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The Ocurrence of Multi-Nucleated Cells in Parathyroid Glands of Sheep and Rats

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Koyun ve Sıçanların Paratiroid Bezlerinde Multi-Nükleer Hücrelerin Oluşumu

Summary: The effects of different fixation methods on the ultrastructure of parathyroid cells was studied in sheep and rats. Electron microscopy of parathyroid (PT) glands of sheep fixed by immersion demonstrated multi-nucleated cells with close to each other irregularly shaped nuclei showing folds and undulations, with remnants of the plasma membrane between them. The chromatin is finely granular and dispersed over the nucleus in irregular clusters. The electron-light cytoplasmic matrix containing few secretory granules (SG), small Golgi complex (GC), rough endoplasmic reticulum (RER) with dilated cisternae were indicated. Mitochondria were markedly swollen. In contrast, the PT glands of the rats fixed by perfusion exhibited uniform cells instead of multi-nuclear cells. The uniform cells containing electron-dense cytoplasmic matrix, tortuous plasma membranes, slightly dilated RER and GC, few SG and inconspicuous dilated mitochondria were observed. From these finding we conclude that the occurrence of the "multi-nucleated cells" in PT glands of sheep depends on fixation methods.

Key Words: Parathyroid gland, multi-nucleated cell, fixation method

Özet: Bu çalışmada, farklı fiksasyon metotlarının koyun ve rat paratiroidlerinin ultrastrüktürü üzerine etkisi incelendi. İmmersiyon-fiksasyonla tespit edilen koyun paratiroid (PT) bezlerinin elektron mikroskopisinde, multi-nükleer hücrelerde düzensiz şekilli çekirdeklerin bir birine çok yakın olduğu ve aralarında plazma membran kalıntılarının bulunduğu belirlendi. Kromatin'in, çekirdek içinde ince granüller ve düzensiz parçacıklar şeklinde dağıldığı görüldü. Yoğunluğu az olan sitoplazmik matrikste, az sayıda salgı granülüne ve Golgi kompleksinin küçük keseciklerine rastlandı. Endoplazmik retikulum sisternaları ve mitokondriyonların oldukça fazla şekilde dilate olduğu tesbit edildi. Perfüzyon-fiksasyonla tespit edilen rat PT bezlerinin elektron mikroskop incelenmesinde ise, multi-nükleer hücreler yerine uniform hücreler gözlendi. Bu hücrelerde plazma membranının kıvrımlı ve sitoplazmik matriksin yoğun olduğu belirlendi. Endoplazmik retikulum sisternaları, Golgi kompleks kesecikleri ve mitokondriyonların önemsiz derecede dilate olduğu ve salgı granüllerine çok az rastlandığı tesbit edildi. Bu bulgulardan, koyun paratiroid bezlerinde multi-nükleer hücrelerin oluşumunun farklı fiksasyon metotlarından kaynaklanabileceği sonucuna varıldı.

Anahtar Kelimeler: Paratiroid bezi, multi-nükleer hücre, fiksasyon metodu

Introduction

Histologically, the parathyroid (PT) cells comprise dark chief cells, light chief cells and oxyphil cells (13). In addition to these cells, occasional mention is made in the literature of multi-nucleated cells or syncytial cells (10). They are reported to occur in parathyroid glands in man (1) and dogs (3, 6, 14, 17) regardless the age or functional stage (12). Multi-nucleated cells are especially found in hyper-parathyroidism (14, 21).

The first electron-microscopical description of parathyroid was by Ekholm (7). Later, there have been many electron-microscopical investigations on the normal, stimulated and inactivated parathyroid glands in different species; these have been well documented by Nilsson (13). At the electron microscopic level, PT glands contained 5 cell variants: dark cells, light cells, atrophic cells, intermediate cells and multi-nucleated cells (22). In dogs and rats, multi-nucleated cells were only observed in PT glands fixed by immersion in glutaraldehyde (19) but never in parathyroid glands after perfusion fixation (22).

In the present study, we first compare the ultrastructure of PT glands of sheep fixed by immersion with that of glands of rats fixed by perfusion using the same fixative and second evaluate the influence of different fixation methods on the ultrastructure of PT cells. The aim of this study was to investigate the occurrence of the multi-nucleated cells in parathyroid glands of sheep and rats whether depends on fixation methods.

