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The Morphological Study of Mitochondria in Rat Cardiac Ganglion Cell

Chun-Hsiang Chen*, Cecilia Hsuan Day**, Kuei-Yu Hsueh***, Jui-Chang Chung****, Tseng-Jung Liu*****

Abstract

The intramitochondrial inclusion was found in the nervous tissue of the central nervous system and the peripheral nervous system of the vertebrate. During our serial studies of the autonomic nervous system from different strains of rat, we found intramitochondrial microcylinder located in the principal neuron of Long-Evans rat's cardiac ganglia. In the enlarged intracristal space of mitochondria, various numbers of intramitochondrial microcylinder were aggregated and parallel arranged. The diameter of hexagonal shaped microcylinder is approximately 25-28 nm, its length is 760-2300 nm. The function and significance of the intramitochondrial microcylinder in the mitochondria in principal neuron of cardiac ganglia are unknown and need further investigation.

Keywords:Intramitochondrial microcylinder, principal neuron, cardiac ganglia, Long-Evans rat

* Instructor, Department ofNursing ,Meiho Institute of Technology
** Instructor, Department of Nursing ,Meiho Institute of Technology
*** Instructor, Department of Nursing ,Meiho Institute of Technology
**** President, Guo-Ren Hospital, Pingtung city, Taiwan.
***** Associate professor, Department of Nursing ,Meiho Institute of Technology
Corresponding author: Tseng-Jung Liu. E-mail: x2009@meiho.edu.tw

Introduction

In the nervous tissue of the mammalian central nervous system, the mitochondrial inclusions have been found in the astrocytes of albino rat, hamster (Blinzinger et al., 1965), cat, dog and monkey (Duncan and Morales 1973), and the pinealocytes of Sprague-Dawley rat (S-D rat) (Heidbhchel 1982)and Long-Evans rat (L-E rat)(Lin 1965). In the peripheral nervous system, the mitochondrial inclusions were found only in the spinal ganglion neurons of Wistar rat (Mugnani 1964). However, till now, no intramitochondrial inclusion was reported in the nervous tissue of the autonomic nervous system.

Recently, in our serial studies of the nervous tissue of the autonomic nervous system, it was found that there were distinct differences of immunocytochemical reactions in the cardiac ganglia of different animals. Under the electron microscopic observations, we also found some ultrastructural differences in the cardiac ganglia between the different strains of rats. In this report, we described the appearance of intramitochondrial inclusions in principal neurons of cardiac ganglia of L-E rats but not in that of S-D rats. This is an original report of the intramitochondrial inclusion observed in the neuron of the autonomic nervous system.

Materials and Methods

In this study, 6 adult S-D rats and 6 adult L-E rats (b.w. 350-400 gm) were used. All animals were anesthetized by intraperitoneal injection of sodium pentobarbitone (Nembutal, 30 mg/kg b.w.). After the chest was opened, the animal was perfused through the heart with a solution consisting of 2% (v/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 0.1M sodium phosphate buffer at room temperature and pH 7.35. Blocks were then taken from the interatrial septum and the posterior walls of the atria of animal's heart. The blocks were then postfixed in 1% osmium tetroxide, dehydrated in ethanol, pass through the propylene oxide, and embedded in the Epikote. Cardiac ganglia were identified in thick plastic sections stained with toluidine blue (1%). Thin sections from these ganglia were cut by the ultramicrotome (Ultracut), stained with lead citrate and uranyl acetate, and observed in a JEOL2000EXII electron microscope.

Results

1. General structure of the cardiac ganglia

In the light microscopic observation, several groups of the cardiac ganglia were

foundin the posterior wall of both atrium, the interatrial septum, and coronary sulcus of the heart. Every cardiac ganglion of the animals examined showed apparently normal morphology of neuronal and non-neuronal structures (Figure 1), which were encapsulated by a thin layer of capsule. Thick section of cardiac ganglia viewed by light-microscopy also showed normal general structures of cardiac principal neurons, small granular cells, satellite cells, nerve fibers, and capillaries (Figure 2).

