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Ultrastructure of atrial and ventricular myocytes of newborn rats: evidence for the existence of specific atrial granule-like organelles in the ventricle

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Summary. The present study examined the ultrastructure of atrial and ventricular myocytes from the heart of newborn rats. It was found that, despite former reports stating that ventricular myocytes in adults do not contain cytoplasmic granules, specific atrial granule-like organelles are present in the ventricles of rats at birth. The presence of these granules together with the relatively underdeveloped contractile apparatus and extensive Golgi complex suggests that the ventricular, like the atrial, myocytes may have an endocrine function before or at birth. Further study is required to determine whether these ventricular cytoplasmic granules contain the same atrial natriuretic peptide species known to be present in the atrial specific granules.

Key words: Newborn rats, Atrium, Ventricle, Specific atrial granule, Atrial natriuretic peptide

Introduction

One morphological characteristic that distinguishes mammalian atrial from ventricular myocytes is the presence of cytoplasmic electron-dense, specific atrial granules. Since the discovery of atrial granules in guinea pig heart (Kisch, 1956), many attempts have been made to determine their functional significance. In 1981, de Bold et al. demonstrated that crude atrial extract, when intravenously injected into assay rats, produced a massive, but short-lived, diuresis and natriuresis, indicating that this extract contains a natriuretic factor. Subsequently, it has been established that the mammalian heart has an endocrine function (Cantin and Genest, 1985- a review), and that a family of atrial natriuretic peptides (ANP) is synthesized, stored in specific granules and released by the atrial myocytes.

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Recently, it was reported that fetal and newborn rat ventricular myocytes are immunoreactive to ANP antibodies (Thompson et al., 1985; Back et al., 1986; Scott and Jennes, 1987; Toshimori et al., 1987) despite earlier studies that failed to demonstrate the presence of either immunoreactivity to ANP or granules in the ventricles (Cantin et al., 1980). The aim of the present study was to examine the ultrastructure of myocardiocytes in newborn rats, and more specifically to ascertain whether or not specific atrial granule-like organelles were present in the neonatal ventricle.

Materials and methods

Experimental animals

Four pregnant Wistar-Kyoto (WKY) rats, 13-15 days gestation, were purchased from Taconic Laboratory Animals and Services (Germantown, New York). Rats were given Purina rat chow and water *ad libitum*, and checked daily for offspring. Within 24 hours after birth, two neonates from each litter were sexed and weighed (5 females and 3 males, birth weight ranged from 5.27 to 7.08g).

Preparation for electron microscopy

Each newborn rat was etherized and its thorax opened by a midline incision. The rats were perfused through the left ventricle with 2.0 ml of fixative consisting of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer, pH 7.5. The hearts were excised and immersed in the above fixative for 4 hours. Under a dissecting microscope, the right and left auricular appendages and portions of the right and left ventricles distal to the atrioventricular valves were removed. The ventricles were divided along the interventricular septum. Samples from each of the four chambers were dissected into four pieces, washed in 5.4% sucrose in 0.1M cacodylate buffer for 1 hour, postfixed in 1% OsO_4

in 0.1M cacodylate buffer for 1 hour, *en bloc* stained with saturated uranyl acetate in 50% ethanol, dehydrated through a graded ethanol series, and flat-embedded in Araldite. Thin (gold) sections were cut on a LKB ultramicrotome with glass or diamond knives and placed on copper grids. Sections were stained with uranyl acetate in methanol for 3 mins and lead citrate for 4 mins, and then air dried, and viewed under an Hitachi 500 electron microscope.

Results

Ultrastructure of myocardiocytes

In general, both atrial and ventricular neonatal myocardiocytes exhibit irregularly arranged bundles of myofilaments in reduced density, with an increased amount of interfibrillar sarcoplasmic space (Figs. 1, 2), as compared with those in adult hearts. Common to both cell types is a relatively large, centrally-located nucleus, bordered by adjacent myofilaments forming a sarcoplasmic core. As in the adult, neonatal myofibrils have typical sarcomeres which delimited by Z lines.

The juxtanuclear sarcoplasm contains numerous mitochondria, elements of rough endoplasmic reticulum, glycogen particles, one or more Golgi complexes, polyribosomes and secretory-type granules. The mitochondria are spherical or elongate, have a bilaminar membrane with tubular invaginations of the inner leaflet, a moderately dense matrix and mitochondrial granules (Figs. 1-5). Scattered between the myofibrils and at the periphery of the cells are various numbers of mitochondria, granules, rough endoplasmic reticulum, free ribosomes, glycogen and rarely, multivesicular bodies.

