ULTRASTRUCTURE OF CARDIAC MUSCLE AND NERVE CONTIGUITIES

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During the past century, light microscope studies have indicated the presence of an extensive plexus of nerve processes within the myocardium of the hearts of frogs and other vertebrates (3, 6, 9, 15, 28, and many others). Because of the relatively low resolving power of the light microscope, the unreliability of the silver and methylene blue staining techniques used, and the complexity of the nerve plexuses within the heart, neuromuscular relationships were not adequately defined in the foregoing studies. In recent years, the structural organization of nerve plexuses within the heart was reinvestigated with the electron microscope. Several investigators (2, 4, 7, 8, 11, 25) were able to show contact between unmyelinated nerve processes and the surfaces of cardiac muscle cells; other workers, however, demonstrated the presence of nerve processes within the myocardium but did not show neuromuscular contacts (5, 12, 17, 19, 20, 24, 26).

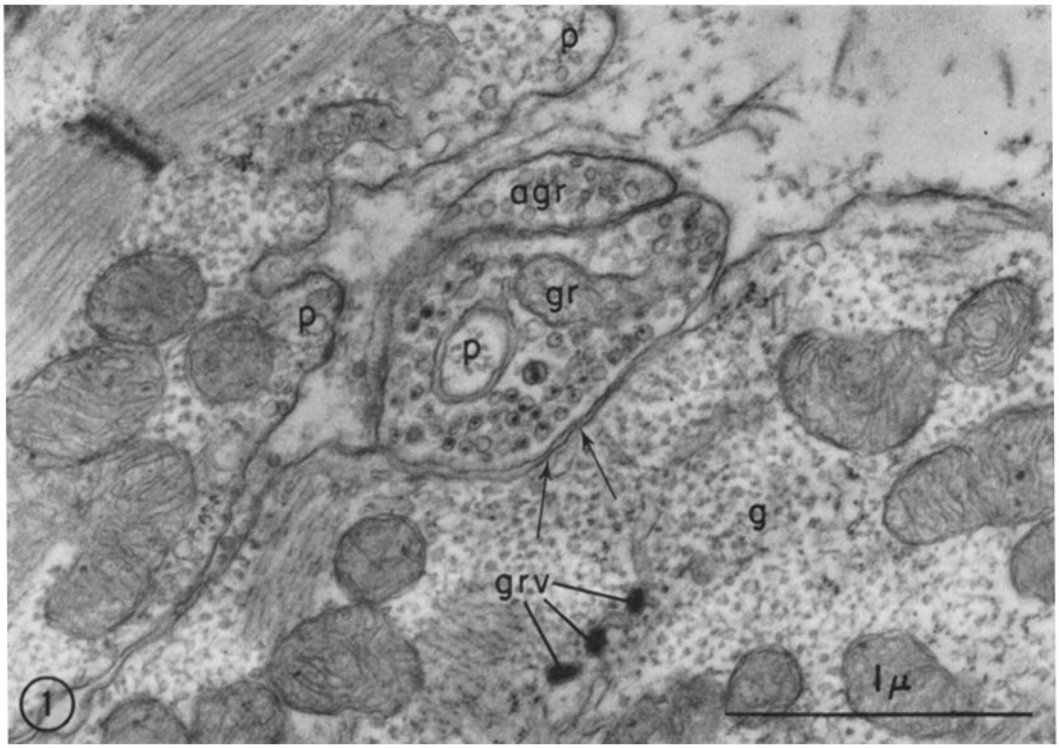

FIGURE 1 This electron micrograph shows a nerve process (*gr*) containing predominantly granular vesicles which makes intimate contact with the surface of a cardiac muscle cell (arrows). A portion of a nerve process (*agr*) containing only agranular vesicles is situated above the other nerve process. Serial sections would most likely show these two nerve processes lying side-by-side. *g*, glycogen particles; *p*, protrusion of cardiac muscle cell; *grv*, large granular vesicles. Myocardium of the apex of the ventricle of frog heart. × 40,000.

The present investigation will demonstrate contiguities between cardiac muscle cells and two types of vesiculated nerve processes. A preliminary account of this work was previously published in abstract (22).