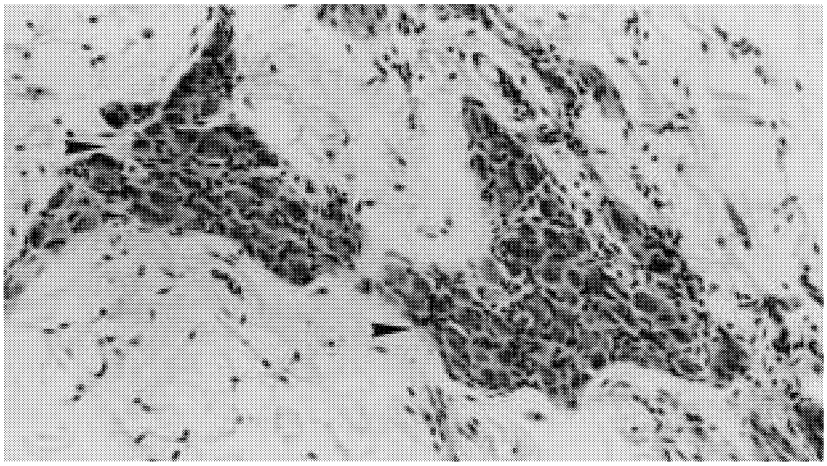

Figure 1 The light microscopic photograph shows two groups of cardiac ganglia (arrowhead) in the interatrial septum of 24-months-old L-E rat. H.E. stain. (225X)

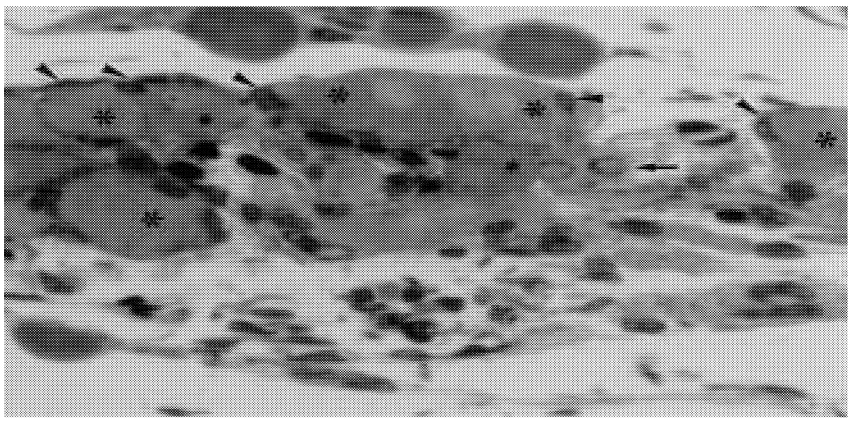

Figure 2 The photograph of thick section of cardiac ganglia shows the principal neuron (star), satellite cell (arrowhead), small granular cell (arrow) and capillary. (Toluidine blue stain, 1120X)

2. Occurrence of microcylinder-bearing mitochondria in the cardiac ganglia

Under the electron microscope, in the cardiac ganglia of L-E rat, the characteristic intramitochondrial microcylinders were frequently found in the perikaryon of the principal neuron (Figure 3, 4). The intramitochondrial microcylinders were occasionally found in the axon of principal neurons. However,

no microcylinder was found in the mitochondria of satellite cells (Figure 5), small granular cells (Figure 6), preganglionic axon terminals (Figure 7), and endothelial cells of the capillary (Figure 8). In the S-D rat, the microcylinder-bearing mitochondria have never been found in any cells of cardiac ganglia.

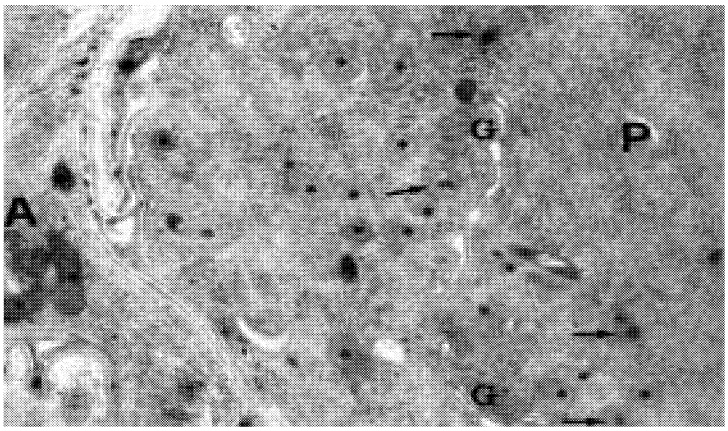

Figure 3 The electron micrograph shows an axon terminal of preganglionic nerve fiber (A) and the cytoplasm of principal neuron (P) in the cardiac ganglia. The cytoplasm of principal neuron is characterized by the presence of microcylinder bearing mitochindria (arrow). G: Golgi complex; star: mitochondria without inclusion. (18000X)

