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Abstract

To clarify the mode of secretion of specific atrial granules, rat atrial muscle cells were examined by transmission electron microscopy. Atrial granule formation and exocytotic features of granules were clearly seen. Abrupt breaks in the unit membrane structure of mature granules were observed in thin sections, but these breaks were not detected in freeze-fracture replicas. These findings support the concept that the granule contents are released to the extracellular space by exocytosis.

KEYWORDS: exocytosis, atrial natriuretic polypeptide, rat atrium, electron microscopy

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Exocytotic Features of Rat Specific Atrial Granules

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To clarify the mode of secretion of specific atrial granules, rat atrial muscle cells were examined by transmission electron microscopy. Atrial granule formation and exocytotic features of granules were clearly seen. Abrupt breaks in the unit membrane structure of mature granules were observed in thin sections, but these breaks were not detected in freeze-fracture replicas. These findings support the concept that the granule contents are released to the extracellular space by exocytosis.

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Granules in atrial muscle cells were first recognized by electron microscopy in the guinea pig (1). Later, atrial muscle cells of other mammals were reported to contain numerous storage granules, which were referred to as specific atrial granules by Jamieson and Palade (2). Although the content of the granules was not identified, their intimate association with Golgi apparatus, thought to be involved in the formation of atrial granules, was pointed out by these authors. Otsuka *et al.*(3) also observed many granules of various sizes around the Golgi apparatus, which were dilated at the ends of the cisternae and occasionally filled with fine granular materials. They stated that proteinaceous secretory granules appeared to be formed in Golgi apparatus of gland cells.

In 1979, de Bold reported that heart atrial granularity appeared to respond to changes in the water-electrolyte balance, and in 1981, de Bold *et al.*(5) observed a rapid and potent natriuretic response upon the injection of atrial myocardial extract. Their discovery led to the purification of human natriuretic polypeptide by Kangawa and Matsuo (6) and to the localization of the hormone in atrial granules (7). Thus, it is apparent that atrial muscle cells produce a polypeptide hormone. However, the mechanism of secretion of these granules has not been elucidated. There are two views regarding the mechanism of atrial granule secretion: *1)* that the granule contents are released to the extracellular space by exocytosis (8, 9), and *2)* that atrial granules do not secrete by exocytosis but the granule contents are released intracellulary after lysis of granule membranes. Abrupt breaks seen in the granule membrane were considered to be evidence of membrane lysis by Theron *et al.*(10).

In this study, atria from rats were ex-

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amined electron microscopically in thin sections and freeze-fracture replicas to clarify the mechanism of specific atrial granule secretion.

Materials and Methods

Thin-section electron microscopy. Wistar rats weighing 250-300 g were anesthetized with ether and killed by decapitation. The hearts were rapidly removed and processed for electron microscopic studies. The atria were placed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and cut into small blocks. The blocks were immersed in the same fixative for 3 h at 4 °C. After they were rinsed with buffered solution, they were postfixed with 1% osmium tetroxide for 1.5 h at 4°C. These blocks were dehydrated in a graded series of acetone and embedded in Epon 812. Ultrathin sections were prepared with an MT 5000 ultramicrotome using a diamond knife, stained with uranyl acetate and lead citrate, and were examined under an electron microscope (JEOL JEM 100 CX).

Freeze-fracture. Wistar rats were anesthetized with ether and killed by decapitation. The hearts were rapidly removed, and the atria were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4°C. Fixed specimens were placed in 30-40% glycerin for 12-24 h. These pretreated specimens were rapidly frozen with Freon cooled with liquid nitrogen. The frozen specimens were fractured in a freeze fracture apparatus (JEOL EE-FED) at -120°C at a vacuum of 1×10^{-7} torr, and etched at -100°C. Then, they were replicated by double shadowing with platinum-palladium and carbon. The replicas were separated from the specimens by immersing them into a commercial sodium hypochlorite solution for 15-60 min. The replicas were washed with distilled water and placed on a grid mesh and observed electron microscopically.

Results and Discussion

Thin-section electron microscopy. Specific atrial granules were predominantly seen in the central sarcoplasmic core, in the sarcoplasmic layers between myofibrils and under the plasma membrane. In the central sarcoplasmic core, well developed Golgi apparatus were present and occasionally associated with numerous specific atrial granules of various sizes. The Golgi apparatus were composed of vacuoles, lamellae and vesicles often containing moderately electron-dense materials (Fig. 1). Abrupt breaks in the unit membrane structure were frequently seen in mature granules (Fig. 2b, 2c).