MATERIAL AND METHODS

All cardiac muscle tissue used for this study was obtained from the apex of the ventricle of the hearts of frogs (*Rana pipiens*). One % osmium tetroxide (pH 7.5), buffered with veronal-acetate, was injected into

the pleuroperitoneal cavity immediately after severance of the spinal cord at the base of the brain. Shortly thereafter, the heart was removed from the opened pleuroperitoneal cavity and placed in cold fixative. While in fixative, the lower portion of the ventricle was removed from the rest of the heart and fixed for an additional 90 min. After fixation, the ventricular tissue was dehydrated in ethyl alcohol and embedded in Vestopal-W with the apex of the ventricle oriented toward the future cutting surface of the block. Each section was individually placed over a 0.2- mm x 1-mm slit in the center of a copper disc (specimen mount) and subsequently stained with uranyl acetate. The methods used to insure that the same area of each tissue section was placed over the slit in the specimen mount and those used to prepare three-dimensional illustrations in perspective are fully described in previous publications (16, 23).

RESULTS

The examination of serial sections of myocardium with the electron microscope reveals that vesiculated nerve processes, lacking Schwann cell investments, make intimate contact with the surfaces of cardiac muscle cells. These contiguities are frequently observed in sections of myocardium. In most instances, the vesiculated nerve processes are prominent within the perivascular space in which they are sandwiched between the walls of blood capillaries and the surfaces of cardiac muscle cells. Here, these nerve processes usually exist in pairs: in one process, only agranular vesicles are present, while in the other process, vesicles of the granular type predominate. The agranular vesicles are, on the average, smaller than the vesicles which contain electron-opaque granules. The following descriptions of the electron micrographs and three-dimensional illustrations depicted in this report characterize the many neuromuscular contiguities observed in this study.

In Fig. 1, a nerve process containing primarily granular vesicles is situated within a crevice formed by two adjoining cardiac muscle cells. Here the nerve process is in intimate contact with the

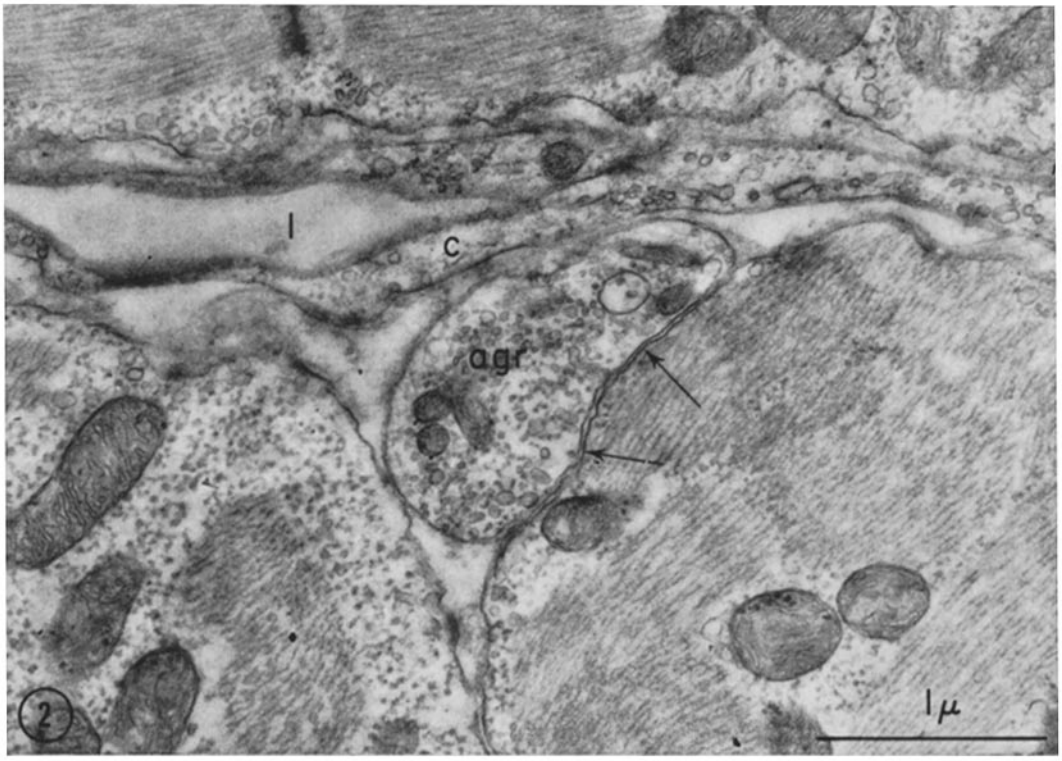

FIGURE 2 Electron micrograph. A nerve process (*agr*) containing agranular vesicles and some glycogen particles is situated within the perivascular space where it is making intimate contact with the surface of cardiac muscle cells (arrows). *c*, capillary wall; *l*, lumen of capillary. Myocardium of the apex of the ventricle of frog heart. × 30,000.

