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Node-like cells in the myocardial layer of the pulmonary vein of rats: an ultrastructural study

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INTRODUCTION

It is well known that a layer of striated muscle consistently occurs in the pulmonary vein in all the 50 or more species of mammals already examined. The extent of the myocardial envelopment varies; it is confined to the extrapulmonary portion of the vein in most mammals including man, and extends to the intrapulmonary region in rodents such as the rat (Takino, 1933; Mukai, 1941; Kramer & Marks, 1965; Nathan & Gloobe, 1970). Light and electron microscopical studies have shown structural similarities between striated muscle in the vein and atrial cardiac muscle (Karrer, 1959, 1960; Policard, Collet & Pregermain, 1959; Heppleston, 1961; Klavins, 1963; Ludatscher, 1968; Spach, Barr & Jewett, 1972; De Almeida, Böhm, Carvalho & De Carvalho, 1975). This similarity is supported by the physiopharmacological responses of the striated muscle cells in the vein to electrical stimulation and to a variety of drugs (Spach *et al.* 1972; De Almeida *et al.* 1975; MacLeod & Hunter, 1967).

Several possible functional roles of the myocardial layer in the regulation of the pulmonary circulation have been proposed (Karrer, 1959). One of these is a valve-like action preventing regurgitation of atrial blood into the pulmonary vein, and another is a milking action to enhance the venous return into the left atrium. It is relevant that in the late 19th century, Brunton & Fayrer (1874) observed the independent pulsation of the pulmonary vein after all motion had ceased in the cardiac cavities of rabbits and cats. This early finding has recently been confirmed in a study of the myocardial cells of the pulmonary vein of the guinea-pig by using electrophysiological intracellular recording techniques (Cheung, 1980). These findings suggest that the pulmonary vein might be capable of an independent pacemaking activity.

The present study was undertaken to re-examine the ultrastructure of the myocardial layer in the pulmonary vein of rats and a structural basis for a generator of independent pacemaking activity was sought. Clear cells possessing every feature of the fine structure of sinus node pacemaker cells could be demonstrated. No other morphological report appears to be available concerning the occurrence of this type of cell in the venous myocardial layer.

MATERIALS AND METHODS

Ten adult male rats (Wistar strain) weighing about 250 g were fixed under nembutal anaesthesia by transcardiac perfusion with 500 ml of 2·5 % glutaraldehyde in 0·05 M cacodylate or phosphate buffer, pH 7·4. After perfusion, portions of the

intrapulmonary veins running from the pulmonary hilus into the middle lobe of the left lung were dissected out together with the surrounding pulmonary tissues. The tissues were cut into small pieces transversely along the vessels and immersed in the same fixative for an additional hour. The tissue blocks were then postfixed with 1% osmium tetroxide for 1 hour. After dehydration in a graded series of ethanol, the tissue blocks were embedded in Araldite. Sections were cut using a Porter–Blum type I microtome. Thick sections, 0·5–1 μm in thickness, were stained with 1% toluidine blue for tissue orientation under the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HU-125DS electron microscope at an accelerating voltage of 100 kV.

RESULTS

Structure of the pulmonary venous wall

Intrapulmonary vein walls of the rat were composed of three layers: the tunica intima, tunica media and tunica adventitia. The tunica intima consisted of endothelial cells and subendothelial connective tissue. This connective tissue was sometimes lacking, and the endothelial cells might then directly appose the smooth muscle cells. The tunica media comprised an inner smooth muscle layer and an outer myocardial layer with an intervening connective tissue layer. Smooth muscles were arranged in a circle around the vein. In the hilar portion of the vein, the smooth muscle cells were in a single layer or were occasionally even lacking, but in smaller veins they were thicker and sometimes formed cushions, especially in the branching portion.

The myocardial layer was 5–8 cells thick near the pulmonary hilus, where myocardial cells tended to form internal circular and external longitudinal bundles.Thin connective tissue spaces intervened among the muscle cells. The myocardial layer became thinner as the vessels penetrated more deeply into the lung, branching repeatedly. It eventually disappeared where the diameter of the vessels was reduced to 100–150 μm.