The plasmalemma of the myocardiocytes is frequently observed either communicating with or attaching to adjacent cells via gap junctions, or desmosomes and fascia adherens, respectively. Tubular invaginations of the plasmalemma, constituting the primordia of the transverse-tubule system may be represented by T-tubules at, or near, the Z line. Elements of the sarcoplasmic reticulum ramify throughout the cell and may be visualized partially investing the myofibrils or coupled with the plasmalemma (Figs. 6-8).

Expanded extracellular spaces are evident in the immature cardiac tissue of the neonate. Bundles of collagen fibers are beginning to occupy such interstitial regions. Autonomic nerve endings may be observed in close proximity to bundles of myofibers (Fig. 9). As well as mitochondria, nerve processes contain numerous vesicles with either clear or dense cores.

Specific granules and Golgi apparatus

Numerous specific granules observed in atrial myocytes are located primarily at the perinuclear poles, but also may be found throughout the sarcoplasm and at the cell periphery. In contrast, similar appearing granules occur at a much lower frequency in ventricular cells. However, the granules from both cardiac chambers are membrane-bound and exhibit an electron-dense, homogeneous core (Fig. 10). This core may be slightly retracted from the inner membrane giving the appearance of a «halo». These specific granules are readily distinguished from the much larger lipid droplets by the presence of a limiting membrane.

Both atrial and ventricular myocytes display an extensive, well-developed Golgi complex consisting of layers of membrane-bound saccules, often closely associated with cytoplasmic vesicles (Figs. 11, 12). The specific granules are frequently observed in close proximity to the maturing face of the Golgi complex (Fig. 13). Mitochondria and glycogen particles are commonly observed at the nuclear pole.

Discussion

Neonatal mammalian cardiocytes have not been as thoroughly studied as those of the adult heart (Simpson et al., 1973). Recent work has suggested the presence of ANP-like immunoreactivity in the fetal and neonatal ventricles (Back et al., 1986; Thompson et al., 1986; Scott and Jennes, 1987; Toshimori et al., 1987). Results from these studies suggest that peptide production begins in the fetal rat heart (Toshimori et al., 1987) and, as such, may have an influence on the maturation of the cardiovascular regulatory mechanisms. This notion is supported by the work of Reale et al. (1987) who demonstrated that the serum ANP level in newborn babies is markedly decreased within a few days after birth. The possibility of early developmental patterns affecting subsequent homeostasis prompted this survey of myocardiocytes in newborn rats aimed at elucidating ultrastructural differences which may support this hypothesis.

The overall appearance of immature myocardiocytes resembles a three dimensional network at the myofibrillar level (Challice and Viragh, 1973). The apparent underdevelopment, in both size and number, of myofibrils suggested by the vast amounts of intercellular sarcoplasm implies that contractility may not be the only primary function of the early heart, as it is in the mature organ. Further, the transverse-tubule system is not completely developed at birth and, thus, tubular elements may be difficult to discern (Hibbs and Ferrans, 1969). The transverse tubules, purportedly involved in enhancement of excitatory transmission to facilitate contractile function, are thought to develop rapidly in the first few postnatal weeks in response to the increased workload of the heart (Hibbs and Ferrans, 1969; Challice and Viragh, 1973; Ayettey and Navratnam, 1978). In contrast, the sarcoplasmic reticulum, thought to be derived from the nuclear envelope, is fairly well developed by the fourteenth gestational day and can be observed extending throughout the sarcoplasm (Challice and Viragh, 1973).

Perhaps the most significant feature of the neonatal heart which supports a role other than contractility, is the relatively large volume of Golgi complex in both atrial

Fig. 1. Neonatal rat atrial myocyte exhibiting specific granules (small arrowhead). Nucleus (N), mitochondria (M), Z line (large arrow), desmosome (large arrowhead), sarcolemma (small arrow) and sarcoplasmic coupling (*). × 18,200

Fig. 2. Neonatal rat ventricular myocyte with a few specific granules (small arrowhead). Nucleus (N), nucleolus (Nu), mitochondria (M), intercalated disc (large arrowhead), rough endoplasmic reticulum (arrow) and desmosomes (*). × 13,000

and ventricular myocytes and the occurrence of progranules in the distended cisternae (Ross and Reith, 1985). This is in direct contrast to observations made in cultured neonatal cardiocytes which demonstrate fewer, if any, specific granules and a less elaborate Golgi complex (Cantin et al., 1980).