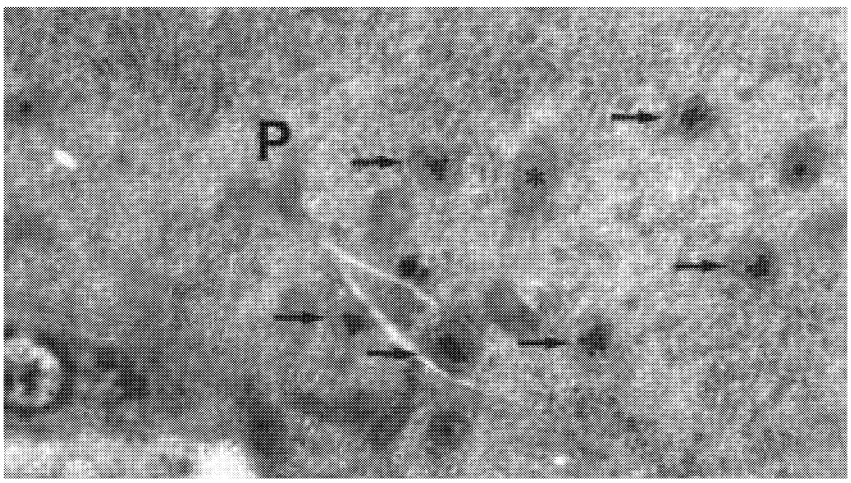

Figure 4 Higher magnification of the electron micrograph of principal neuron in the cardiac ganglia (P). The aggregated microcylinders in the enlarged intracristal space of mitochondria (arrow) and some mitochondria without inclusion (star) distribute in the cytoplasm of principal neuron. (24000X)

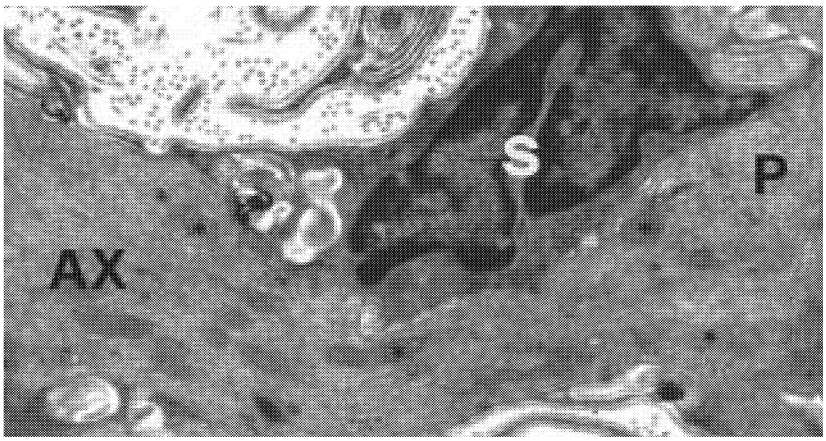

Figure 5 The electron micrograph shows the axonal process (AX) extended from the cell body of the principal neuron (P) which is surrounded by the satellite cell (S). No intramitochondrial inclusion is observed in the mitochondria of principal neuron (star) and satellite cell (m). (24000X)