The tunica adventitia was composed of loose connective tissue with some capillaries and lymphatics. All these layers of the pulmonary vein were plentifully supplied by vasa vasorum. Especially in the myocardial layer, there were numerous capillaries and small arterioles among the muscle cells.

Unmyelinated nerve fibres and terminals enveloped by Schwann cells were common in the myocardial layer and were occasionally present in the connective tissue between the smooth muscle layer and the myocardial layer. Most of the nerve fibres in the

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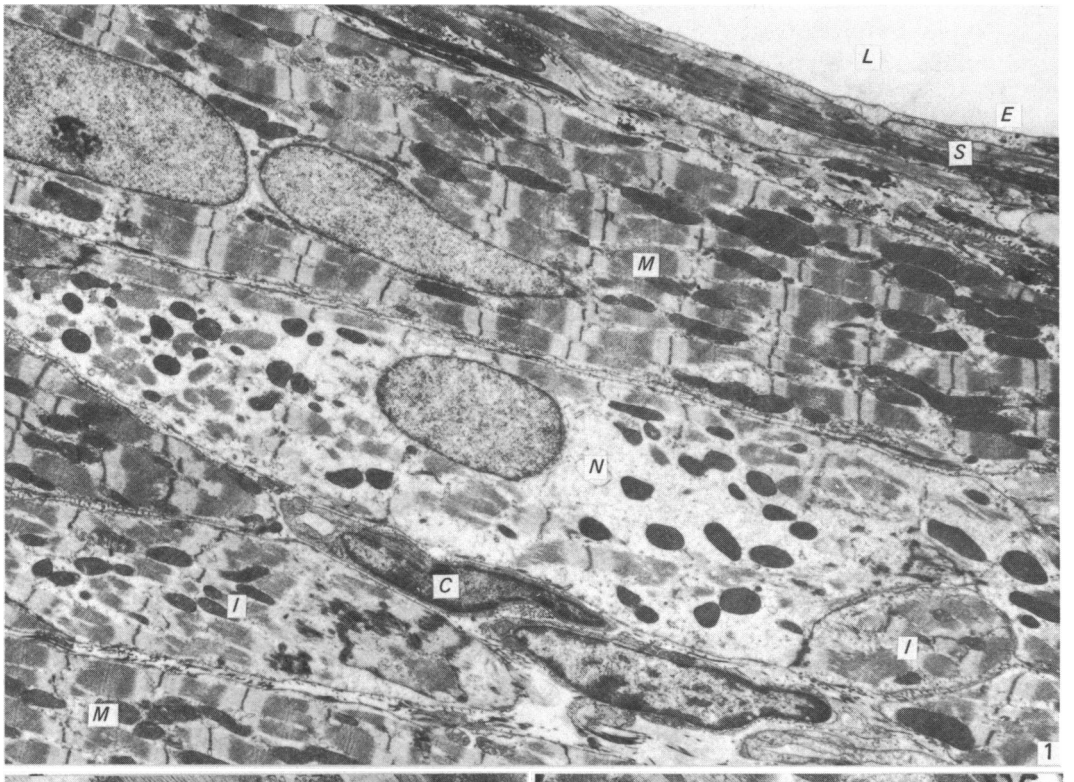
Fig. 1. A clear, node-like cell (*N*) interposed among ordinary myocardial cells (*M*) comprising the pulmonary venous wall. The node-like cell can be easily recognised by its clear cytoplasm with a paucity of myofibrils and round or oval mitochondria, in contrast to ordinary myocardial cells. Some of the myocardial cells show an intermediate feature of myofibrillar content (*I*). *C*, capillary within the myocardial layer; *E*, endothelial cells of the pulmonary vein; *S*, smooth muscle layer; *L*, lumen of the pulmonary vein. × 6000.

Fig. 2. Longitudinal section of ordinary myocardial cells of the pulmonary venous wall. Myofibrils are well developed and regularly arranged. Elongated mitochondria are arranged along the myofibrils. Note the typical intercalated disc (*) composed of fasciae and maculae adherentes in the place of Z bands, and gap junctions (arrows) at side-to-side junctional sites. × 17000.