Materials and Methods

Parathyroid glands for electron-microscopical examination were obtained immediately after 3 sheep were slaughtered. The glands were immediately placed into ice cooled phosphate buffered saline (PBS), dissected into small blocks and immersed in 2.5 % glutaraldehyde (GA) in 0.1 M Na/K-phosphate for 2 h at 4 °C under gentle shaking. Three adult rats (200-300 g.) were deeply anesthetized by intraperitonaeal injection of Ketalar (65 mg/kg). Parathyroid glands were removed (under the aid of a stereomicroscope) after perfusion with 2.5 % glutaraldehyde in 0.1 M. Na/K-

phosphate at room temperature for 15-20 min. with a pressure of approximately 120 cm H_2O through the left cardiac ventricle. Then, the glands were dissected and immersed into ice-cooled 2.5 % glutaraldehyde in the same buffer for 1 h under gentle shaking. After immersion-and perfusion fixations tissue blocks were then washed in the same buffer for 1 h at 4 °C or stored at 4 °C overnight, postfixed with 1 % osmium tetroxide (OsO_4) in 0.05 M same buffer for 1 h at 4°C, dehydrated in a graded series of ethanol starting at 70 % each step for 7 min. and after two changes in propylene oxide. The tissues were embedded in Epon 812. Semithin sections were stained with methylene blue and ultrathin sections with Mg-uranylacetate and lead citrate.

Results

Light microscopic examination of methylene blue stained semithin section revealed a heterogeneous appearance of PT cells in glands fixed by immersion whereas in perfusion fixed PT glands the parenchymal cells showed a high uniformity.

Electron microscopy of PT glands of sheep fixed by immersion demonstrated multi-nucleated cells with close to each other irregularly shaped nuclei showing folds and undulations, with remnants of the plasma membrane between them. The chromatin is finely granular and dispersed over the nucleus in irregular clusters. The electron-light cytoplasmic matrix containing none or only few secretory granules (SG), small Golgi complex (GC), rough endoplasmic reticulum (RER) with dilated cisternae were indicated. Mitochondria were markedly swollen (Fig. 1). In contrast, the PT glands of rats fixed by perfusion did not exhibit any multi-nucleated cells. In addition, perfusion fixed PT cells were very uniform so that the distinction between dark and light chief cells were impossible. The uniform cells containing electron-dense cytoplasmic matrix, tortuous plasma membranes, slightly dilated RER and GC, few SG and inconspicuous dilated mitochondria were observed (Fig. 2).

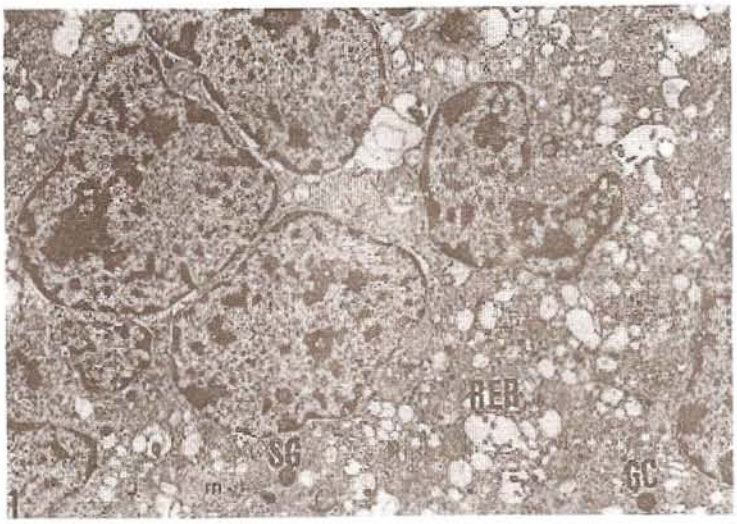

Figure 1. Sheep PT cells fixed by immersion with 2.5 % GA in 0.1 M Na/K-phosphate for 2 h at 4 °C and postfixed with 1 % OsO_4 in 0.05 M Na/K-phosphate for 1 h at 4 °C. Part of a multi-nucleated cells with close to each other irregularly shaped nuclei showing folds and undulations,with remnants of the plasma membrane between them. The chromatin is finely granular and dispersed over the nucleus in irregular clusters. The electron-light cytoplasmic matrix containing none or only few secretory granules (SG), small Golgi complex (GC), rough endoplasmic reticulum (RER) with dilated cisternae and markedly swollen mitochondria (m). (EM x 7400).

