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Ultrastructure of Thyroid and Parathyroid Bodies in Case of Hypocalcemia and Hypercalcemia

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To analyze ultrastructural transformations of parafollicular cells of thyroid body and main cells of Parathyroid body in case of experimental blood hypocalcemia and hypercalcemia.
Place and Duration of Study: Tashkent State Dental Institute, between April 2015 and October 2015.
Methodology and Study Design: White outbreed male rats with the mass of 130-140 g were split by 3 groups based on content of the free calcium ions in the blood serum: normal (benchmark, n=10); low (hypocalcemia, n=40); high (hypercalcemia, n=40). Experimental intraperitoneal administration of 2,5% ethylenediominotetraacetic acid and 10% Calcium gluconate during 1, 3, 6 and 10 days resulted in hypocalcemia and hypercalcemia consequently, in elevation and reducing the parathormone contained in the blood.
Results: In case of the normal content of the calcium ions in the blood, the main, oxyphilic, intermediate and cambial cells were identified. The main cells as the most numerous ones are characterized with oblong form and small size. The nucleus is large, located eccentrically, the nucleolem may be even, sulcated, with invagination, and nucleoplasm is rich with euchromatin.

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Conclusion: Hypocalcemia after ethylenediominotetraacetic acid introduction in 1, 3, 6 and 10 days is activating B type cells. Hypercalcemia after calcium gluconate introduction in 1, 3, 6 and 10 days causes hy Parathyroid body po-function.

Keywords: Hypocalcemia; hypercalcemia; thyroid body; parathyroid body.

1. INTRODUCTION

According to the numerous research works, about half of calcium in the blood plasma is associated with organic phosphates, therefore its free ions amount to about 1.2 mM/l. As for the cell internal content, the calcium ion concentration in its cytoplasm is by 10,000 less – about 100 nM/l. Despite it is actively involved in the neurotransmission, segmentation, an astalsis, control many metabolic processes, its concentration both in the blood and in the cell cytoplasm may fluctuate with small amounts [1-5]. It is achieved due to the existence of sophisticated enzymatic and transmission structures in the cell membrane, reverse correlation in the functional system, comprising the main cells (CC) of Parathyroid body (PB) and parafolicular cells (PC) of thyroid body (TB), absorbing cells of intestine and kidney, osteoblastic cells and osteoclasts of bony tissues [3,6,7]. Increase of the PB parathormones (PH) is causing several effects oriented to the keeping the phosphorus and calcium balance: both elements increase in the bony tissues in case of blood hypomineralization in different correlation, phosphorus reabsorbtion in the kidney decreases and calcium reabsorbtion increases, and, to the contrary, the phosphorus absorption in the narrow intestine increases, and the calcium absorption decreases [8]. Given this fact, we have set a GOAL: to analyze ultrastructural transformations of parafollicular cells of thyroid body and main cells of Parathyroid body in case of experimental blood hypocalcemia and hypercalcemia.

2. MATERIALS AND METHODS

White outbreed male rats with the mass of 130-140 g were split by 3 groups based on content of the free calcium ions in the blood serum: normal (benchmark, n=10); low (hypocalcemia, n=40); high (hypercalcemia, n=40).

Hypocalcemia was achieved through the daily intraperitonial introduction of 2.5% disodium salt water solution of ethylenediominotetraacetic acid (EDTA 1.0 ml per 100 g of the animal's mass). EDTA with the calcium ions of the blood serum is forming insoluble salt and decreases calcium concentration. On the contrary, hypercalcemia was achieved through the daily intraperitonial introduction of 10% solution of calcium gluconate (1.0 g per 100 g of the animal's mass). Removal of the animals from experiment was accomplished in line with the European Convention for the Protection of Animals Used in the Scientific Research (1984), within 1, 3, 6 and 10 days. Calcium ion concentration in the blood serum was identified using atomic sorption spectrophotometer (Beckman, USA). For the mother solution preparation $CaCO_3$ was dissolved in the minimal amount of hydrochloric acid in an amount of O.5 g of $CaCO_3$ in 1 ml. The mother solution of lanthanum chloride was received through solution of 58.65 g of salt in 250 ml of concentrated hydrochloric acid. After that solution was amended to 1,000 ml with distilled water. The blood serum was diluted in correlation 1:25. The serum solutions were compared with the reference solution of the calcium ions through spectrophotometry.