In this study we observed numerous specific granules in the atria, but a much lower frequency of specific atrial granule-like organelles in the ventricles. Cytolocalization of granules using ANP antibodies has lead to reports that the auricles contain the greatest density of granules (Jamieson and Palade, 1964; Cantin et al., 1979; Back et al., 1986; Scott and Jennes, 1987). This is in keeping with the concept that the auricles are remnants of the primitive atria (Moore, 1977). However, it is questionable whether there is a difference in density of granules between left and right atria (Jamieson and Palade, 1964; Hibbs and Ferrans, 1969; Cantin et al., 1979; Back et al., 1986; Scott and Jennes, 1987). Scott and Jenes (1987) demonstrated no difference in the fetal rat atria, whereas Jamieson and Palade (1964) suggested the adult right atrial myocytes have an apparent greater density than those of the left atrium. Some recent reports showed little or no immunoreactivity in the right ventricle but a higher intensity in the left ventricle (Back et al., 1986; Thompson et al., 1986; Toshimori et al., 1987). Nemer et al. (1986) demonstrated that in adult rats both the atrium and ventricle contain ANP, and express the ANP gene, although the level is much higher in the atria.

This apparent differential development of specific granules raises several questions about a) the function of the granules during development; b) the subsequent disappearance of ventricular granules; c) the reported reduction in left atrial granular density; and d) the apparent regression of the ventricular Golgi apparatus.

Further investigation is required to determine the form and function of ventricular granule content. Confirmation of ventricular reactivity to ANP antiserum and cDNA may add further credibility to the suggestion that the immature heart may serve in a developmental endocrine capacity.

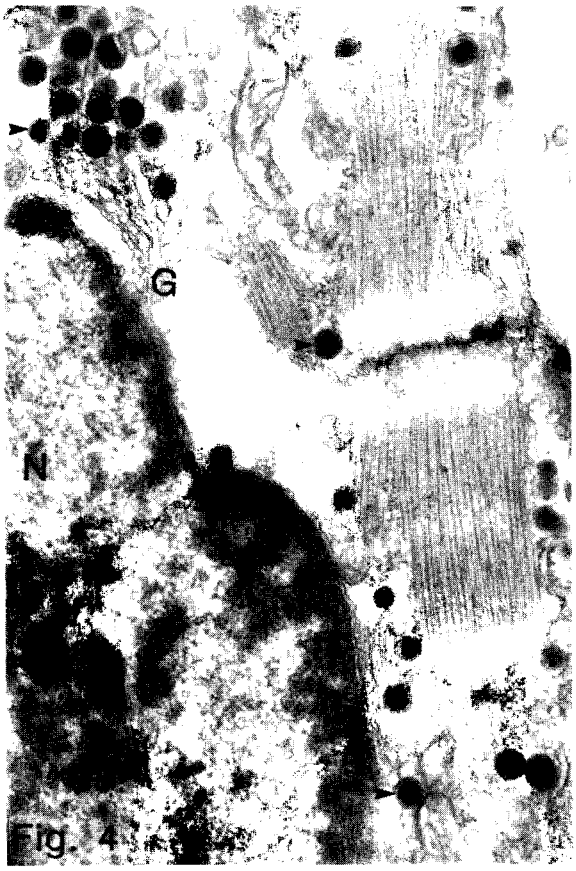

Fig. 3. Ventricular perinuclear area. Golgi apparatus (G), granules (small arrowhead), nucleus (N) and mitochondria (M). × 27,500

Fig. 4. Atrial perinuclear area. Golgi apparatus (G), granules (small arrowhead) and nucleus (N). × 17,100