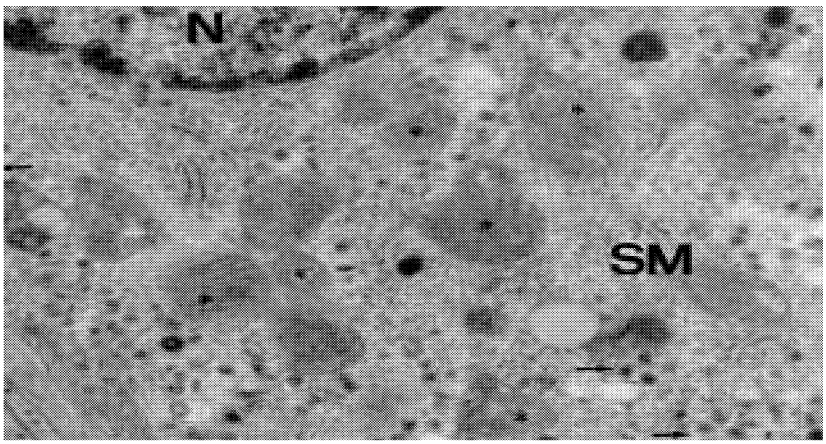

Figure 6 The electron micrograph shows that in the cytoplasm of small granular cell (SM), numerous dense-core granules (arrow), rough endoplasmic reticulum, mitochondria (star) and lipid droplet are observed around the nucleus (N). No intramitochondrial inclusion is found in the mitochondria. (18000X)

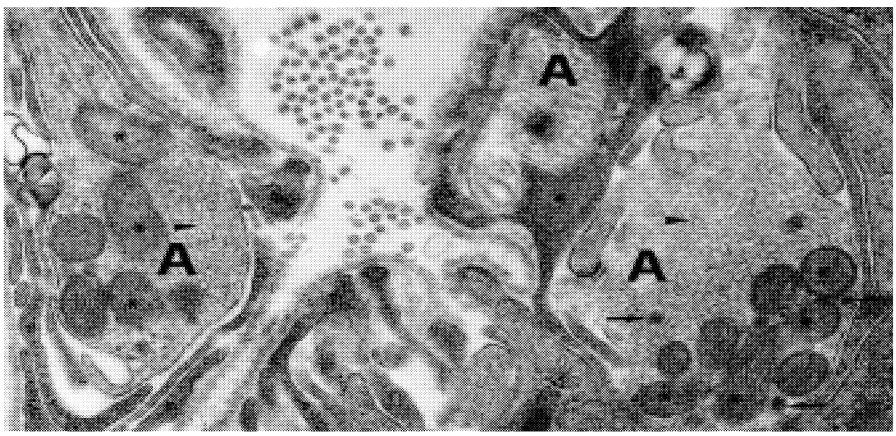

Figure 7 The electron micrograph of the axon terminal of preganglionic nerve fiber (A) shows numerous clear vesicles (arrowhead), dense-core vesicles (arrow) and mitochondria (star) are observed. Whereas no intramitochondril inclusion is observed in the mitochondria. P: principal neuron of cardiac ganglia. (36000X)

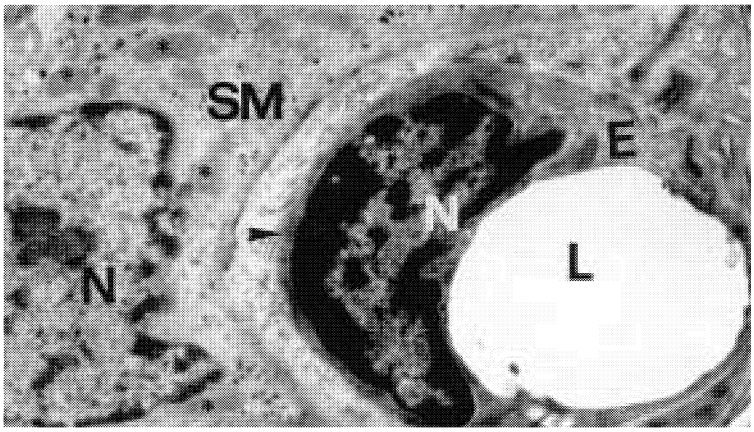

Figure 8 The electron micrograph of small granular cell (SM) and capillary (arrowhead) in the cardiac ganglia. The mitochondria (star) in the small granular cell and endothelial cell (E) are without inclusion. N: nucleus. (18000X)

3. Localization and number of microcylinders in mitochondria

In the cytoplasm of principal neuron, the size of mitochondria bearing the microcylinders was generally slightly larger than those without the microcylinders (Figure 4, 9). In those mitochondria, one, two or more dilated intracristal spaces were filled with parallel arrays of microcylinders (Figure 9-13). Simultaneously, the localization of the microcylinders in mitochondria was exclusively within the outer compartment. In the outer compartment of mitochondria, the microcylinder tended to be much more common and abundant in dilated intracristal spaces than in the space between the outer and inner membranes of the mitochondrial surface (Figure 10, 12, 13). The intramitochondrial microcylinders occurred in numbers varying from 1 to 10 or more per cut profile of the dilated intracristal space (Figure 10-14) The number of microcylinders was closely related to the extent of dilatation of the crista (Figure 10, 13,15).

