Correlative Ultrastructural and Electrophysiological Study of the Purkinje System of the Heart

BY S. A. BENCOSME*, A. TRILLO†, J. ALANIS‡, AND D. BENÍTEZ‡

SUMMARY

To delineate major morphological differences between Purkinje and working myocardial fibers, a comparative electrophysiological and ultrastructural study of the Purkinje system and working myocardial cells was undertaken. To this effect, the false tendon, papillary muscle, trabeculae carneae and segments of the Purkinje extramural network from hearts of dogs, rabbits and cats were studied. The cell populations of the false tendon and of the extramural Purkinje network have similar characteristics and can be, therefore, easily differentiated from the papillary muscle, trabeculae carneae and working myocardial fibers of the ventricular wall. The main distinguishing features of Purkinje fibers are the absence of triads and the presence of peculiar small mitochondria. In addition, the intercalated discs are straighter and thus less prominent than in working myocardial fibers. Some of the current discrepancies on the ultrastructure of the Purkinje system and working myocardial fibers have been resolved by this correlated electrophysiological and morphological study.

INTRODUCTION

The existence of the specialized conductive system of the mammalian heart has been well established by light microscopy4,7. Recently, investigators have undertaken the task of distinguishing Purkinje cells from working myocardial fibers by ultrastructural differences. In this respect, species and regional differences have been reported5,6,10,20,23,24,29,36. It can be agreed with Johnson and Sommer19 that there are very few ultrastructural features which could be used to determine the nature of a given myocardial cell. This is in sharp contrast with the well established electrophysiological properties of different myocardial cells. Their distinctive transmembrane potentials are well defined and used routinely to identify the types of myocardial cells11,12,16. Analysis of the electrophysiological properties of the false tendon and extramural Purkinje network have contributed valuable information on the propagation of impulses within these structures and from them to the ventricular wall5,37. Correlative morphological and electrophysiological studies of nodal and atrial tissues have been undertaken8,13,32. Similar information is lacking with respect to the false tendon and the extramural Purkinje network.

The present work was undertaken to determine the nature and ultrastructure of the muscle fibers present in the false tendon and in the extramural Purkinje network of dogs, rabbits and cats. To this end, the nature of cardiac fibers in these structures were first identified electrophysiologically and tissues so explored were then studied with the electron microscope. It was found that the cardiac muscle fibers present in the false tendon and in the extramural Purkinje network are exclusively of Purkinje type and that these fibers differ clearly from the working myocardial cells of papillary muscle, trabeculae carneae and ventricular wall.

MATERIAL AND METHODS

Thirty-six mongrel dogs of both sexes, including six puppies (six weeks old) were used. In addition, seven rabbits (2 kgs) and three cats (2.5 kgs) were also used. Dogs were anaesthetized with Nembutal, cats with ether. Rabbits were sacrificed by a blow on the neck. The hearts were quickly removed and placed into a glass jar with Tyrode solution at 37°C. The Tyrode solution composition was: NaCl 137 mM; KCl 2.7 mM; CaCl₂ 1.8 mM; MgCl₂ · 2H₂O 0.5 mM; NaH₂PO₄ 0.4 mM; NaHCO₃ 12 mM and dextrose 8.25 mM. Ventricles were opened and their false tendons with cor-
responding papillary muscles still attached were isolated (false tendon-papillary muscle preparation). Tissues explored electrophysiologically were pinned to a piece of soft wood and placed in a lucite chamber containing flowing Tyrode solution (37°C aerated with a gas mixture (95% O2, 5% CO2) as described by Hoffman and Suckling17). Transmembrane potentials of all tissues studied with the electron microscope were recorded through glass micropipettes (3M KCl) in order to identify electrophysiologically the type of cells existing in these tissues except when otherwise specified.

Experiments on dogs. False tendon-papillary muscles, trabeculae carneae and portions of extramural Purkinje network obtained from dogs were divided into two equal groups.

Group 1. Immediately after removal these tissues underwent electrophysiological studies. Subsequently they were fixed for electron microscopic observations as described below.

Group 2. The same type of structure without previous electrophysiological exploration were fixed for electron microscopy as in Group 1.