Fig. 3. Atrial specific granules in the paranuclear zone and the intermyofibrillar spaces of the ordinary myocardial cell in the pulmonary vein. Note the conspicuous variety in granule size and density. × 14000.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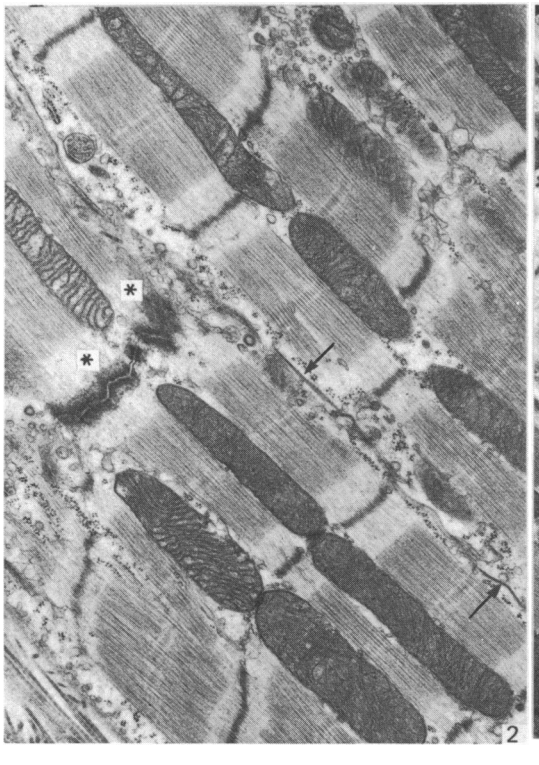
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myocardial layer were in close proximity to capillaries and small arterioles. They varied in thickness, 0·2–1·0 μm, and contained small and large vesicles, respectively about 50 and 100 nm in diameter, with and without a core.

General architecture of the myocardial cell layer

Most of the myocardial cells in the pulmonary vein were cylindrical in shape, 7–18 μm in the largest diameter; a round or oval nucleus with prominent nucleoli was located in the cell centre. Their cytoplasm was filled with dense masses of myofibrils composed of both thick and thin filaments. The myofibrils were 0·5–1·3 μm in thickness and most of them were arranged parallel to each other. The myofibrils appeared to be in a relaxed state and the A, I, H and Z bands and M line were clearly seen (Figs. 1, 2).

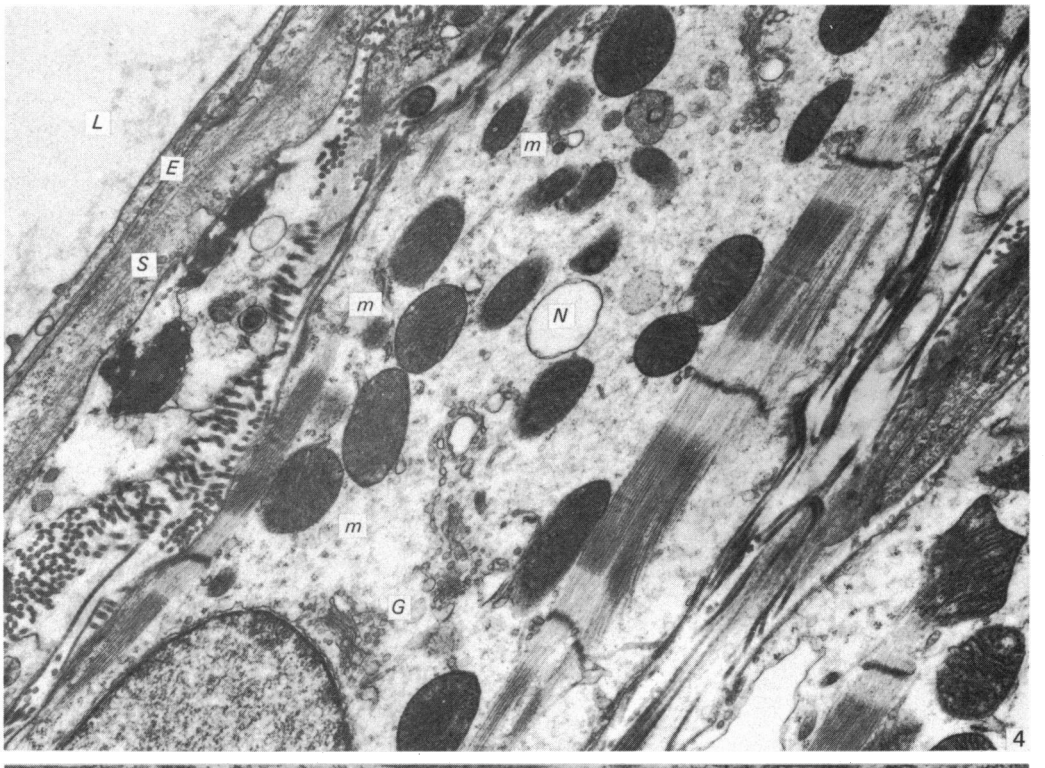
There were abundant mitochondria in the interfibrillar space, the perinuclear regions and the subsarcolemmal space. They were elongated in shape with numerous transversely orientated cristae. Subsurface vesicles and sarcoplasmic reticulum were well developed and glycogen granules were dispersed in the cytoplasm among the mitochondria. Membrane-bound granules were present near the Golgi apparatus (Fig. 3). They varied in size, ranging from 200 to 400 nm in diameter, and had a more or less electron-dense core surrounded by a thin submembranous halo. These features were similar to those of the atrial specific granules. Fasciae and maculae adherentes and gap junctions were formed at the end-to-end junctional sites between adjacent myocardial cells, representing intercalated discs, and gap junctions were formed at the side-to-side junctional sites (Fig. 2).

