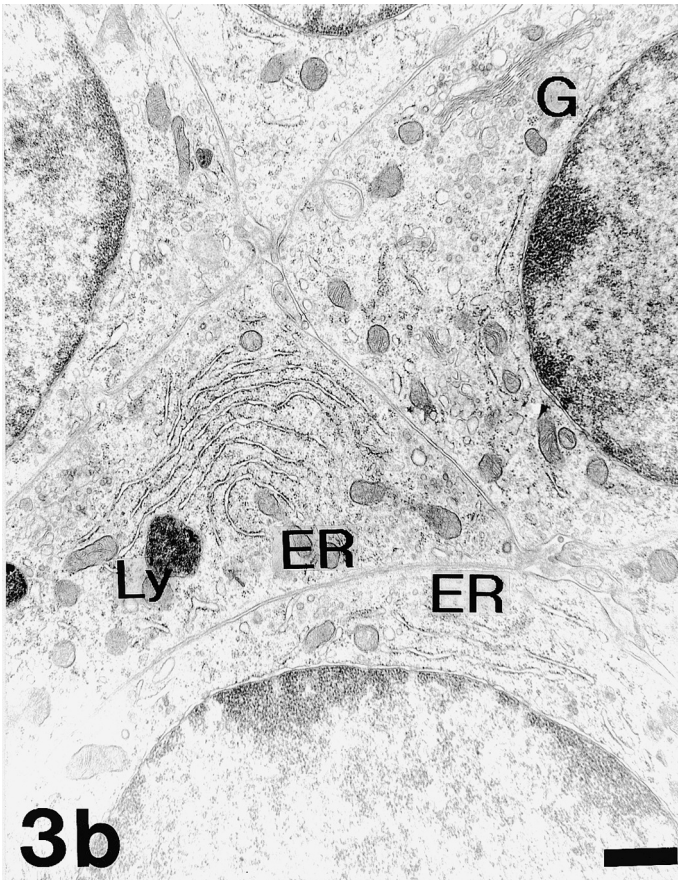


Fig. 2. a. Parathyroid chief cells of a control hamster at 2 months of age. The chief cells contain a moderately developed Golgi apparatus (G), cisternae of the rough endoplasmic reticulum (ER) and lipid droplets (LD). Scale bar: 1 μm. **b.** Parathyroid chief cells of a UM-X7.1 hamster at 2 months of age. The Golgi apparatus (G) and cisternae of the rough endoplasmic reticulum (ER) are similar to those of the control animals. Ly: lysosome; LD: lipid droplets. Scale bar: 1 μm.





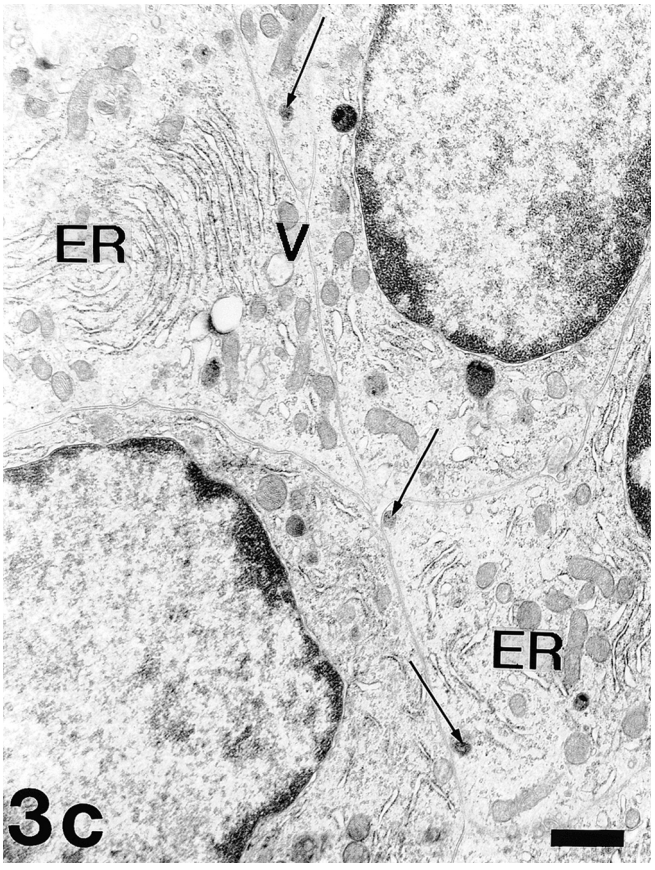


Fig. 3. a. Parathyroid chief cells of a control hamster at 6 months of age. G: Golgi apparatus; ER: cisternae of the rough endoplasmic reticulum. Scale bar: 1 μm. **b, c.** Parathyroid chief cells of a UM-X7.1 hamster at 6 months of age. The well-developed cisternae of the rough endoplasmic reticulum (ER) are randomly distributed and arranged as curvilinear whirls. G: Golgi apparatus; Ly: lysosome; V: large vacuolar body; Arrow: secretory granule. Scale bar: 1 μm.