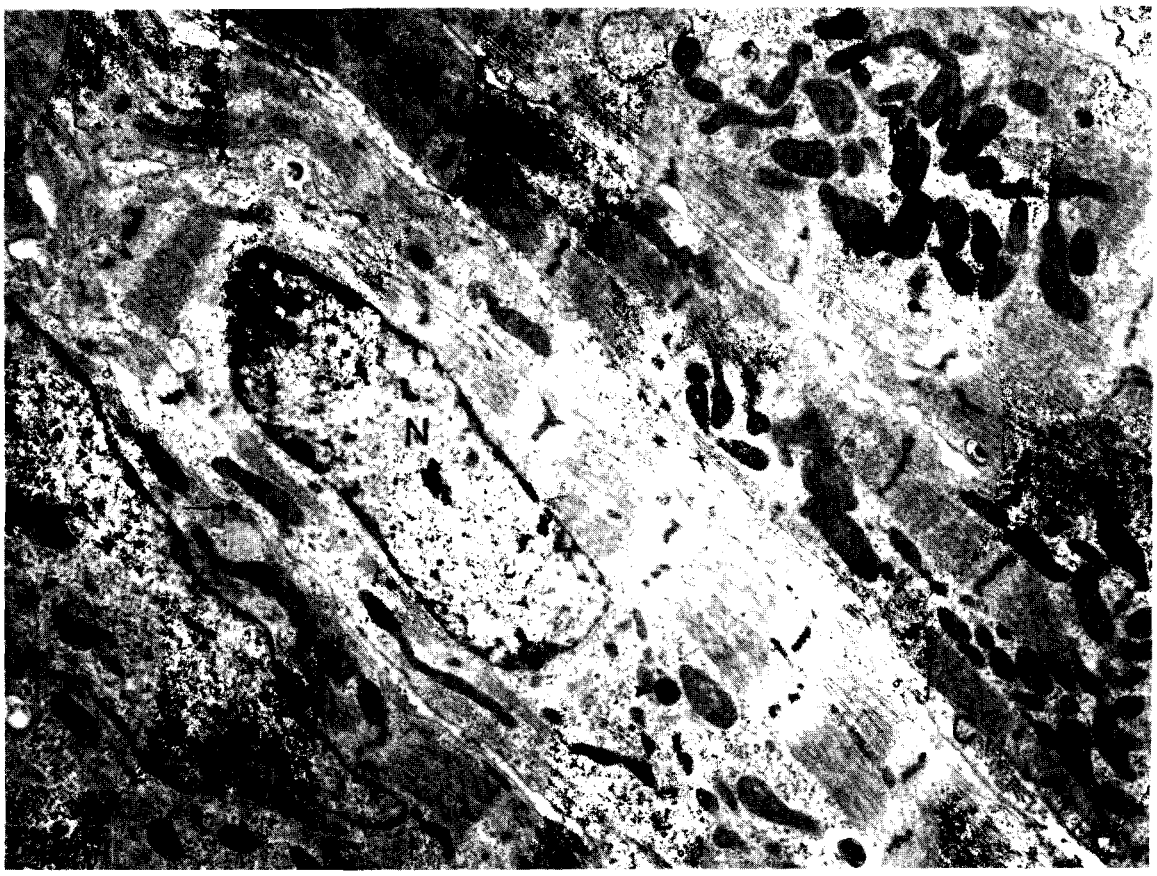

Fig. 5. Ventricular myocyte exhibiting typical ultrastructure at birth. Nucleus (N), mitochondria (M), atrial-like granules (small arrowhead), desmosome (arrow) and collagen fibrils (C). × 9,100

