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Parathyroid Cell Variants May be Induced by Different Fixatives II. An Electron Microscopic Study

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Farklı Tespit Sıvılarının Paratiroid Hücre Çeşitliliği Üzerine Etkisi II. Elektron Mikroskobik İnceleme

Summary: In this study, the roles of the different combination of aldehydes on the ultrastructure of parathyroid (PT) cells were investigated. Parathyroid glands of rats were fixed by perfusion with different combinations of aldehydes in sodium phosphate buffer, and post fixed osmium tetroxide (OsO_4) in the same buffer. The specimens, parenchymal cells of PT glands, fixed in 2.5% glutaraldehyde (GA) were uniform cells with markedly enlarged intercellular spaces (IS), tortuous plasma membranes, electron-dense cytoplasmic matrix containing slightly dilated cisternae of rough endoplasmic reticulum (RER), large or small Golgi complex (GC), many mitochondria and few secretory granules (SG). PT glands fixed with 4% paraformaldehyde (PFA) led to intermediate cells with electron-lucent cytoplasmic matrix containing markedly dilated cisterna of RER, large or small GC, few mitochondira and few SG. However, the use of fixatives consist of 1%GA - 2% formaldehyde (FA) led only to light cells with highly electron-lucent cytoplasmic matrix containing disrupted membranes of RER or GC. The results thus show that parathyroid cell variants arise during improper immersion or perfusion fixation and that different combinations of aldehydes are important factors provoking parathyroid cell variants.

Key Words: Rat, parathyroid gland, parathyroid cell variants, different fixatives

Özet: Bu çalışmada, farklı aldehit kombinasyonlarının, paratiroid hücrelerinin ince yapısı üzerine etkisi incelendi. Sıçan paratiroid bezleri, sodyum fosfat tamponu varlığında farklı aldehit kombinasyonları kullanılarak perfüzyon-fiksasyon metodu ile ön tespite, osmik asit kullanımı ile de ikinci tespite tabi tutuldular. Elektron mikroskobik incelemelerde; % 2.5 glutaraldehit ile tespit edilen doku örneklerinde paratiroid dokusu parankimal hücrelerinin üniform hücre yapısında olduğu görüldü. Bu hücrelerde, intersellüler mesafenin oldukça geniş, hücre zarının katlantılı, sitoplazmik matriksin yoğun, granüllü endoplazmik retikulumun hafif dilate, büyük ve küçük Golgi kompleksleri ile çok sayıda mitokondriyon ve az sayıda salgı granüllerinin varlığı dikkati çekti. Paraformaldehitin %4'lük solusyonu ile tespit edilen paratiroid dokusunun intermediyer hücrelerden oluştuğu ve bu hücrelerde sitoplazmik matriksin açık, granüllü endoplazmik retikulumun şiddetli dilate, büyük ve küçük Golgi kompleksleri ile az sayıda mitokondriyon ve salgı granüllerinin varlığı belirlendi. %1 GA-%2 FA ile tespit edilen doku örneklerinde ise, paratiroid dokusunun sadece açık hücrelerden oluştuğu ve bu hücrelerde sitoplazmik matriksin oldukça açık, granüllü endoplazmik retikulum ve Golgi kompleksi membranlarının parçalanmış olduğu gözlendi. Bu çalışmada, paratiroid hücre çeşitliliğinin, yetersiz immersiyon -veya perfüzyon- fiksasyon esnasında oluşmuş olabileceği ve farklı aldehit kombinasyonlarının bu hücre çeşitliliğinde önemli rol oynadığı sonucuna varıldı.

Anahtar Kelimeler: Sıçan, paratiroid bezi, paratiroid hücre çeşitliliği, farklı fiksatif

Introduction

Light and dark cells of parathyroid (PT) glands are believed to represent different functional stages of PT cells which undergo cyclic morphological changes in the course of parathyroid hormon secretion (1,2,8). This idea was put forward, first because of the occurence of so called dark and light cells differing in the stainability of the cytoplasm, in the size and distribution of organelles, and in the course of the plasma membrane (8,12). Second, it was found that the appearance of PT cells varied in relation to the calcium concentration used for stimulation or suppression secretory activity of PT cells (9). Stöeckel and Porte (11), however, have suggested that the different morphology in rat PT cells is the result of fixation artefacts rather than biological events. This possibility may be supported by the finding of a more uniform electron density in parathyroid glands fixed by perfusion than in those fixed by immersion (10). Comparison of immersion fixed PT glands with those fixed by perfusion of Mongolian gerbils (4), rats (6,14), dogs (10,15), mice and cats (15) revealed strong evidence to assume that PT cell variants arise during immersion fixation.

The aim of this study was undertaken to investigate the roles of the different combination of aldehydes on the ultrastructure of PT cells in rats using vascular perfusion technique.