The pieces of PB and TB with the size 1 mm3 for electronic and microscopic examination were fixed in the buffered 2.5% solution of gluteraldehyde (20 min.) with the post-fixation in 1% solution of osmic acid (1.5 hours). After generally accepted follow-through the spirits with increasing concentration the tissue was introduced in araldite. Ultrathin sections were received on LKB-4800 ultramicrotome (Sweden) and observed on IEOL-100S microscope (Japan). PH was identified with radioimmunoassay technique with the help of the bull PH and set of antiserums produced by RIA-RTG-100 Company (Belgium).

3. RESULTS AND DISCUSSION

In case of the normal content of the calcium ions in the blood (Table 1), the main, oxyphilic, intermediate and cambial cells were identified.

The main cells as the most numerous ones are characterized with oblong form and small size. The nucleus is large, located eccentrically, the nucleolem may be even, sulcated, with invagination, and nucleoplasm is rich with

Table 1. Dynamics of the calcium ion concentration after EDTA and calcium gluconate introduction (M+m; mEq/L; n=10)

Type of experiment	Benchmark	Experiment periods, days			
		1	3	6	10
EDTA introduction	3.48±0.08	3.05±0.09*	2.92±0.14*	3.01±0.14*	3.12±0.08*
Ca gluconate introduction	--«--«--	5.51±0.12**	7.32±0.16**	5.50±0.17**	5.48±0.19**

*Note: * -- P<0.05; ** -- P<0.01, compared to the benchmark*

euchromatin. Cytoplasm contains large number of oblong profiles of scabrous endoplasmic reticulum (SER) forming flattened cisterns, vacuoles and vesicles. Golgi apparatus is located near the nucleus, is interacting with the smooth and scabrous reticulum and has moderate development. Chondriosomes are small and evenly distributed along entire cytoplasm amount. Geterochronic condition of the main cells makes it possible to observe A and B type CCs in PB: A is characterized with more oblong form, plasmolemma lines, and interdigitation with adherent cells. SER profiles have oblong form and actively interact with the nuclear membrane. Ribosomes and polysomes are numerous ones, Golgi apparatus is moderately developed and comprises mainly cisterns; lysosomes with various form and size, in moderate quantities with the certain localization. In some sections of cytoplasm residual bodies can be identified. Lipidic granules (LG) in CCc of A type are identified in large quantity with the medium electron density. Secretory granules (SG) are electron dense, with diameter 150-200 nm, not numerous and are located along the cell periphery. Apparently, their secretion is continuous and controlled by the calcium ion concentration in interstitium. In normal condition CC B type is observed relatively seldom, and their correlation with A type is equal to 1:3-5. Their nucleuses are round with the surface invagination; SER profiles are short ones, forming expanded cisterns and vacuoles. Compared to A type, they have single SGs, less lysosomes and LGs, mitochondrial matrix is more dense. Cell membrane is strongly plated with numerous interdigitations.

Oxyphil cells of PB, singly or in form of compact clumps are observed among the main cells; they are large and have singly oblong electron dense SGs with diameter 6-10 nm. Oxyphil cells have large nucleus with approximately equal content of euchromatin and heterochromatin; 1-2 of dense nucleuses are located excentrically. Oblong and round mitochondria occupy significant volume of cytoplasm; SER profiles are single ones and short. Golgi apparatus is underdeveloped and is usually located near nucleus; SGs are almost not found in its structures. Lipidic drops, different in terms of form and size, are identified along entire cytoplasm and in CCs as well. Presumably, oxyphil cells are carrying out the synthesis and secretion of citric acid or the other tricarboxylic acids of the Krebs' cycle [4], as is well-known also involved in control of the calcium ions in the blood via formation of chelates.