Occurrence and histological characteristics of clear muscle cells

Although hardly discernible by light microscopy, examination with the electron microscope demonstrated a population of clear muscle cells, different from ordinary myocardial cells, in parts of the veins which contained 2–4 myocardial cell layers; they were never found in portions close to the pulmonary hilus or in extra-pulmonary portions possessing a thicker myocardial layer (Fig. 1). The clear cells appeared either singly or as a small group of cells, interposed among ordinary myocardial cells. Sometimes they attached to each other, but bundles of clear cells joining with left atrial musculature could not be found. The clear muscle cells had a slender cytoplasmic outline and the nucleated portion measured 7–12 μm in diameter. The nuclei were oval in shape with one or two prominent nucleoli and contained dispersed chromatin. Myofilaments were much more scanty than in ordinary myocardial cells (Figs. 1, 4). Most of them were arranged in bundles corresponding to ordinary myofibrils and A, I and Z bands were clearly seen in the bundles. Each myofilament bundle, however, was thinner and shorter than the

Fig. 4. High magnification electron micrograph of a node-like cell (*N*) in the myocardial layer of the pulmonary vein. Note the few myofibrils, small, oval and randomly distributed mitochondria and wide cytoplasmic matrix filled with a flocculent substance. Mitochondria having longitudinal, parallel cristae are frequently seen (*m*). *E*, endothelium of the pulmonary vein; *G*, poorly developed Golgi apparatus; *L*, lumen of the vein; *S*, smooth muscle layer of the vein. × 16500.

Fig. 5. A portion of a node-like cell (*N*) with adjacent ordinary type muscle cells (*M*). Myofibrils in the node-like cell are irregularly arranged, and different aspects of myofibrils cut longitudinally (*l*), obliquely (*o*) and transversely (*c*) may appear in a single section of the cell. Note the end-to-end junctional specialisation with the ordinary myocardial cell, which is cut obliquely(*). × 19000.