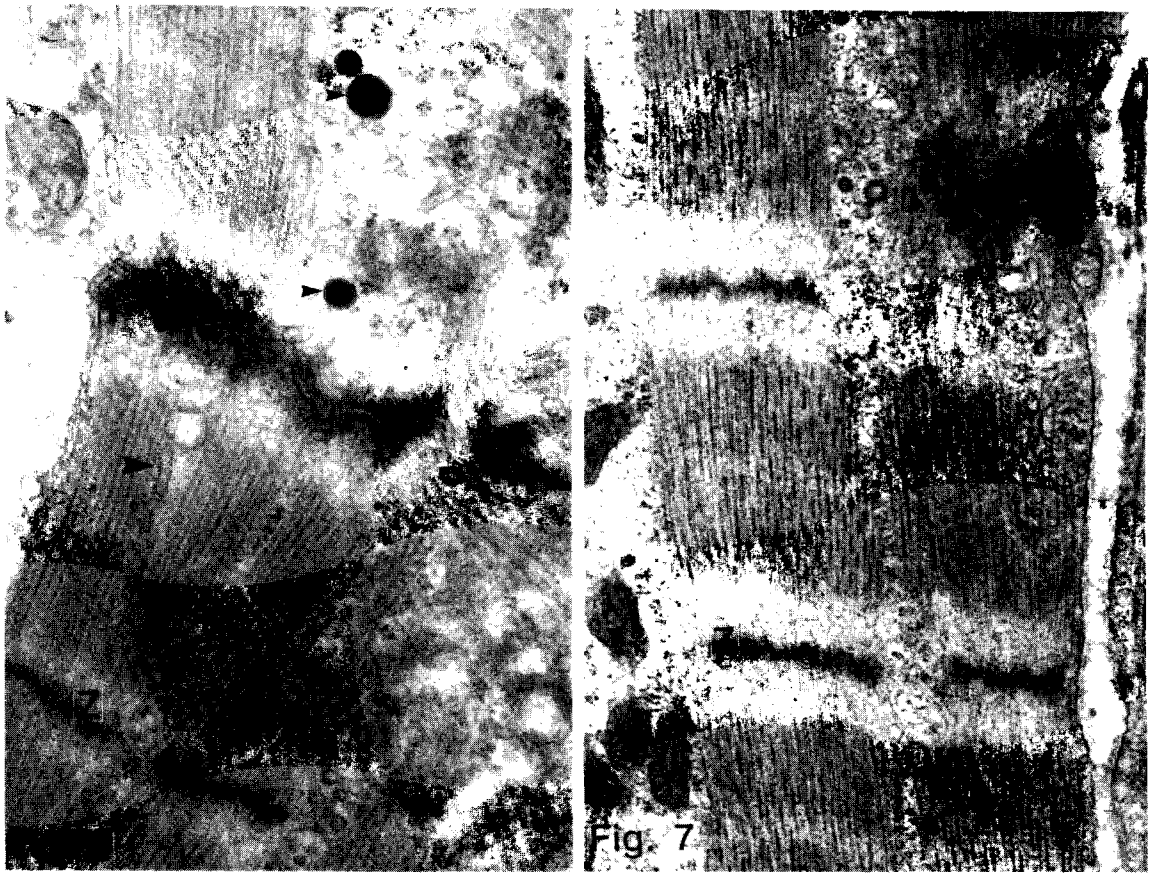

Fig. 6. Portion of an atrial myofiber exhibiting an intercalated disc (ID) and elements of sarcoplasmic reticulum (large arrowhead). Granules (small arrowhead) and Z-line (Z). × 30,000

Fig. 7. Portion of a ventricular myofibril exhibiting an intercalated disc (ID) and elements of sarcoplasmic reticulum (large arrowhead). × 24,000

Fig. 8. Cell-to-cell attachment of ventricular myocyte. Fascia adherens (large arrowhead), gap junction (small arrowhead), desmosome (arrow), coated vesicle (Cv) and pinocytotic vesicle (v). × 36,700