To estimate the number of Purkinje cells present in a given segment of the false tendons, recording micropipettes were used. The false tendon with its papillary muscle was stimulated at a constant frequency with square waves applied through either platinum needles or glass micropipettes filled with 3M KCl. In some instances, regular spontaneous activity was recorded. Recording microelectrodes had a tip diameter of 0.5-1.0 μ and a resistance of 10-25 megohms. The microelectrodes were connected to one end of a P6 DC Grass preamplifier through a cathode follower (grid current 10-13 Amps.). The records were taken from a dual-beam oscilloscope (Tektronix 565). Cells were identified according to the shape, magnitude and duration of their transmembrane potentials. Once a microelectrode was on the surface of the false tendon, it was displaced downward until a cell was impaled. After the first impalement the micropipette was slowly advanced until the false tendon was explored in its whole thickness. When this exploration was completed, at least three other segments of the same false tendon were similarly studied. For each segment explored a new micropipette was used. By means of a calibrated micromanipulator the number of cells in depth as well as the separation between them were measured. The abrupt appearance and disappearance of the resting membrane potentials were used to measure the thickness of each Purkinje cell. In some experiments the tips of the recording micropipettes were left within the false tendon for better localization of explored areas. The electrophysiological studies were completed in an hour or less. At this time, the Tyrode solution was replaced by a 6.25% glutaraldehyde solution29 and the tissues were fixed in the lucite chamber for one hour at 4°C. Small fragments of papillary muscle, trabeculae carneae, false tendon and portions of extramural Purkinje network were transferred to a glutaraldehyde solution and kept at 4°C for another two hours. After glutaraldehyde fixation, tissues were post-fixed in buffered osmium tetroxide23 and embedded in Epon 8122. Section 1-2 μ thick were cut and stained with toluidine blue for observation under the light microscope for orientation purpose. Thin sections cut with an MT-2 ultramicrotome were doubly stained with uranyl acetate27 and followed by lead citrate28 and examined with either Hitachi HU-11 or a Carl Zeiss EM-9 electron microscope.

The following tissues were taken from the rabbit and cat hearts: a) false tendon; b) papillary muscle; c) portions of extramural Purkinje network32 and d) trabeculae carneae. In all instances tissues were studied electrophysiologically and ultrastructurally following the procedures described for tissues obtained from dogs.

RESULTS

Experiments on dogs

Electrophysiology: The cells from the false tendon of dogs are of the Purkinje type, since their action potentials show the typical shape, magnitude and duration described for these fibers (Fig. 1A). On the other hand, the cells of the papillary muscle gave action potentials similar to those described for the working myocardial fibers of the ventricles (Fig. 1B). Regarding the number and spatial distribution of Purkinje cells in the false tendons, we shall express the individual figures as mean of the measurements obtained from the different segments of the false tendons studied. In seventy segments of false tendons explored, the first layer of Purkinje cells was found to be located at 114 μ from the surface (minimum 7 μ and maximum 197 μ). For each segment the average number of impaled cells was 7 with a
of endothelial cells. Abundant bundles of collagen and elastic fibers, oriented longitudinally to the long axis of the tendon, form a matrix into which bundles of Purkinje fibers are found. The number of cells in these bundles varies from a few to fifty or more. These cells measure an average of 25 μ in width and 75 to 100 μ in length. Among the collagenous matrix, there are abundant fibroblasts, some smooth muscle fibers and small arterioles and capillaries (Figs. 2 and 3).

Purkinje fibers resemble working myocardial cells; however, there are some distinctive features. The former are wider, shorter, and their myofibrils are less abundant and peripherally located. Frequently Purkinje fibers have either large paranuclear or subsarcolemal areas which appear buff-colored when stained with the toluidine blue.

Electron microscopy. The subendocardial space of the false tendons contains collagen, elastic fibers, fibroblasts and scanty smooth muscle cells (Fig. 4). The Purkinje fibers occupying the centre of the false tendon are in close contact with one another; however, they may diverge leaving intercellular spaces containing collagen fibers and occasional fibroblasts. Purkinje fibers are covered by a basal lamina. At somewhat regular intervals, their plasma membrane and basal lamina show shallow indentations in relation to the Z-bands (Fig. 5). Pinocytotic vesicles are often found in relation to the plasma membrane (Fig. 5).

The number and general arrangement of myofibrils within Purkinje fibers varies; usually, they contain fewer myofibrils than working myocardial fibers (Fig. 6). Occasionally myofibrils are randomly oriented and myofilaments lack the typical parallel arrangement (Fig. 6). Normally arranged myofibrils manifest the usual sequential pattern of bands and lines comprising a sarcomere (Figs. 5 and 6). Deposits of an electron dense substance, here referred to as Z-band-like material, is frequently found in areas where myofilaments are randomly oriented (Fig. 6). At a higher magnification this material is formed mainly by dense filaments, approximately 130 Å in diameter, separated by a space of approximately 240 Å (Fig. 7). Transverse sections show a grid pattern. Similar material was described by Fawcett14.

Large paranuclear and subsarcolemmal areas devoid of myofibrils with abundant glycogen granules and mitochondria are frequently observed (Figs. 6 and 8).
The sarcoplasmic reticulum is present in the intermyofibrillar spaces (Figs. 8 and 9), and may also be found in relation to the Z-band. Although triads were not found, peripheral couplings were frequently observed (Fig. 5). In areas devoid of myofibrils, the sarcoplasmic reticulum is formed by numerous small tubules (Figs. 8 and 9) some of which have irregular bulbous dilatations filled with a coarsely fibrogranular material of moderate electron density (Figs. 8 and 9). Similar bulbous dilatations of the sarcoplasmic reticulum are rarely seen in other myocardial cells. When found, their content was considerably less prominent.