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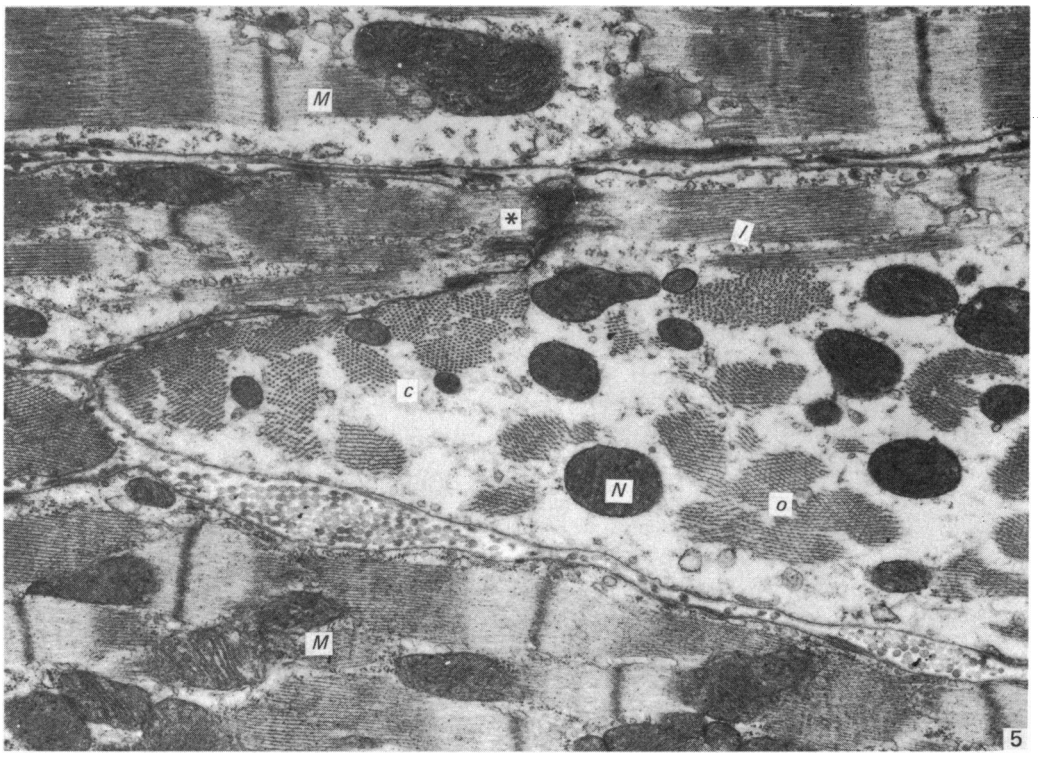

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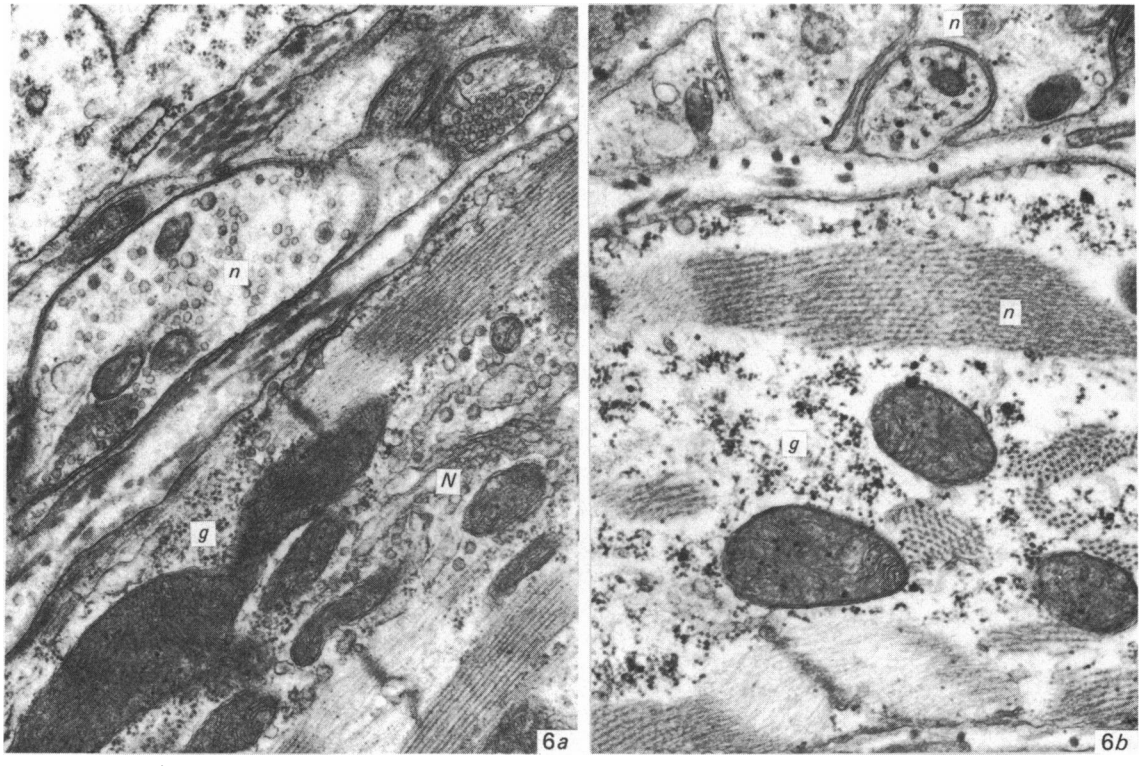