Direct evidence of granule formation was occasionally seen. Electron-dense materials similar to those in mature granules were contained at the ends of the cisternae of the well developed Golgi apparatus in the central sarcoplasmic core (Fig. 2a). Mature granules connected to the Golgi lamellae were also observed (Fig. 2b). Exocytotic features of atrial granules were rarely seen. Figs. 2c and 2d show the typical exocytotic process in these cells : fusion of the limiting membrane of the granules and the cell membrane, and continuity of the cell membrane with the granule membrane. The granular contents were present under the basement membrane (Fig. 2d).

Freeze-fracture. Well-developed Golgi apparatus were present in the central sarcoplasmic core. They were occasionally associated with numerous specific atrial granules of various sizes. Many membrane-associated particles were seen on the protoplasmic fracture face (PF face) of the granules, but structures corresponding to the abrupt breaks observed in the thin sections were not detected (Fig. 3).

The findings that the ends of Golgi cisternae contained electron-dense material similar to that in mature granules and that mature granules were connected to Golgi lamellae support the suggestions of other investigators that atrial granules are formed in the Golgi apparatus (2, 3, 9, 10). The exocytotic release of the granules was dem-

Fig. 1 Atria from rats. Specific atrial granules (g) are seen near the nucleus (N) in the central sarcoplasmic core, sarcoplasmic layers and under the plasma membrane. Golgi apparatus (G) and specific atrial granules (g) of various sizes are seen in the central sarcoplasmic core. Abrupt breaks were seen in mature granules. Bar=1μm. ×14,000.