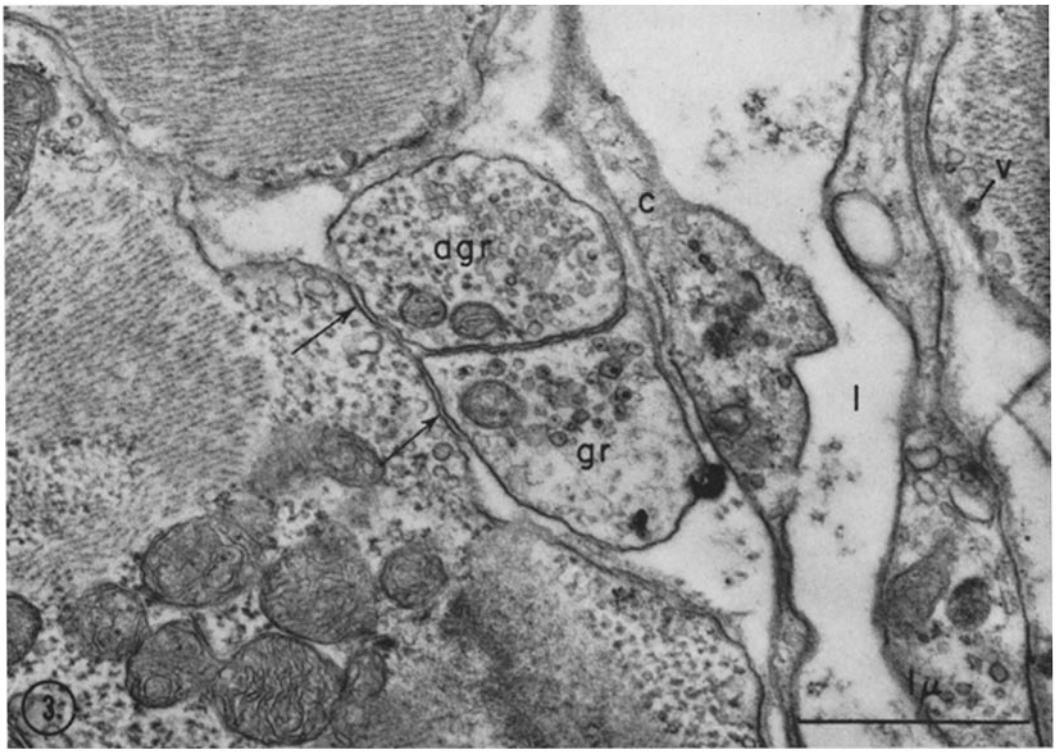

FIGURE 3 Electron micrograph. Vesiculated nerve processes, one (*agr*) containing only agranular vesicles and some glycogen particles and the other (*gr*) endowed predominantly with granular vesicles, are situated within the perivascular space where they make intimate contact with the surface of a cardiac muscle cell (arrows). *c*, capillary wall; *l*, lumen of capillary; *v*, granular vesicle in sarcoplasm of muscle cell. Myocardium of the apex of the ventricle of frog heart. × 30,000.

surface of the muscle cell in the lower portion of the field. It appears that a muscle cell protrusion has penetrated the nerve process. Three large granular vesicles which lie deep within the sarcoplasm of the muscle cell are surrounded by glycogen particles. Vesicles of this type have been previously reported within the sarcoplasm of ventricular muscle of the toad (18). The wall of a capillary lies just out of the field beyond the upper right corner of the micrograph. A nerve process containing only agranular vesicles is shown in Fig. 2. It is closely applied to the sarcolemma of a cardiac muscle cell. Myofilaments are present directly beneath the sarcolemma in the area of contact. This nerve process is also situated within a crevice between two muscle cells, and a capillary wall lies above it. In Fig. 3 a pair of nerve processes, situated the same way as the one in Fig. 2, are contiguous with a cardiac muscle cell. The lower nerve process contains vesicles predominantly endowed with granules, whereas the upper process contains only agranular vesicles in addition to some glycogen particles which may be misinterpreted as electron-opaque granules within vesicles when they happen to overlie vesicles in relatively thick sections. Mitochondria of small diameter are present in both nerve processes. Occasionally, granular vesicles very similar to those in nerve processes can be found within the sarcoplasm of muscle cells. Such a vesicle can be seen directly beneath the sarcolemma of the muscle cell in the upper right corner of Fig. 3. The functional significance of these granular vesicles and of the larger ones shown in Fig. 1 is unknown at this time.