Fig. 6(*a–b*). Nerve fibres (*n*) containing small clear vesicles (6*a*) and small cored vesicles (6*b*) in close apposition to the node-like cell (*n*) with a narrow intervening space. *g*, glycogen granules. ×32000.

ordinary myofibrils, and bundles extending over no more than three sarcomeres appeared in a single section. The bundles were arranged irregularly in the cells, and two adjacent bundles often ran at right angles to each other in a single cell (Fig. 5). Some short bundles of myofilaments without any distinct bands might occur in the marginal areas of the cytoplasm. Some clear cells contained increased amounts of rather regularly arranged myofibrils, representing intermediate forms between the typical clear cells and the ordinary atrial cells of the pulmonary vein (Fig. 1).

Abundant small mitochondria were dispersed throughout the cytoplasm. They were round or oval in contrast to the elongated forms seen in the ordinary myocardial cell, and possessed well developed cristae and a moderately dense matrix. Mitochondria with longitudinal, parallel, cristae were frequently encountered. The Golgi apparatus was small and located near the nucleus. No membrane-bound granules similar to atrial specific granules were found in the Golgi area. Surface vesicles and sarcoplasmic reticulum were poorly developed in comparison with the ordinary myocardial cells. The cytoplasmic matrix among the myofilament bundles, mitochondria and the various membranous structures was conspicuously wide and occupied by amorphous, flocculent substances together with glycogen granules (Figs. 4, 6).

End to end junctional specialisations between the clear cells and adjacent ordinary myocardial cells were formed at the tapered, peripheral region of the clear cells. Most parts of the clear cell surface were covered with a basal lamina and apposed to

the interstitial space containing collagen fibrils (Fig. 5). The junctional specialisations ran obliquely to the long axis of the cells. They were composed of small fasciae adherentes and a few maculae adherentes and gap junctions.

Nerve fibres and terminals were located at a distance of more than 200 nm from the clear muscle cells and the ordinary myocardial cells, where an extracellular space and basal laminae of both muscle cells and nerve fibres intervened between the two cellular elements (Fig. 6). No obvious difference in frequency of occurrence of nerve fibres was found between regions around the clear muscle cells and around the ordinary myocardial cells.

DISCUSSION

The present study confirms the close resemblance between the striated muscle of the pulmonary vein and the atrial muscle. This musculature is smaller in diameter, having sparse T tubules and less complicated intercalated discs than ventricular cardiac muscle as has been noted by several authors. The morphological features of membrane-bound granules observed in this study are similar to those of atrial specific granules (Jamieson & Palade, 1964). Atrial-like granules in the myocardial layer of the pulmonary vein have been described only in dogs, where the short myocardial sleeve of the pulmonary vein is limited to its extrapulmonary portion (Spach *et al.* 1972). In mammals, atrial granules are specific to ordinary atrial muscle (Jamieson & Palade, 1964) and the existence of the granules in the pulmonary vein cells again emphasises the similarity of these cells and atrial cells.

In striking contrast to the ordinary myocardial cells forming the outer wall of the pulmonary vein, the clear muscle cells revealed for the first time in the present study are characterised by their paucity of myofilament bundles, irregular disposition of the bundles, small and oval mitochondria with a moderate electron density, absence of atrial specific granules and the ample amounts of sarcoplasm between intracellular organelles. Since the ordinary myocardial cells next to the clear muscle cells exhibit well preserved ultrastructural features, and because mitochondria and other membranous organelles in the clear muscle cells are also preserved well, the differences in the ultrastructure of the clear cells from that of the ordinary myocardial cells are not fixation artifacts.