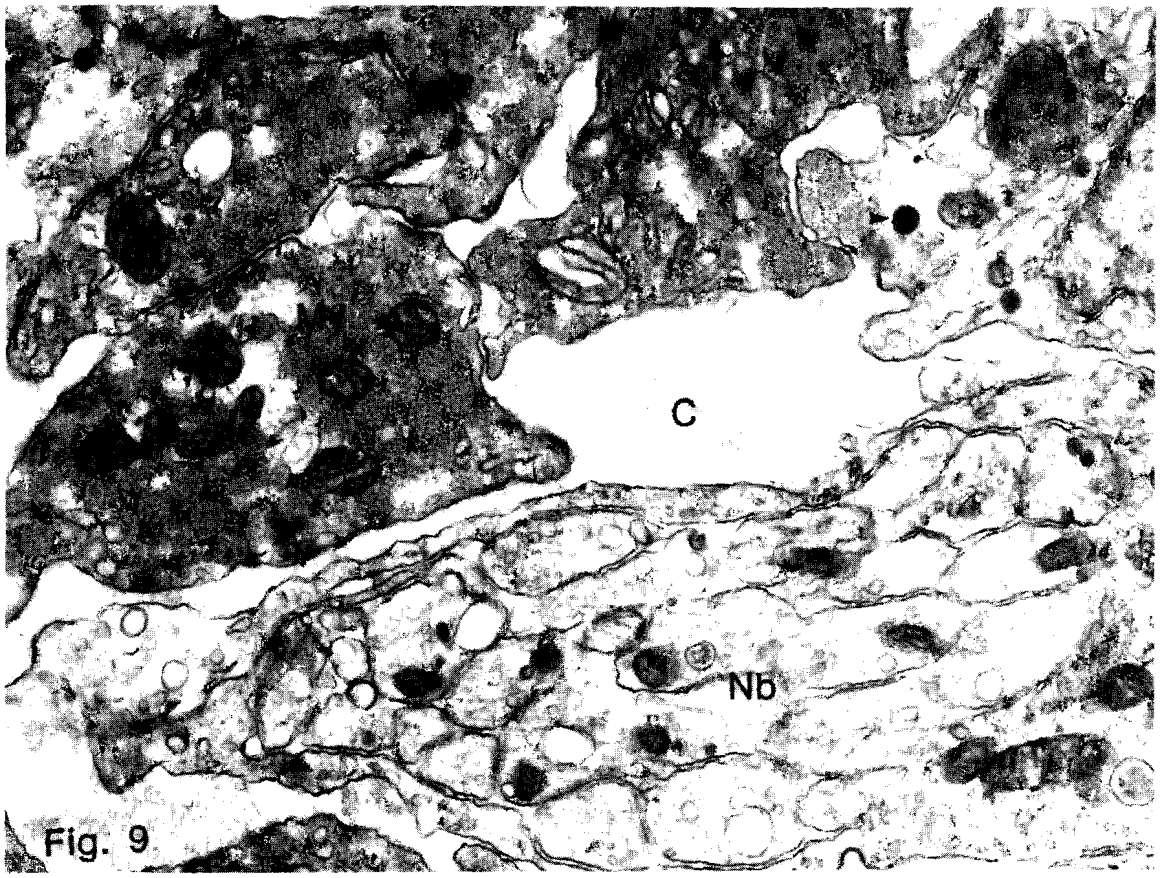

Fig. 9

Fig. 9. Nerve bundle (Nb) adjacent to atrial myocytes (My). Granules (small arrowhead) and collagen fibers (C). × 21,000