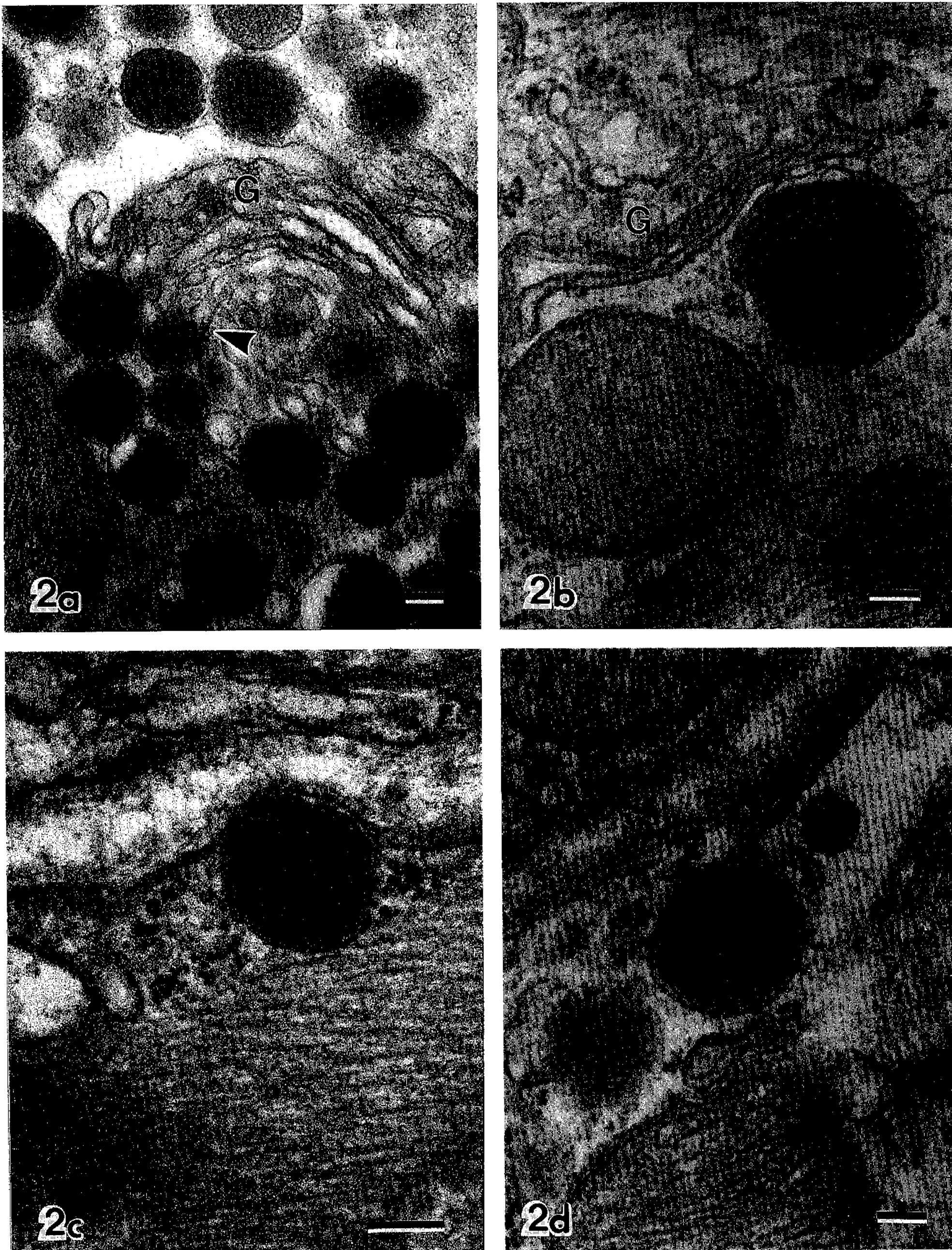

Fig. 2 Granule formation and secretion by the atrial muscle cell. a) The intimate association of the granules with Golgi apparatus (G) is seen. The cisternae were swollen and contained electron dense materials similar to those in mature granules. Bar=0.1 μm. ×45,000. b) A mature granule is associated with the Golgi membrane. An abrupt break is seen in the granule. Bar=0.1 μm. ×58,000. c) The granule membrane is in close contact with the plasma membrane. An abrupt break is seen in the granule. Bar=0.1 μm. ×98,000. d) Exocytosis occurred by the fusion of the granules with the plasma membrane. Bar=0.1 μm. ×64,000.

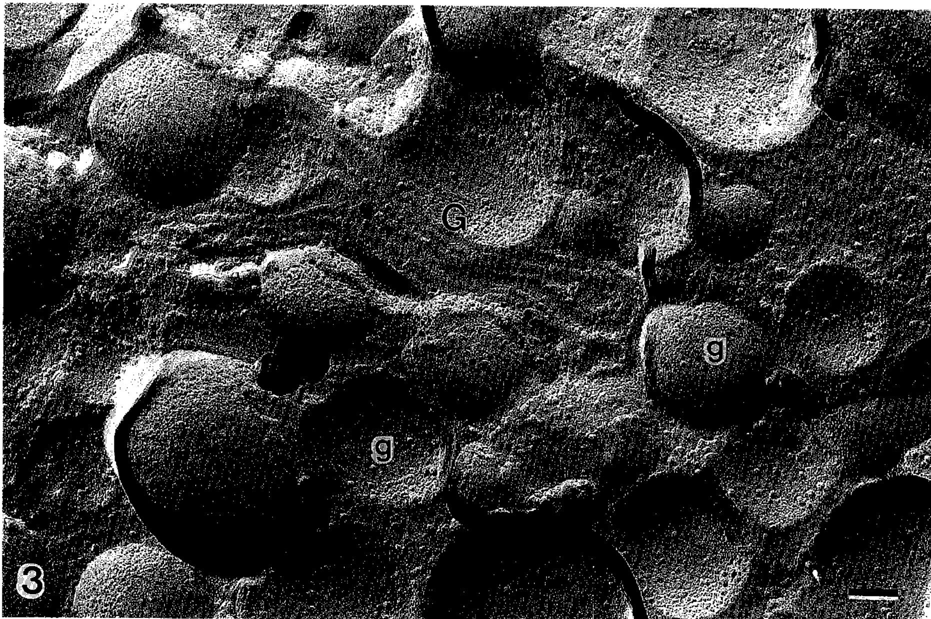

Fig. 3 Freeze-fracture replica from rat atria. The Golgi apparatus (G) and numerous specific atrial granules (g) of various sizes in the central sarcoplasmic core. Many membrane-associated particles were seen on the PF face of the granules. Abrupt breaks were not detected in these granules. Bar=0.1 μm. ×60,000.

onstrated by an ingenious method recently developed by Page *et al.* and Sugawara. Also in the present study, exocytotic figures were clearly seen. The abrupt breaks in the unit membrane structure of mature granules, which were observed by Theron *et al.* (10), were also observed in this study.

Theron *et al.* concluded that atrial granules did not secrete by exocytosis but core material was released intracellularly after lysis of granule membranes, based on the result that no evidence of the exocytosis of granule contents was seen and that membrane lysis appeared to occur in atrial granules.

It is well known that the use of the freeze-fracture technique reveals the three-dimensional surface structure of biological membranes which can not be revealed by thin section electron microscopy. Accordingly, we used the freeze-fracture technique in this study to clarify the unit membrane structure of specific atrial granules. In freeze-fracture replicas of atrial muscle cells, no structure was detected corresponding to the abrupt breaks in the unit membrane structure of mature granules, which were observed in thin sections. This fact suggests that the apparent granule membrane lysis seen in thin sections correspond to the cut surface of slack in the unit membrane of the granules.

Page *et al.* and Sugawara demonstrated exocytotic granule release in the atrial muscle cell. However, they did not mention the presence of the abrupt breaks in the unit membrane structure seen in their figures. The present result that abrupt breaks were not detected in freeze-fracture replicas support the suggestion of Page *et al.* and of Sugawara that the granule contents are released to the extracellular space by exocytosis.

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