Introduction of EDTA in 24 hours causes reduction of the calcium ions in the blood, in average, by 112%. In 3, 6 and 10 days it decreases, in average, by 116, 113.5 and 110%, respectively (Table 1; P<0.01), compared to the level of control animals. PH is significantly growing after EDTA introduction in all periods of experiment and is equal in 1, 3, 6 and 10 days, in average, 127, 370, 450 and 400%, respectively, compared with its control level (Table 2). Accordingly, the strong synchronization of the active state of CC PB is morphologically observed in all periods of research: their B type prevails (Fig. 1). SER profiles increase, the gap in cisterns with medium electron density substrate extends. Golgi apparatus becomes hyperplasic and occupies significant area near the nucleus. Mitochondria are polymorphic with different dense matrix. Number of SGs and LGs in cytoplasm sharply decreases and euchromatin prevails in the nucleus. Cell membrane has numerous with different interdigitation intensity significantly increasing surface of its interaction with interstitium, adherent cells and numerous fenestrated blood channels. The nerve terminals identified near the gland cells almost do not have SGs. Therefore, decrease of the calcium ions in the blood after EDTA introduction correlates with morphological characteristics of PH CC PB.

If EDTA intraperitonial introduction in all periods of research, in average, has decreased concentration of calcium ions by 110-116%, the

calcium gluconate, in average, increases it by 150 (1, 6 and 10 days of introduction), 210% (3 days), compared to its value with control animals (Table 1). PH concentration in 1, 3, 6 and 10 days, in average, decreases by 108, 204, 131, 120%, respectively (Table 2).

When comparing PH dynamics after introduction of EDTA and calcium gluconate, the different extent of PB reactivity should be noted: hormone concentration increases more strongly in case of hypocalcemia than reduces in case of hypercalcemia. It should be thought that influence on osteoclasts and osteoblasts and the further osteocitarian remodeling requires significant amount of PHs [1]. Consistently, due to this fact the change of PB CCs A and B types correlation is reasonable: B type increases after EDYA introduction and, to the contrary, A type – after calcium gluconate introduction. In dynamics, identified changes of A and B type correlation correlate with concentration of calcium ions in the blood.

In case of hypercalcemia from period to period the number of LGs in PB CC cytoplasm increases and the length of SER membranes decreases; Golgi apparatus reduces, mitochondria becomes smaller (Fig. 2), the high dense particles, presumably, calcium appear in their matrix [4]. The concentration of the large number of electron dense secretory granules is typical of cytoplasm near the cell membrane. The cell membrane almost does not form the lines and interdigitations. Therefore, hypercalcemia causes CC hypo-function and main cell A type domination.

TB is a vital organ producing not only triiodothyronine (T3), thyroxin (T4), but also calcitonin playing certain role in regulation of the calcium homeostasis in the blood [5]. In physiological content of the calcium ions (3.48+0.48 mEq/L; Table 1) tireocytes and parafollicular cells can be found in the wall of each follicular gland, which in terms of ultrastructure can be of two types: a) similar to tireocytes and b) parafollicular cells (PCs). PCs in the wall of the follicular gland or among follicular glands are located singularly or in a form of small groups of 2-3 cells. These cells with round, oblong or cubic shapes in the wall of follicular gland may contact with colloid located in the follicular gland gap; they form dense contact, thin desmosomes and low-grade interdigitations with adherent cells. PC's base is constantly contacting with 1-3 blood channels through the thin homogenic basal lamina. PC's nucleus with 1-2 compact plasmosomes are excentrically located and are rich with euchromatin. SER profiles in cytoplasm are forming 2-3 flattened cisterns, Golgi apparatus is identified near the nucleus and occupies rather large area with all structures. The substance with moderately dense matrix and number of crista is located inside some of them. Mitochondria are not numerous, small with moderately dense matrix and number of crista. Free ribosomes and polysomes are evenly disseminated along entire cytoplasm. The existence of significant number of single type round electron dense SGs with thin light ring in cytoplasm near cytolemme adherent to the blood capillary is typical of PC. In case of hypocalcemia (Table 1) PCs become larger and receive mainly conical shape. The nucleus has uneven surface and unevenly expanded perinumain space; nucleoplasm contains approximately equal number of eumatine and heteromatine. SER profiles are in moderate quantity, they form the different size vacuoles with the moderate density substance. Mitochondria are small with dense matrix and some crista; Golgi apparatus is with hypoplasia and contains the moderate number of vesicoles and single vacuoles (Fig. 3). In case of hypocalcemia SHs are large, have high density and much greater quantity, compared the rats with the normal concentration of calcium ions in the blood.