4. Morphological characteristics and dimension of intramitochondria microcylinders

In sections cut parallel with the long axis of the microcylinder, profile of the microcylinder often appeared in a paralleled straight-lines or twilled oblique-line pattens (Figure 12, 15). When the section was cut transversely, the microcylinders appeared as hexagonal rings of approximately 25 – 28 nm in diameter and was composed of six peripheral subunits subunits surrounding a central one (Figure 10). The length of microcylinders varied from 760 –2300 nm.

Though in the dilated intracristal space, groups of microcylinders were monolayered or multiplayer parallel arranged in a regular array with a nearly constant center-to-center distance (Figure 10,15). However, groups of microcylinders in the enlarged crista are not always parallel arranged, the perpendicular arranged intramitochondrial microcylinders are also can be found in some mitochondria (Figure 11, 12).

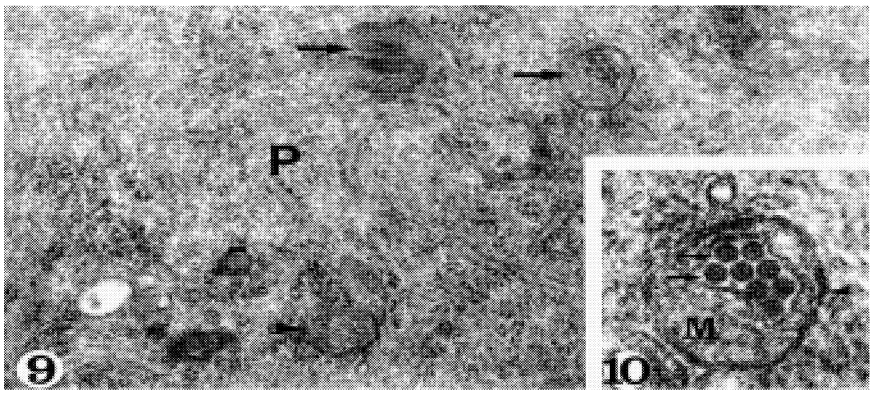

Figure 9 The electron micrograph shows the microcylinder bearing mitochondria (arrow) and mitochondria without inclusion (arrowhead) are observed in the cytoplasm of principal neuron of cardiac ganglia (P). (36000X)

Figure 10 Higher magnified electron micrograph show the cross sectioned hexagonal array of microcylinder (arrow) in the dilated intracristal space of mitochondria (M). The regular hexagonal array of microcylinder is made up of 6 peripheral subunits and a single central one. Arrowhead indicates the outer membrane of mitochondria. (100000X)

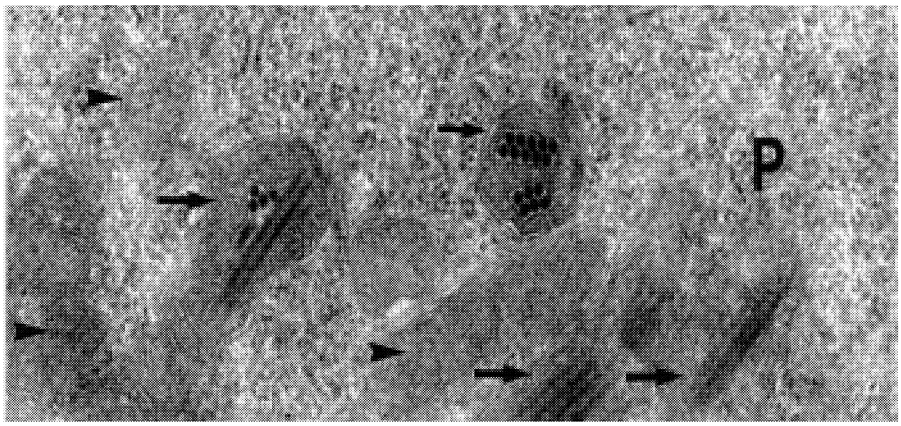

Figure 11 The electron micrograph shows different pattern of aggregated microcylinder in microcylinders are sectioned in various planes to the long axis of mitochondria. Arrowhead indicated the mitochondria without inclusion. (36000X)