Materials and Methods

Six adult rats (200-300g) kept on a standard diet were deeply anesthetized by intraperitoneal injection of Ketalar (65mg/kg). Five thousand units of sodium heparin dissolved in 1ml saline was injected directly into the left ventricle. The right atrium and the apex of the heart were cut, and a cannula was inserted through the left ventricle into the aorta. Physiological saline proceeded the flow of fixatives: a) 2,5 % gluteraldehyde, b) 4 % paraformaldehyde,

c) 1 % gluteraldehyde + 2 % formaldehyde; respectively in 0.1 M sodium phosphate buffer (pH:7.2). The flow rate was maintained at approximately 6 ml/min for 15 min. for both the saline and the fixative solutions. Following perfusion, PT glands were carefully removed and immersed in the same fixatives for 1 h at 4 ^{o}C, washed in the same buffer for 1 h at 4 ^{o}C, and post-fixed with 1 % OsO_4 in sodium phosphate buffer for 1 h at 4 ^{o}C. The tissues were then dehydrated in a graded series of ethanol starting at 70% each step for 10 min and, after two changes in propylene oxide. The tissues were embedded in Araldite. Ultrathin sections were stained with Mg-uranyl acetate and lead citrate for the electron microscopic (Zeiss EM-10) examination.

Results

PT cell variants in rats as revealed by perfusion fixation with different combinations of aldehydes in same buffers are summarized in Table 1. The specimens, parenchymal cells of PT glands, fixed in 2.5% GA were uniform cells with markedly enlarged IS, tortuous plasma membranes, electron-dense cytoplasmic matrix containing slightly dilated cisternae of RER, large or small GC, many mitochondria and few SG. (figure 1). PT glands fixed with 4% PFA led to intermediate cells with electron-lucent cytoplasmic matrix containing markedly dilated cisterna of RER, large or small GC, few mitochondira and few SG. (figure 2). However, the use of fixatives consist of 1%GA - 2%FA led only to light cells with highly electron-lucent cytoplasmic matrix containing disrupted membranes of RER or GC. (figure 3).

Tablo 1. PT cell variants in rats as revealed by perfusion fixation with different combinations of aldehydes in same buffers.

Fixatives	Buffers	PT cell variants	
%2.5 GA	Sodium phosphate	uniform cells	electron-dense cytoplasmic matrix, slightly dilated cisternae of RER, large or small GC, many mitochondria, few SG, markedly enlarged IS, tortuous plasma membranes, focally free ribosomes.
%4 PFA	Sodium phosphate	intermediate cells	electron-lucent cytoplasmic matrix, markedly dilated cisterna of RER, large or small GC, few mitochondira, few SG.
%1 GA - %2FA	Sodium phosphate	light cells	highly electron-lucent cytoplasmic matrix, disrupted membranes of RER or GC.

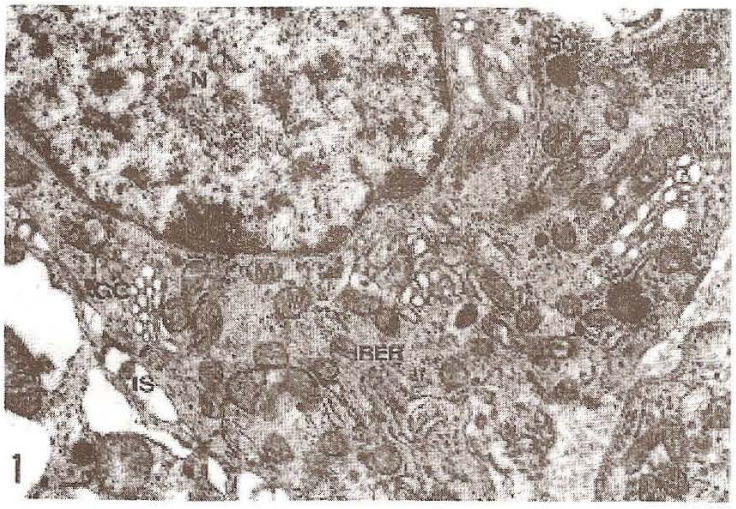

Figure 1. Rat PT cells fixed by perfusion with 2.5% GA in 0.1 M sodium-phosphate and postfixed with 1% OsO_4 in 0.1 M sodium-phosphate. Uniform cells with markedly enlarged intercellular spaces (IS), tortuous plasma membranes, electron-dense cytoplasmic matrix containing slightly dilated cisternae of rough endoplasmic reticulum (RER), large or small Golgi complex (GC), many mitochondria and few secretory granules (SG). (EM x 13000).