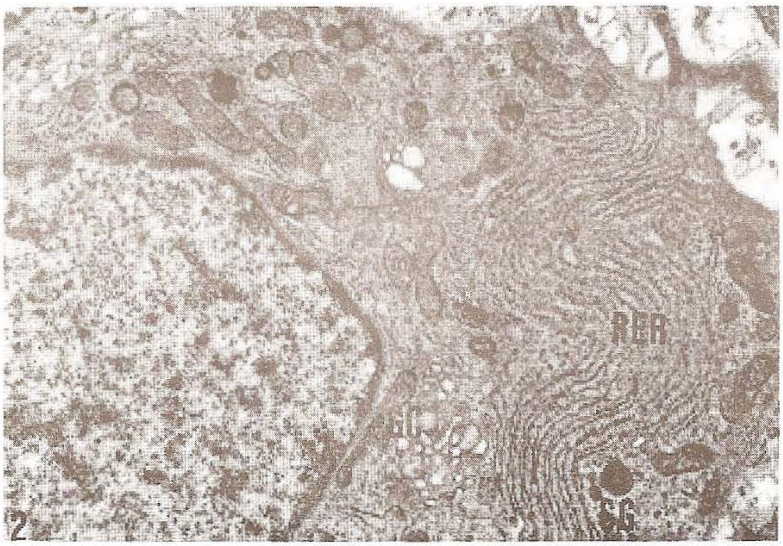

Figure 2. Rat PT cells fixed by perfusion with 2.5 % GA in 0.1 M Na/K-phosphate for 15-20 min. at room temperature and postfixed with 1 % OsO_4 in 0.05 M Na/K-phosphate for 1 h at 4 °C. Uniform cells containing electron-dense cytoplasmic matrix, tortuous plasma membranes, slightly dilated RER and GC, few SG and inconspicuous dilated mitochondria (m). (EM x 13000).

Discussion

Multi-nucleated cells in parathyroids were first described at the beginning of this century (10). Their abundant presence in the present material is typical of parathyroid hyper-function as described by Dämmerich (6). It is understandable that the number of multi-nucleated cells increases with age, as reported by Bargmann (1) and Setoguti (17).

There has been much discussion about the development of multi-nucleated cells in animals. The most common polykaryocytes are foreign body giant cells and osteoclasts, which develop by fusion of several mononuclear cells (5, 15). The syncytic trophoblasts in the placenta (4) are morphologically similar to the multi-nucleated cells in the parathyroid, observed by both light and electron-microscope. The syncytiotrophoblasts are presumed to be formed through fusion of several trophoblasts (16, 9). However, contrary opinions are also found in the literature: it is suggested that they are a result of nuclear division of one cell (2). Oksanen (14) suggested that mitoses are not seen in the multi-nucleated cells or in the chief cells. The multi-nucleated cells are considered to be living and secreting cells and not degenerating chief cells (6). They were regularly seen in hyper-functioning parathyroids (14).

In morphometric studies on the behaviour of cell membranes involving more than 200 rat PT glands which were fixed by perfusion under carefully controlled conditions, multi-nucleated cells were never observed (18, 20, 23). Since this cell type exhibit largely distended RER cisternae and remnants of plasma membranes (3, 12), it is believed that multi-nucleated cells are the result of disintegration of cell membranes taking place during fixation or subsequent dehydration and that they are not normal constituents of canine PT glands.

Glutaraldehyde reacts rapidly but penetrates slowly (11). FA, on the other hand, needs 24 h for fixation but it has the advantage of rapid penetration (8). Therefore, immersion of precooled PT glands into a formaldehyde-glutaraldehyde mixture seems to reduce the formation of "multi-nucleated cells" (19). However, this procedure does not prevent the development of other fixation artefacts in dog and rat PT glands such as shrinkage of nuclei, dilation of RER cisternae, distribution of Golgi lamellae, swelling of mitochondria and whole cells.

At present it is not clear how multi-nucleated cells arise. However, the undulated nuclei, the swollen mitochondria, the dilated RER cisternae and the remnants of plasma membranes indicate that "multi-nucleated cells" are the result of a rapid degenerative process. This process can take place within minutes before or during immersion in glutaraldehyde. From these finding we conclude that the occurrence of the "multi-nucleated cells" in PT glands of sheep depends on fixation methods.

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