In a preliminary three-dimensional study, serial sections have revealed findings such as are demonstrated in the perspective reconstructions in Figs. 4 and 5. The sections upon which the illustration in Fig. 4 is based amount to a combined thickness of

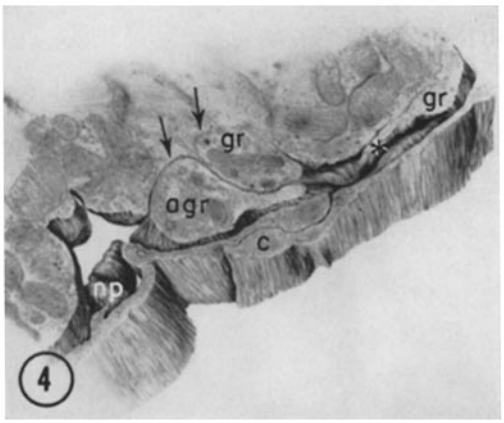

FIGURE 4 Three-dimensional illustration drawn in perspective from serial sections of the apex of the ventricle of frog heart. It shows two nerve processes within the perivascular space and sandwiched between the capillary wall and the surface of a cardiac muscle cell. The nerve processes become constricted between two expanded portions. Note the close apposition of the nerve processes to the surface of the cardiac muscle cell (arrows). The total thickness of all sections represented in this figure is 800 mμ. *agr*, expanded portion of nerve process with agranular vesicles; *gr*, expanded portion of nerve process with granular vesicles; *, constricted portion of nerve process; *np*, nerve process thought to be continuous with the expanded portion of the nerve process containing agranular vesicles; *c*, capillary wall.

800 mμ. In Fig. 4, one can see the capillary wall in the foreground and two vesiculated nerve processes sandwiched between it and the surface of a cardiac muscle cell. The two nerve processes, one containing a few granular vesicles and a number of glycogen particles and the other containing only agranular vesicles, are embedded within the depressed surface of the muscle cell and each of them shows a constricted segment between two expanded portions or varicosities. The expanded portions of the processes contain the vesicles. The structure immediately to the left of the nerve process containing agranular vesicles, which is also sandwiched between the capillary wall and the surface of the cardiac muscle cell, is apparently a continuation of this nerve process. However, the sections required for showing this continuity were not obtained. The thick slice of tissue as illustrated in Fig. 4 can be altered by deleting some of its parts and by drawing the perceptible details of subsequent sections in addition to those of the top section. This is the case in the illustration shown in Fig. 5. In this instance, the capillary wall was deleted and the remaining slice of tissue was divided horizontally into two equally thick slices. These two slices were then moved out of register with each other and separated so that one slice is situated above the other. This procedure permits the demonstration of two surfaces, which, in this case, are represented by the two electron micrographs at the right of the figure. It also permits the demonstration of the extent of the contiguity of processes and cardiac muscle cells beyond that which can be discerned from the observation of electron micrographs.

DISCUSSION

The electron micrographs in this study demonstrate the profiles of two types of vesiculated nerve processes in contact with the surfaces of muscle cells of frog myocardium. From the present evidence, it is assumed that nerve processes within the myocardium retain their characteristic vesicular structure throughout their courses. The processes which display only agranular vesicles are thought to represent nerve processes which never contain granular vesicles, and the ones that display granular vesicles show a predominance of them wherever vesicles are present. The possibility exists, however, that both kinds of nerve processes may consist of alternate areas (or varicosities) of agranular and granular vesicles, or that these two types of vesicles may be distributed haphazardly within both kinds of nerve processes. It appears that the latter alternatives are unlikely, since in most pairs of processes seen, a process containing granular vesicles was situated adjacent to one containing only agranular vesicles. Furthermore, in the pairs of vesiculated processes traced in serial sections, each process retained its individual vesicular character. In the several longitudinally sectioned nerve processes observed, which extended for distances up to 5 μ, no evidence of irregular distribution of the two types of vesicles was seen. It is hoped that this problem will be resolved by the three-dimensional study of the origin and termination of vesiculated nerve processes within the myocardium which is now in progress.