Table 2. Dynamics of parathormone in the blood serum after EDTA and calcium gluconate introduction (M+m; ng/ml n=10)

Type of experiment	Benchmark	Experiment periods, days			
		1	3	6	10
EDTA introduction	1.21±0.05	1.54±0.09**	4.54±0.08 ***	5.45±0.09**	4.95±0.09**
Ca gluconate introduction	--«--«--	1.11±0.06*	0.591±0.092**	0.921±0.120*	1.01±0.092

*Note: *--P<0.05; **--P>0.001; ***--P<0.001*

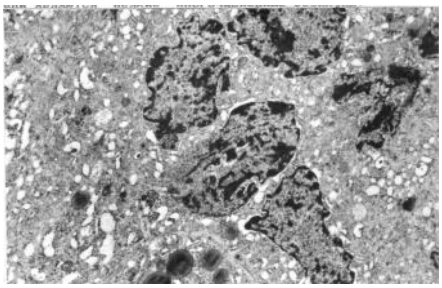

Fig. 1. Vacuolization of the numerous profiles of Scabrous Endoplasmic Reticulum (SER), reduction of Lipidic Granules (LGs), uneven surface of Nucleus (N) in the main cells of B type of thyroid body in case of 3-Day intraperitoneal introduction of EDTA. Scale 15,000

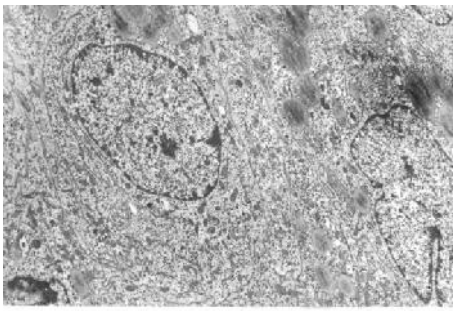

Fig. 2. Reduction of the length and flattening of profiles of Scabrous Endoplasmic Reticulum (SER), Golgi Apparatus (GA) hypoplasia, Large Lipidic Granules (LGs), single interdigitations of the lateral plasmolemma of the main cells A type in case of 3-Day intraperitoneal introduction of calcium gluconate. Scale 20,000

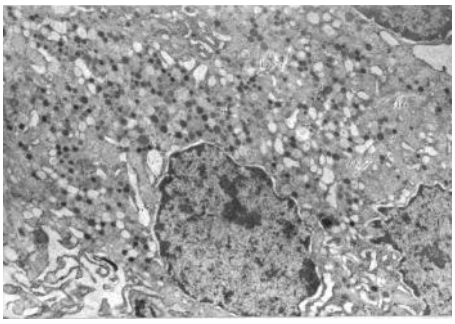

Fig. 3. Numerous Secretory Granules (SGs), fragmented profiles of Scabrous Endoplasmic Reticulum (SER), expansion of Perinumain Space (N) in parafollicular cells of thyroid body in case of 3-Day intraperitoneal introduction of EDTA. Scale 10,000