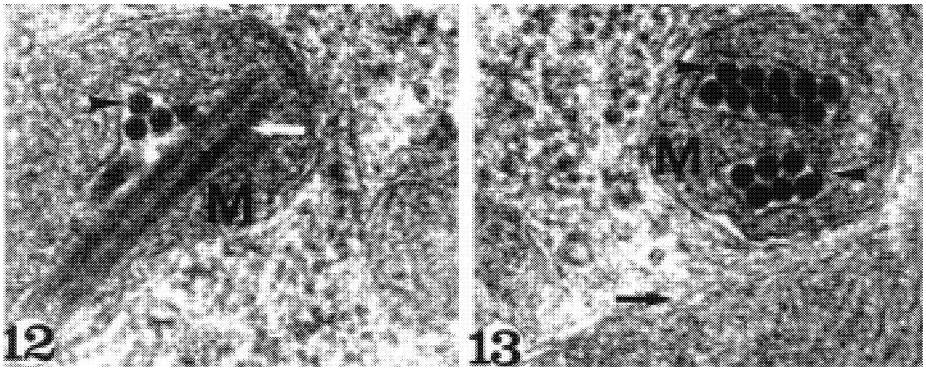

Figure 12 The electron micrograph shows that in the enlarged intracristal space of mitochondria (M), both the cross sectioned (arrowhead), longitudinal and oblique sectioned (arrow) microcylinders are observed. (100000X)

Figure 13 Higher magnified electron micrograph of the mitochondria (M) shows that there are two groups of parallel aggregated microcylinders (arrowhead) in the dilated intracristal space. The number of microcylinders in each intracristal space are varied and forming multilayered aggregates. Arrow indicates another mitochondria. (100000X)

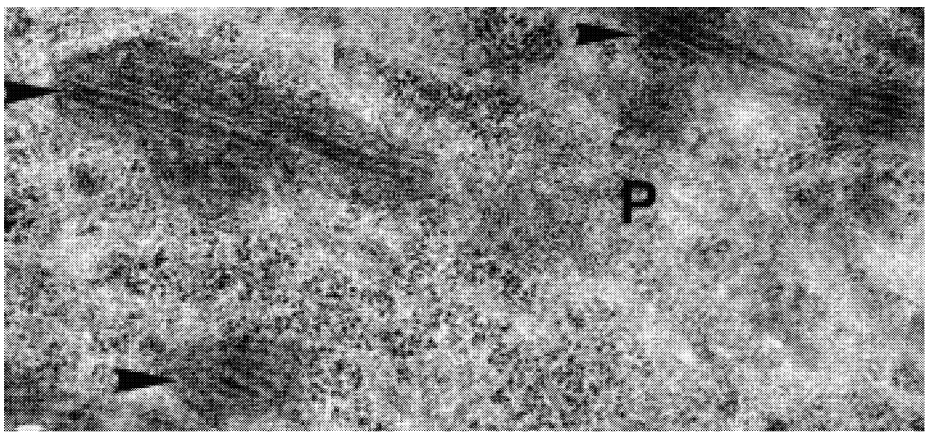

Figure 14 The electron micrograph shows several large mitochondria with intramitochondrial microcyliners (arrowhead) locate in the cytoplasm of principal neuron (P) of cardiac ganglia. In the dilated intracristal space, various number of longitudinal sectioned microcylinders parallel arranged (open arrow). The arrow indicates 4 parallel aggregated microcylinders in the enlarged intracristal space of a mitochondria. (36000X)