of lysosomes in the UM-X7.1 hamster at 12 months of age was significantly higher than that in the control hamster. The mean value of lipid droplets was significantly higher at 2 months of age than at 1 and 6 months of age. The mean value of lipid droplets in the UM-X7.1 hamster at 1 and 6 months of age was significantly lower than that in the control hamster. The mean value of large vacuolar bodies in the UM-X7.1 hamster was significantly higher at 2 months of age than at 12 months of age and showed no significant differences from the control hamster. The mean value of secretory granules in the UM-X7.1 hamster was significantly higher at 6 and 12 months of age than at 1 and 2 months of age. The mean value of secretory granules in the UM-X7.1 hamster was significantly higher at 6 months of age and significantly lower at 1 and 12 months of age than that in the control hamster.

Discussion

The rER, the site of PTH synthesis, the Golgi

apparatus, where PTH is packaged into secretory granules, secretory granules, which store and transport PTH to the cell surface, and other organelles (lysosomes, lipid droplets and large vacuolar bodies) concerned with regulating overproduction of PTH are known to be the morphological parameters associated with the functional status of chief cells.

In this study, we observed that the activity of the PTG of the UM-X7.1 hamster at 1 month of age decreased in many chief cells, showing the poorly developed Golgi apparatus and a low number of the secretory granules compared with those of the control hamster. Since the UM-X7.1 hamster at this age showed a light body weight and a low degree of abnormality in the calcium-regulating system compared with the control hamster (Togari et al., 1989), it is thought that the observed decrease at this age may be due to the disturbance of growth in the UM-X7.1 hamster.

At 2 months of age, the activity of the PTG of the UM-X7.1 hamster was similar to that of the control hamster. Jasmin and Eu (1979) mentioned that the UM-X7.1 hamster urinary excretion of norepinephrine was significantly increased at 50 days of age and that the sympathetic nervous system was markedly activated. On the other hand, not only plasma calcium concentration, but also the autonomic nervous systems have been known to play an important physiological role in the regulation of PTH secretion (Atwal, 1981; Shoumura et al., 1983).

Therefore, it is assumed that at 2 months of age, the PTG of the UM-X7.1 hamster activated by the increased sympathetic nervous activity caught up with that of the control hamster.

The PTG of the UM-X7.1 hamster at 6 months of age showed a well developed Golgi apparatus and rER and many secretory granules compared with the control hamster. The large amount and/or size of the Golgi apparatus, rER and secretory granules in the UM-X7.1