It was noticed that the ultrastructural characteristics of the clear cells in the myocardial layer of the pulmonary vein are similar to those of the specialised node cells in the sinus node which have been described in various species including the rat (Virágh & Porte, 1961; Cheng, 1971; Merrillees, 1974; Taylor, 1980). Although developing cardiac muscle cells in the embryo also exhibit ultrastructural features similar to those of clear muscle cells (Leak & Burke, 1964; Okamoto, Satow & Ikeda, 1969; Challice & Virágh, 1973), it is unlikely that the clear cells represent immature elements because of the rather advanced age of rats used in this study.

The close resemblance of the clear muscle cells to sinus node cells suggests that the former may have a potential pacemaking activity. The clear cells, termed node-like cells here, are found solely in the preterminal portion of the myocardial layer of the intrapulmonary vein, and not in the thicker, more proximal, portion of the vein close to the pulmonary hilus. In relation to this topographical distribution, some previous physiological findings should be noted. Cheung (1980) reported that a population of cells showing electrophysiological traces with a steep diastolic depolarisation, a smooth concave transition from diastolic depolarisation to the upstroke of the action potential and a lower magnitude of the resting potential, was limited to the

distal end of the myocardial layer of the pulmonary vein of guinea-pigs. These cells also showed a biphasic chronotropic response to the stimulation of perivascular nerves; these electrophysiological characteristics coincided with those of the sinus node cells. In another experiment by De Almeida *et al.* (1975) using rats, no electrophysiological responses resembling those of sinus node cells were obtained in the myocardial layer of the pulmonary vein in portions lying between the pulmonary hilus and the left atrium. These earlier findings favour the hypothesis that the node-like cells may act as a potential pacemaker. Electrophysiological examination of clear muscle cells in the intrapulmonary venous wall of rats is crucial to the confirmation or otherwise of this hypothesis.

Possible connecting pathways between the sinus node and the atrioventicular node and between the right and the left atrium have recently been examined by structural methods as well as electrophysiologically (Emberson & Challice, 1970; Berger & Rona, 1971; Virágh & Porte, 1973; Sherf & James, 1979). Microscopical studies have revealed that node-like cells occur in various sites such as the Eustachian ridge near the ostium of the inferior vena cava and Bachmann bundle (Sherf & James, 1979) and the ostium of the coronary sinus (Virágh & Porte, 1973). The occurrence of ectopic node-like cells is compatible with physiological evidence of the existence of ectopic pacemakers in the atria. It is also known that destruction or exclusion of the primary pacemaker centre may result in an atrial rhythm originating from these ectopic node-like cell foci (Sealy, Bache, Seaber & Bhattacharga, 1973; Jones *et al.* 1978). It is possible, therefore, that node-like cells in the pulmonary vein could initiate pacemaking activity in the absence of normal myocardial activity following cardiac arrest. This could account for the independent pulsation of the pulmonary vein observed by Brunton & Fayrer (1874). However, to what extent the node-like cells may contribute to the function of the myocardial layer in the pulmonary veins and of the entire myocardial system under normal conditions remains to be elucidated.

SUMMARY

The myocardial layer of the pulmonary vein of adult rats was examined by electron microscopy. Among ordinary myocardial cells resembling those of the atrial myocardium, clear cells with structural features similar to those of sinus node cells were identified. They were distributed in the intrapulmonary, preterminal portion of the pulmonary vein. They appeared singly or in small groups among the ordinary myocardial cells. Their cytoplasm was characterised by a paucity of myofilaments, irregular disposition of myofilament bundles, small and oval mitochondria, absence of atrial specific granules and a wide cytoplasmic matrix between intracellular organelles. The intercalated discs of node-like cells were composed of small junctional specialisations. Nerve fibres containing small and large vesicles with and without dense cores were juxtaposed to the node-like cells over an intercellular space of more than 200 nm. Taking into consideration physiological data, the possibility is discussed that the node-like cells may have a potential pacemaking activity and represent an ectopic pacemaker centre in the pulmonary vein.

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