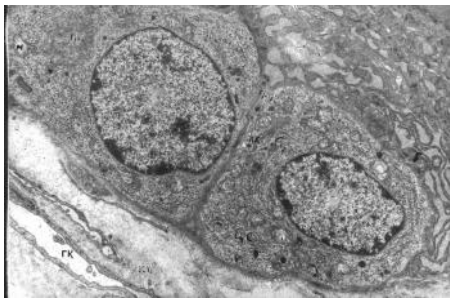

Fig. 4. Single Secretory Granules (SGs), Lightening of Mitochondria Matrix (Mx), flattening and reduction of Scabrous Endoplasmic Reticulum (SER) in case of 3-day intraperitoneal introduction of calcium gluconate. Scale 10,000

In case of hypercalcemia (Table 1) PCs have the cubic shape (Fig. 4), they are located as groups of 2-3 cells, are lightened and almost do not contain SHs. Single germinating cells can be found in the hyperplastic structures of Golgi apparatus closely interacting with unstriated reticulum and nuclear membrane. The nucleus has a round shape, relatively large and contains mainly euchromatine. Perinumain space is unevenly expanded. SER profiles have significant length and are unevenly expanded. Mitochondria have round and oblong shape and are in the functional stress condition. PCs almost do not form interdigitations with adherent cells. The nerve terminals, blood channels sent by the flattened endothelin and penetrated with the large number of pores are permanently identified under PCs. PCs' basal membranes and channels endothelin are lying close to each other and separated just with narrow and almost invisible interstitium.

Therefore, in case of hypocalcemia and hypercalcemia PCs in the blood serum are in the condition of hypo-function and hyper-function, respectively. In hyper-functional condition the process of SH removal is more accelerated, compared to it generation at the stages of intracellular synthesis and aging. In hypo-functional condition SH removal is hardly slowed down while the stages of its synthesis and aging do not experience visible modifications. Though the secretory cycle presents the chain of consequent and interrelated processes simultaneously taking place in the cytoplasm nucleus and cell organ, in condition of hypo-function and hyper-function in PC its final stage when the mature product is synthesized from the cell and interstitium and blood should be considered as the most reactive.

According to the researches, extracellular concentration of the calcium ions [1,4,5,8], plays an important role in the blood coagulation, bony tissue remodeling, neural impulse transmission, etc. It is under the strict control by calcitonin, parathormone and calcitriol hormones which have the reverse correlation among them. Receptors of the calcium ions are membrane proteins and activated in case of increase of its concentration in the blood and interstitium. They "include" the secondary messengers – inozitoltriphosphate (IP3) and diacylglycerol (DAG), which are causing increase of the calcium concentration inside PCs and exocytosis of SHs containing calcitonin. From the other hand, in the main cells of PB its high level decreases secretion of PH increasing calcium in the blood. This activity is mediated by DAG and protein kinase C, and, probably, by decrease of cAMP concentration in the result of G-protein activation. Receptors of the calcium ions are also localized in nephritic and intestinal epithelium [4,5,8]. The mechanisms facilitating keeping calcium ion homeostasis in the blood are controlling protein – receptor on membrane of various cells of internals.

4. CONCLUSION

1. Hypocalcemia after EDTA introduction in 1, 3, 6 and 10 days is activating B type cells, facilitates secretory material removal from them, and increase of PH in the blood. At that, retention of the secretory product removal is observed in PC.
2. Hypercalcemia after calcium gluconate introduction in 1, 3, 6 and 10 days causes PB hypo-function: secretion removal from the gland cells is slowed, the share of the main A type cells increases and PH content in the blood is decreased. Secretion process in TB PC is accelerated.
3. In case of hypocalcemia and hypercalcemia the structural changes of TB PC and main cells of PB are the morphological equivalent of existence of the reverse correlation among them, their functioning association in regulation of the calcium ion concentration in the blood.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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