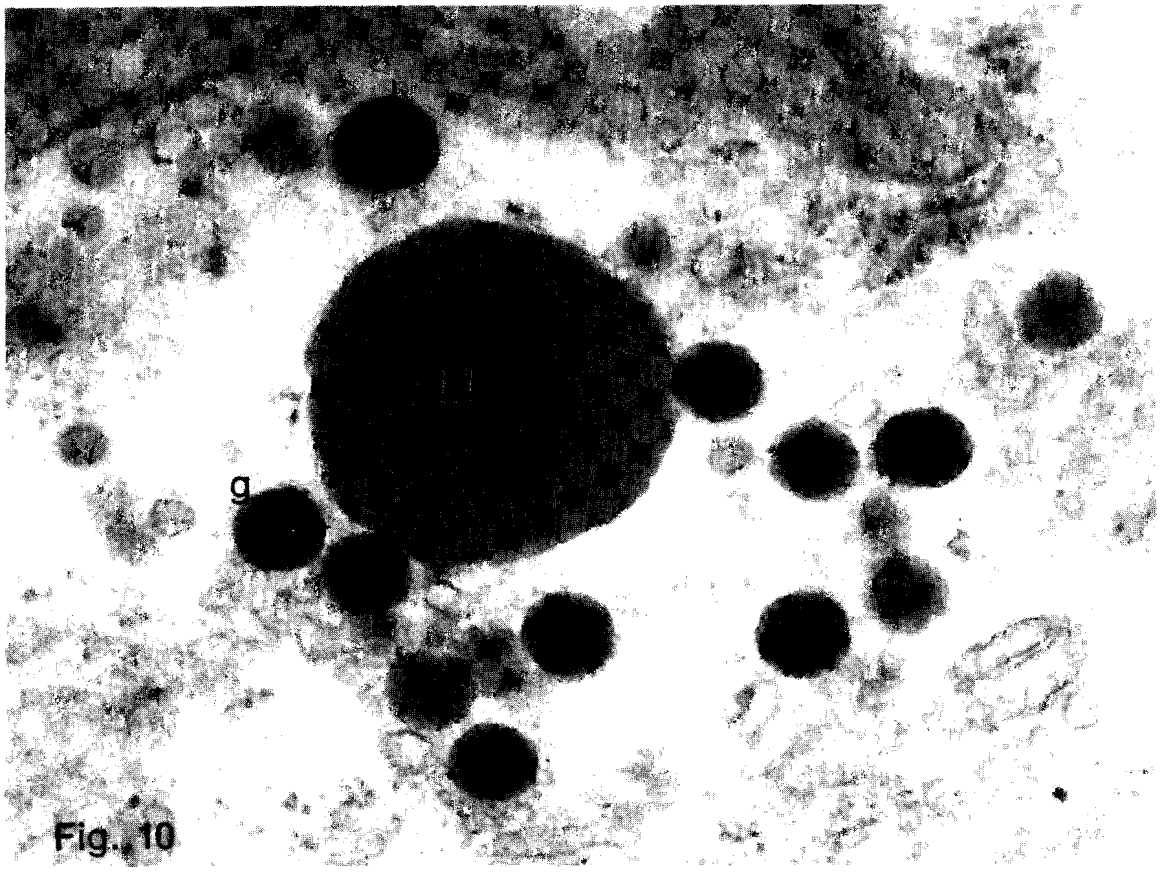

Fig. 10

Fig. 10. Atrial granules (g) relative to a lipid droplet (L). × 57,700

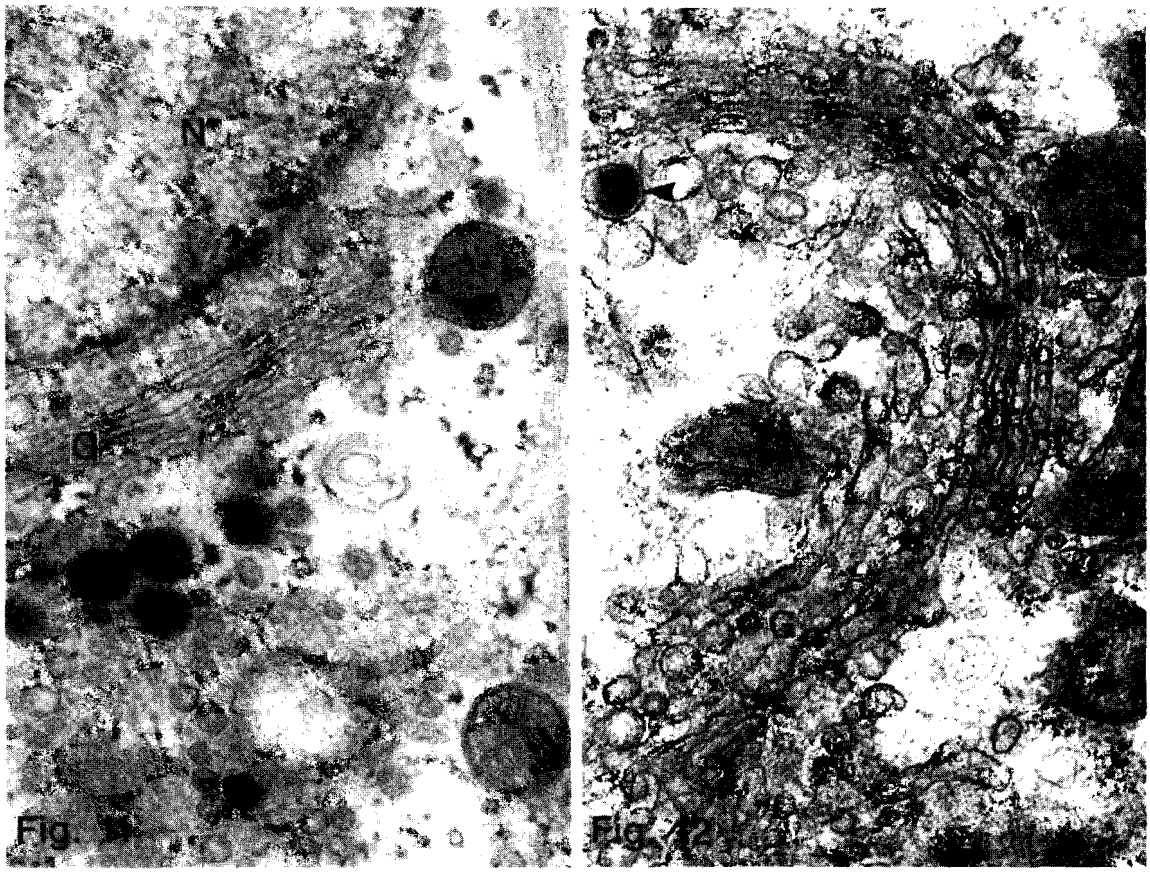

Fig. 11. Atrial nuclear pole demonstrating a well-developed Golgi complex (G) with adjacent specific granules (arrowhead). Nucleus (N). × 19,250