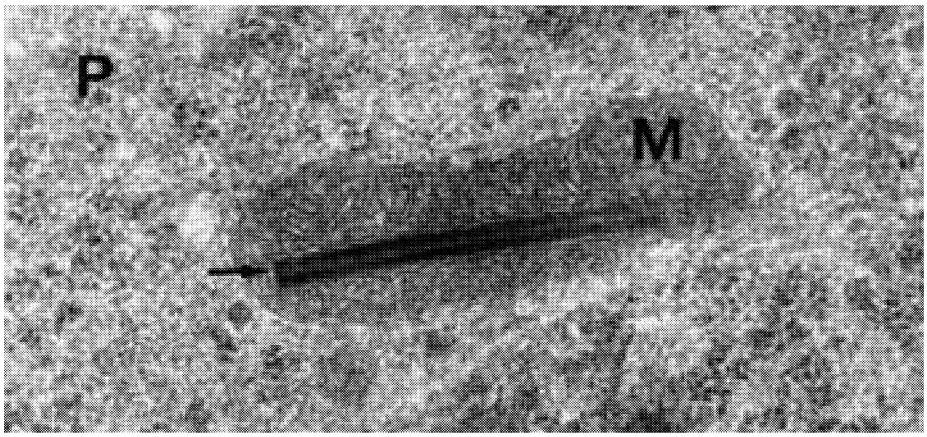

Figure 15 The electron micrograph presents a large microcylinder-containing mitochondrion (M) in the cytoplasm neuron (P). There are two longitudinal sectioned parallel microcylinders (arrow) in the intracristal space. (54000X)

Discussion

In mammals, several groups of cardiac ganglia were found in the posterior wall of atrium, the interatrial septum and coronary sulcus of the heart. The localization of the cardiac ganglia observed in the adult L-E rat and S-D rat of this report is similar to that of our previous publication (Liu et al., 1984).

Under the electron microscopic observations, the intramitochondrial microcylinder was found in the principal neuron of cardiac ganglia of L-E rat but not found in that of S-D rat. The presence of intramitochondrial inclusions have been reported in the thyroid follicular cell of the rat (Fujita and Machino 1964) and human (Matias et al., 1991); seromucous secretory cell of the long-haired fruit bat (Tandler and Phillips 1993); astrocyte of the dog, cat, monkey (Duncan and Morales 1973), hamster (Blinzinger et al., 1965) and Wistar rat (Mugnaini 1964); cell of the renal tubules of the Wistar rat (William 1967), snake (Kurosumi et al., 1966), S-D rat (Suzuki and Mostfofi 1967; Sasaki and Suzuki, 1989), and L-E rat (Ouyang and Lin 1991); duct cell of the salivary gland of S-D rat (Hand AR 1970); the pinealocyte of S-D rat (Heidbuchel 1982) and L-E rat (Lin 1965); the adrenocortical cell of the bovine (Kai et al., 1977) and S-D rat (Saito and Fleischer 1971); the liver cell of the human (Willis 1965) and S-D rat (Sasaki et al., 1990); the skeletal muscle cell of human (Bonilla et al., 1980, Marbini et al., 1987); the oncocytic adenoma of thyroid gland of human (David and Kim 1983); the cybrid cell lines of human Parkinson’s and Alzheimer’s diseases (Trimmer et al., 2000); the plasma cell in human multiple myeloma disease (Kurabayashi et al., 1991); the corpus lutem cell

of the pregnant bovine (Takahana et al., 1997); the colonic neuron of the postoperative delirium patient (Hashimoto et al., 1997).

It was reported by Sasaki and Suzuki (1989) that three types of intramitochondrial inclusions i.e. intramitochondrial filamentous bodies (IMFB), were found in the matrix of mitochondria of the medullary tubules of normal rat kidneys, and many helical filaments and the three types of IMFB were often found independently in different mitochondria. Whereas, in our observations of cardiac ganglia, only one type of intramitochondrial inclusion was found in the mitochondria of principal neurons of the L-E rat.

In the cytoplasmic matrix of the principal neuron of cardiac ganglia of L-E rat, the size of mitochondria bearing the microcylinder was generally slightly larger than those without that. Dilated intracristal spaces were filled with monolayered or multilayered parallel aggregate of the microcylinders. In each dilated intracristal space, the number of microcylinders varying from 1 to 10 or more, which was closely related to the extent of dilatation of the crista. Similar structure of intramitochondrial microcylinders was reported first in the pinealocyte of L-E rat by Lin (1965), however, in his observation, the co-existence of glycogen-like particles and microcylinders within the crista of mitochondria in the pinealocyte, was not observed in the principal neurons of the same strain rat's cardiac ganglia. The difference between Lin's (1965) and our results may be explained by Sasaki and Suzuki (1989), that the intramitochondrial inclusions are not only animal specific but also organ unique.