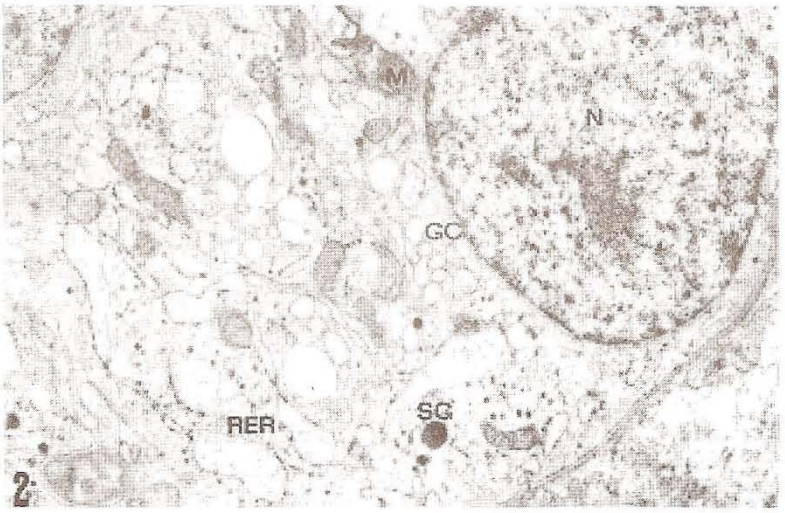

Figure 2. Rat PT cells fixed by perfusion with 4% PFA in 0.1 M sodium-phosphate and postfixed with 1% OsO_4 in 0.1 M sodium-phosphate. Intermediate cells with electron-lucent cytoplasmic matrix containing markedly dilated cisternae of RER, large or small GC, few mitochondira and few SG. (EM x 13000).

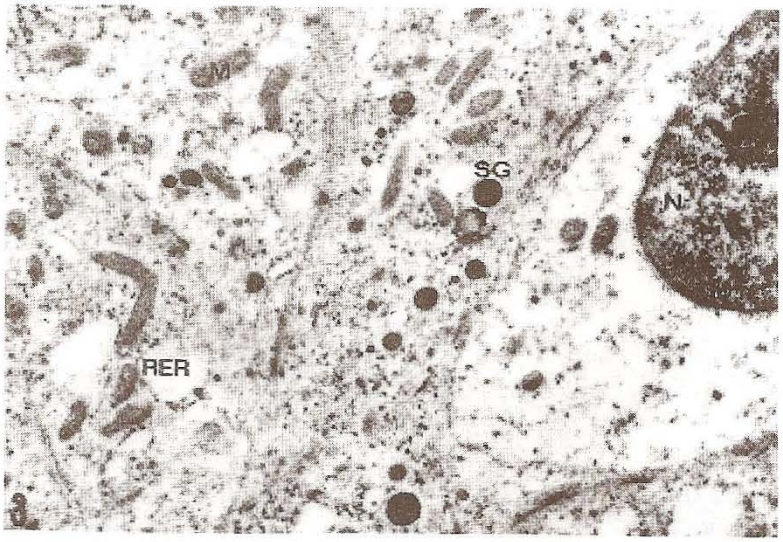

Figure 3. Rat PT cells fixed by perfusion with 1% GA-2%FA in 0.1 M sodium-phosphate and postfixed with 1% OsO_4 in 0.1 M sodium-phosphate. Light cells with highly electron-lucent cytoplasmic matrix containing disrupted membranes of RER or GC. (EM x 10000).

Discussion

In PT glands, the cells concerned with parathormon (PTH) secretion are believed to undergo cyclic changes. The dark chief cells represent the active stages whereas the light chief cells represent the resting stages of a secretory cycle. Intermediate cells are in transitional stage

either from active to resting stages or vice versa (1,2,8,9). In normal PT glands, the dark cells are characterized by prominent rough endoplasmic retikulum (RER) and Golgi apparatus, an increased tortuosity of the plasma membrane. In light cells RER, Golgi apparatus, secretory granules and the tortuosity of the plasma membrane are reduced (1,2,7,8,12).

Interestingly, Stöeckel and Porte (11) mentioned that the differences in PT cell morphology may arise during fixation. Lever (5), who first described the ultrastructure of PT cells, argued that many of the cell membranes lack integrity. Comparative studies have revealed that PT cell variants which regularly occured in immerison-fixed samples were absent in perfusion-fixed PT glands (4,10,14,15) or occured only in perfusion-fixed glands in areas of incomplete fixation (10).

The concept of the existence of a secretory cycle in PT cells was recently called into question by the work of Larsson et al. (4). They demonstrated that, in PT glands of Mongolian gerbils, the occurrence of light chief cells and atrophic cells depended on improper fixation.

PT cell variants occurred in all species examined when tissue was fixed in 2.5% GA. Diversity in PT cell morphology was, however, largely avoided by immersion in mixtures containing 1%GA and 1.5%-2% FA and 2.5-5% acrolein (AC) in bovine, feline and murine PT glands (13). In an attempt to clarify the formation of PT cell variants in dogs, cats, mice and rats (13) and in cattle, sheep, goat, horse and pig (3), it was found that aldehydes and buffers play important roles.

In this study, the specimens, parenchymal cells of PT glands, fixed in 2.5% GA were uniform cells with markedly enlarged IS, tortuous plasma membranes, electron dense matrix containing slightly dilated cisternae of RER, large or small GC, many mitochondria and few SG. PT glands fixed with 4% PFA led to intermediate cells with electron lucent matrix containing markedly dilated cisterna of RER, large or small GC, few mitochondira and few SG. However, the use of fixatives consist of 1%GA - 2%FA led only to light cells with highly electron-lucent matrix containing disrupted membranes of RER or GC. The results support the idea, arising after examination of perfusion-fixed parathyroid tissue, that parathyroid cell variants occur during improper fixation rather than that express functional diversity.

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