The presence of granular and agranular vesicles within nerve processes has prompted several investigators to seek their functional significance. Wolfe et al. (27), using radioautography and electron microscopy, have shown that, after the injection of tritiated norepinephrine into rats, sec-

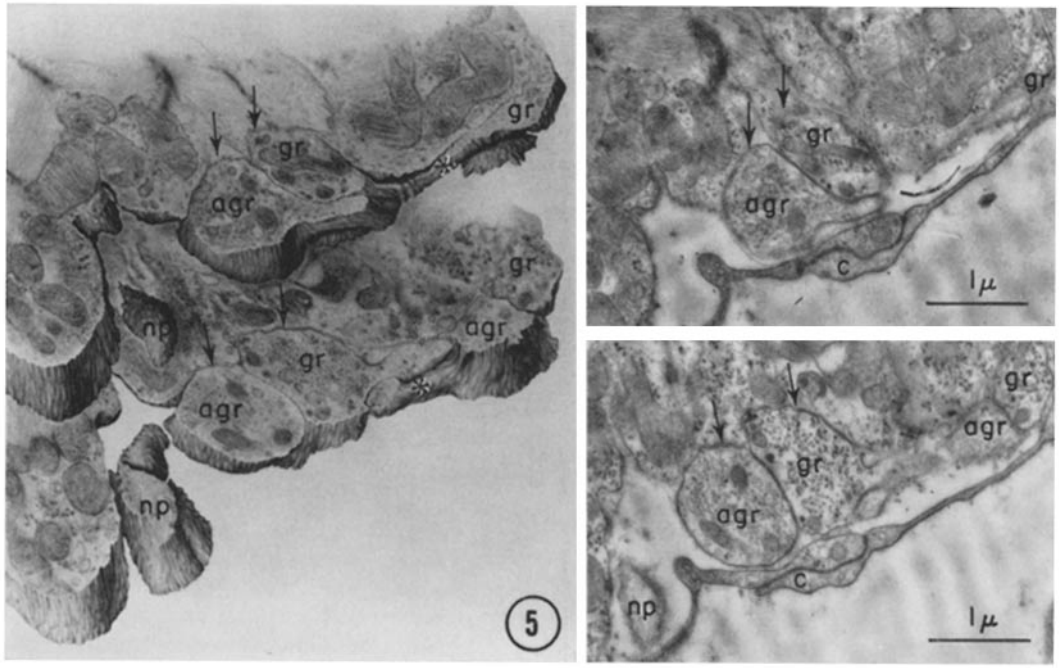

FIGURE 5 Three-dimensional illustration drawn from same serial sections used for the illustration of Fig. 4. The capillary wall was deleted, the remaining slice of tissue divided, and the two separated parts moved out of register with each other. *agr*, expanded portion of nerve process with agranular vesicles; *gr*, expanded portion of nerve process with granular vesicles; *, constricted portion of nerve process; *np*, nerve process thought to be continuous with the expanded portion of the nerve process containing agranular vesicles. The two electron micrographs on the right represent the two surfaces shown in the illustration. Each micrograph, × 13,000.

tions of the pineal glands revealed that only the nerve processes containing granular vesicles accumulated the injected norepinephrine. This prompted these investigators to conclude that norepinephrine resides in the electron-opaque core of the granular vesicle and that the presence of granular vesicles can be used as one criterion for the identification of adrenergic sympathetic axons in electron micrographs. In an electron microscope study of the microsomal fraction of rat hearts, Michaelson et al. (14) determined that vesicles 50 mμ in diameter were present within the fraction. A few of the vesicles contained electron-opaque cores. This evidence seems to implicate vesicles in the storage of catecholamines within the tissues of the heart. With the use of fluorescence methods for the histochemical demonstration of catecholamines, Angelakos et al. (1) have shown that nerve processes which contain catecholamines are present within the ventricular wall of the heart. This information, when compared with the previous studies (14, 27) and the findings of the present investigation, implies that nerve processes which exhibit fluorescence are predominantly endowed with granular vesicles. In addition to the fluorescence methods for the identification of adrenergic fibers, the thiocholine method for the detection of cholinergic fibers has been used with some success in studying the anterior segments of the rabbit eye (13). Heavy staining for nerve processes containing acetylcholinesterase was seen in the sphincter pupillae and ciliary muscle. According to electron microscope studies, these structures contain nerve processes which are predominantly endowed with agranular vesicles (10, 21).

It appears from the foregoing discussion that it may be possible to distinguish between sympathetic and parasympathetic nerve processes on the basis of their vesicular content. The neuromuscular contiguities observed in the present investiga-

tion may represent morphological evidence for the existence of both adrenergic and cholinergic innervation of the muscle cells of the ventricular myocardium.

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