Fig. 12. Prominent Golgi apparatus (G) of a ventricular myocyte with specific granules (arrowhead) adjacent to cisternae of maturing face. × 52,500

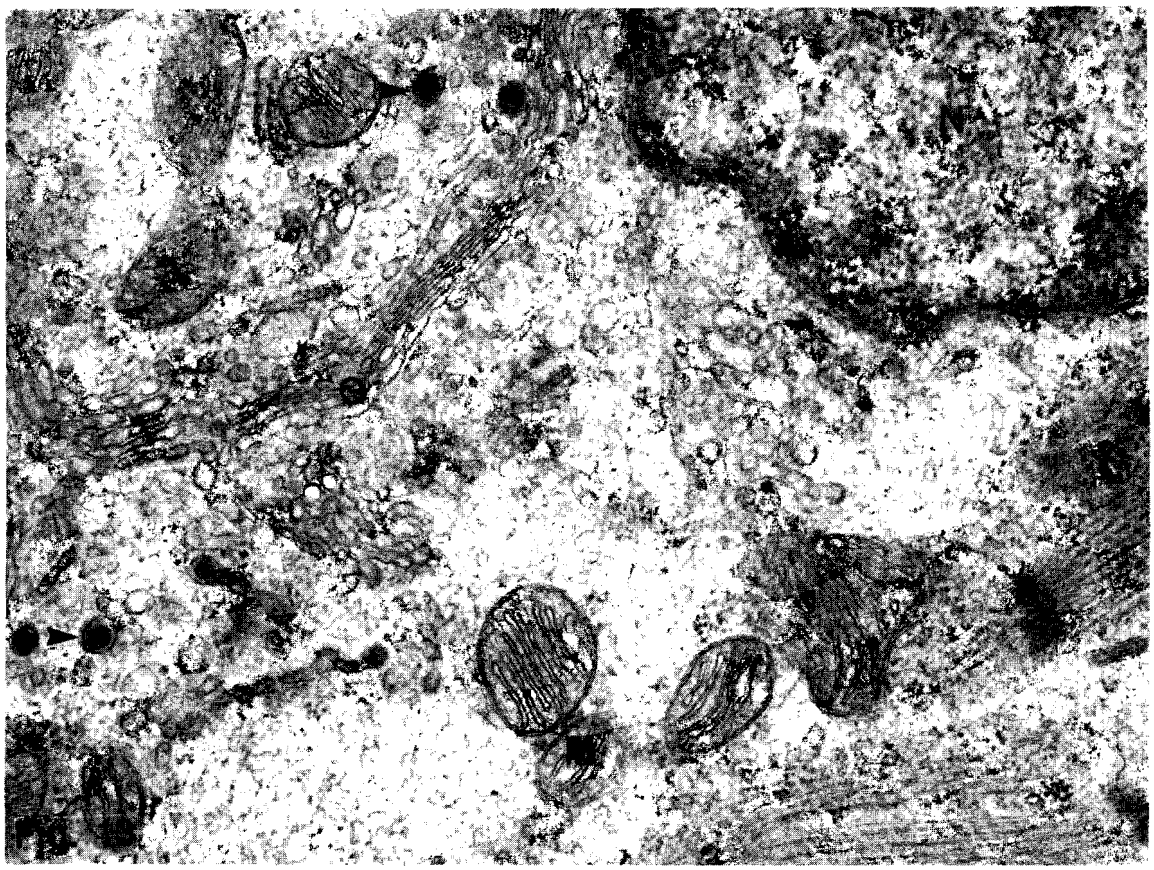

Fig. 13. Nuclear pole of ventricular myocyte displaying an extensive Golgi complex (G) with granules (arrowhead). × 35,500

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