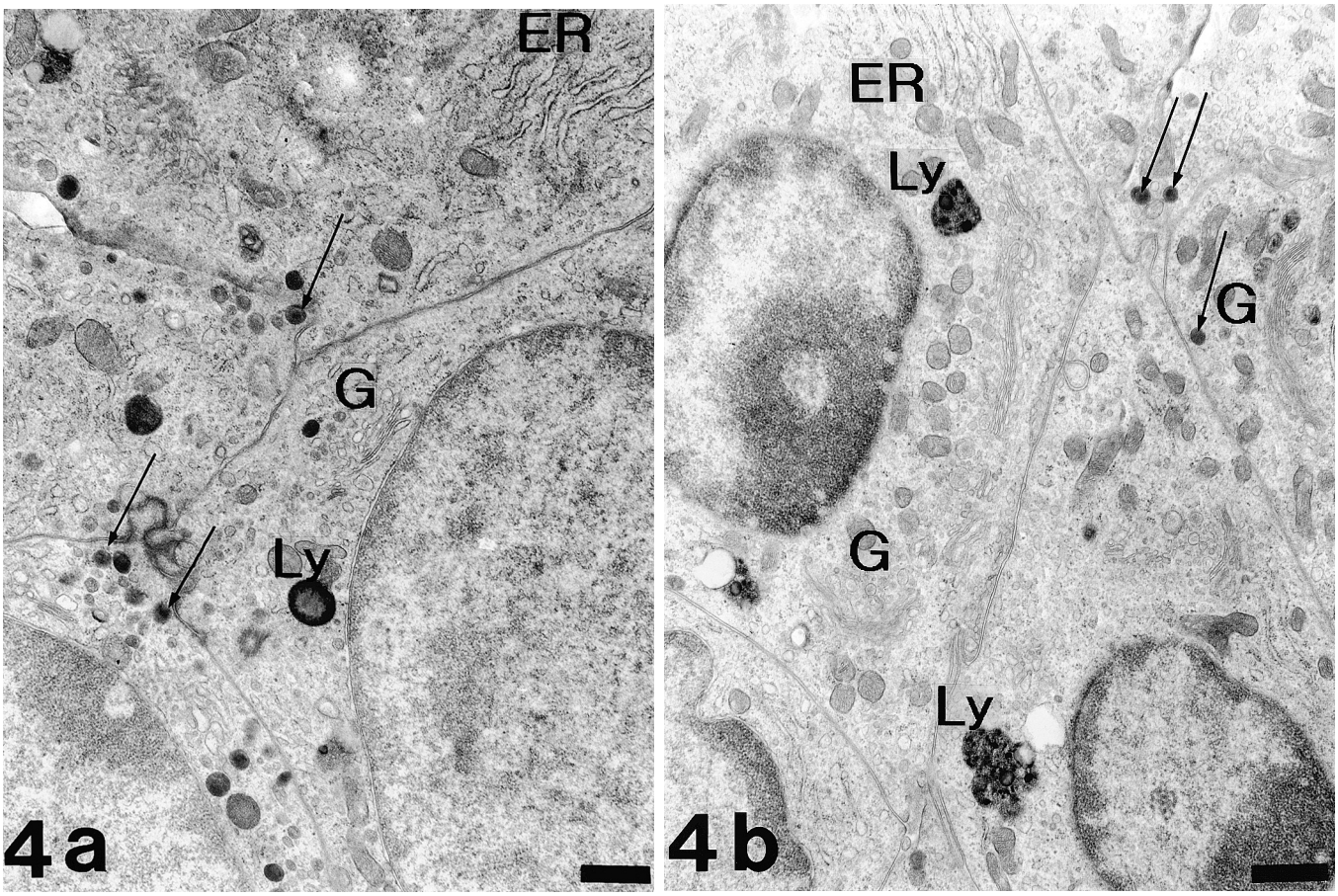


Fig. 4. a. Parathyroid chief cells of a control hamster at 12 months of age. The tortuous course of the plasma membranes of adjacent chief cells is occasionally observed. Many secretory granules (arrows) are situated close to the plasma membrane. G: Golgi apparatus; ER: cisternae of the rough endoplasmic reticulum, Ly: lysosome. Scale bar: 1 μm. **b.** Parathyroid chief cells of a UM-X7.1 hamster at 6 months of age. The well-developed cisternae of the rough endoplasmic reticulum (ER) and Golgi apparatus (G) are seen. The number of secretory granules (arrows) are small as compared to that of the control animals. Some lysosomes (Ly) are noted. Scale bar: 1 μm.

hamster at 6 months of age suggested an increased capacity for synthesis and packaging of PTH (Chen et al., 1990; Shoumura et al., 1990). Togari et al. (1989) reported that the plasma calcium level in the UM-X7.1 hamster was significantly higher than that in the control hamster at 10 and 20 weeks of age and progressed in severity with increasing age. These results suggest that the functional activity of the PTG in the UM-X7.1 hamster was higher than that in the normal hamster.

The reason for the change in the PTG activity in the UM-X7.1 hamster observed at 12 months of age is not clear at present. After about 12 months of age, most of them usually die from cardiac failure and several sites of cellular dysfunction have been identified. Furthermore, the plasma calcium level in the UM-X7.1 hamster increased gradually in an age-related fashion (Togari et al., 1989). Therefore, it is assumed that the tendency for the decrease in the PTG activity compared with the UM-X7.1 hamster at 6 months of age may be due to the malfunction of parathyroid chief cells and/or the influence of feedback on the PTG.

Lysosomes in the PTG increased in number with growth, and might function in the regulation of overproduced secretory granules (Fujii et al., 1975). Our finding is generally consistent with the observation described above.

In the PTG of UM-X7.1 hamster from 1 to 12 months of age, the change in lipid droplets was the opposite to the activity of the rER. According to Castleman and Roth (1978), lipid droplets that originate from autophagosomes (Thiele et al., 1988) are maximal in resting chief cells and reduced in the active state.

Large vacuolar bodies in the UM-X7.1 hamster decreased from 2 to 12 months of age. Emura et al. (1992) reported that large vacuolar bodies decreased with aging and increased when the secretory activity of the PTG was suppressed in response to acute hypercalcemia. It is thought that large vacuolar bodies might be related to a lysosomal digestion in the regulation of overproduction of the PTH. However, additional study is required to clarify the origin and role of large vacuolar bodies in the PTG.

In conclusion, ultrastructurally we have suggested that the synthesis and release of PTH in the UM-X7.1 hamster at 6 months of age were activated, and that the synthesis of the PTH decreased at 12 months of age. However, the precise mechanisms of the calcium regulating system in the UM-X7.1 hamster remain obscure.

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