The intramitochondrial microcylinder is about 25 – 28 nm in diameter and of various lengths, and is composed of six peripheral subunits subunits surrounding a central one. Our observations were different from those reported in the mitochondria or proximal tubules of human (Sasaki and Suzuki 1989). In their report, the intramitochondrial inclusion of helical filaments was 4nm in diameter, with a helix of 18nm width and 18nm pitch.

As to the component of the intramitochondrial inclusions, in the report of Sasaki and Suzuki (1989), the cytochrome oxidase activity was negative in IMFB and helical filaments themselves but positive in all the mitochondrial cristae including longitudinally arranged and prismatic ones. Digestion tests by DNase and RNase were both negative in both IMFB and helical filaments in the mitochondria. They estimated that the helical filaments in hepatic mitochondria consisted mainly of lipid or phospholipid. However, Ouyang and Lin (1991) showed a positive histocytochemistry reaction of cytochrome oxidase and monoamine oxidase appeared on intramitochondrial microcylinders of the L-E rat. They concluded that the chemical composition of microcylinders were proteinaceous. According to the

paper of Sasaki and Suzuki (1989) it was possible that all intramitochondrial inclusions found in the various cell types of different animals not only have the same morphology but also have the same material composition and significance. So it can be suggested that the intramitochondrial microcylinder in the principal neuron of L-E rat's cardiac ganglia was also composed by proteinaceous substance as the conclusion of Ouyang and Lin (1991).

The origin, nature and significance of these intramitochondrial inclusions are not known. It was reported that the intromitochondrial inclusion represented abnormal metabolic function, such as disorders of intramitochondrial protein synthesis (Herrea-Goepfert et al., 1986) or resulted from the environmental lead poisoning (George et al., 1983). Suzuki and Mostofi (1967) observed the intramitochondrial inclusions not only in pathologic conditions but in control animals as well. They suggested, however, that the appearance of IMFB does not necessarily indicate some pathologic phenomenon. The intramitochondrial inclusions should be considered as a variant of normal mitochondrial structure (Willis 1965). Their presence in some cells but not in others may be a reflection of a functional heterogeneity among the cells within the same structure (Willis 1965).

Because the intramitochondrial microcylinders are apparently not observed in the principal neuron of cardiac ganglia of other strains of rat, it seems likely that they are not intrinsic components of the cardiac ganglia, and they may represent proteinaceous products of an unusual mitohcondrial activity. Simultaneously, it seems unlikely that the microcylinders are functionally analogous with both the microtubules and the flagellar fibrils (Lin 1965).

It is a reasonable suggestion that the intramitochondrial microcylinder are macromolecules of a material, probably of protein nature, synthesized under normal conditions within the principal neuron's mitochondria of the cardiac ganglia of L-E rat.

As to the function and significance of the presence of intramitochondrial microcylinders in the principal neuron reported here remain unknown and need further investigations.

Acknowledgements

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大白鼠心臟神經節細胞粒線體之型態學研究

陳春香*、戴瑄**、謝桂鈺***、鍾瑞嶂****、劉增容*****

摘要

許多不同形式之粒線體之內含體均曾在中樞神經系統及週邊神經系統之脊髓神經節的神經元被發現。迄今，在自律神經系統之神經組織內尚未有任何關於粒線體之內含體存在的研究。在我們對不同種的鼠類自律神經系統連續性地研究探索中，我們發現只有在 L-E 大白鼠〈Long-Evans rat〉 的心臟神經節之主要神經元內粒線體的擴大內嵴空間〈intracristal spaces〉常可發現典型的微柱。在這些粒線體，其擴大內嵴空間有不同數目且平行之微柱；在微柱之橫切片呈現直徑 25-28nm 之規則六角環狀；其長度爲 760-2300nm。關於心臟神經節主要神經元內之粒線體微柱與粒線體之關係，甚至與主要神經元之間的關係有待進一步研究探索。

關鍵詞：粒線體內微柱、主要神經元、心臟神經節、L-E 大白鼠。

*美和技術學院護理系講師

**美和技術學院護理系講師

***美和技術學院護理系講師

** **屏東國仁醫院院長

*****美和技術學院護理系副教授、

通訊作者：劉增容，x2009@meiho.edu.tw