Two types of mitochondria were identified in Purkinje fibers (Fig. 9). The first type resembles those seen in working myocardial fibers, whereas...
the second type appears as slender mitochondria with usually only one crista (Fig. 9). This type of mitochondria has a random distribution but accumulations of them can be found near the nucleus (Fig. 10). They measure 3 to 6 μ in length and 1 to 2 μ in width. Their matrix is very dense, and contains mitochondrial granules.

Intercalated discs of Purkinje fibers are formed by desmosomes, fascia adherens and tight junctions as in working myocardial cells. Cell membranes of adjacent Purkinje fibers, however, show considerably fewer interdigitations, thus their intercalated discs appear somewhat less complex than those of working myocardial fibers (Figs. 11 and 12).

The ultrastructure of the extramural Purkinje network is essentially similar to that of the false tendon just described. The fine structure of trabeculae carneae is similar to that of papillary and ventricular muscles. Since the fine structure of papillary and ventricular muscles are well documented we shall not elaborate further on this subject.

Experiments on rabbits and cats

Electrophysiology. The false tendon and the ex-

---

Fig. 6. Dog false tendon. Purkinje fiber cut longitudinally. Myofibrils are scarce and randomly oriented. In some areas, myofilaments lack the normal arrangement and the banding pattern is not observed. In these areas, deposits of a Z band-like material is observed (arrow). (M) Conventional type mitochondria; (M′) slender mitochondria. Glycogen granules (GL) are abundant in the paranuclear zone, and in the internyofibrillar spaces. (N) Nucleus. X 6,200.
Tramural Purkinje network show similar action potentials to those generated by the Purkinje cells of the false tendon of the dog (Fig. 1E). Whereas the action potentials of the papillary muscle and trabeculae carneae were similar to those potentials characteristic of myocardial fibers from the ventricular walls (Fig. 1F).

**Morphology.** Macroscopically the trabeculae carneae and papillary muscles are reddish in colour in contrast with the whitish appearance of the false tendon and of the extramural Purkinje network.

There was no appreciable difference in the fine structure of tissues fixed immediately after their removal and those used for electrophysiological studies. The Purkinje cells of the false tendon and of the extramural Purkinje network closely resemble those of the dog (Figs. 13 and 14). The same was found with respect to the papillary muscle and trabeculae carneae which are also morpho-

---

**Fig. 7.** Dog false tendon. Detail of a zone with deposits of Z band-like material (arrows) longitudinally and cross sectioned. Enlargement of a cross section of this material (framed area) is shown in the inset. *mf*, myofibrils. X 48,000. Inset X 144,000.

**Fig. 8.** Dog false tendon. Paranuclear zone of a Purkinje fiber showing abundant sarcoplasmic reticulum (SR) with bulbous dilatation containing a fibrogranular material (arrows). A detail of the framed area is seen in the inset, showing the connection of one of these dilatations with the sarcoplasmic reticulum (arrow). X 18,200. Inset X 47,000.
logically similar to the myocardial cells of the ventricular wall.

DISCUSSION

In the present work the identification of Purkinje cells has been made following the accepted criteria\(^\text{15,16}\) which distinguish the specialized conductive-tissue from the rest of the myocardial fibers. Our results show that the false tendon in the dog was composed mainly of Purkinje cells and strongly suggests that the same is also valid for rabbits and cats. It should be mentioned that one of us (Alanís, unpublished observations) however, found in the course of electrophysiologically exploring about 300 canine false tendons, in only three instances a few working myocardial fibers covering the Purkinje cell population of the false tendon. As a matter of practical interest, false tendons containing working myocardial fibers appear thicker and show a

---

Fig. 9. Dog false tendon. Intermyo fibrillar region of a Purkinje fiber. Dilatations of a network of tubules (SR) are marked with arrows. Slender mitochondria (Ms) in two different planes of section are also shown. (M) conventional type mitochondria. $\times$ 37,500.

Fig. 10. Dog false tendon. Paranuclear area of a Purkinje fiber with numerous slender mitochondria (Ms). These mitochondria have usually only one crista. (N) nucleus. $\times$ 60,800.
faint reddish coat. These are to be avoided if one wishes to study a pure population of Purkinje fibers. The peculiar distribution and abundance of collagen in the false tendon may be of functional significance. One is tempted to speculate that the bundles of Purkinje fibers are shielded by collagen and protected against excessive stretching during diastole by virtue of their collagenous and elastic elements.

Johnson and Sommer studied in detail a trabecula carnea and referred to it as "a strand of cardiac muscle." Based on morphological studies, these authors concluded that there are at least two different populations of muscle fibers in the rabbit ventricle. The first corresponds to the working fibers, while the second was considered, because of its location, to represent Purkinje fibers. The diffuse spread of trabeculae carneae and their close topographical association with the extramural Purkinje network makes macroscopic distinction between these structures difficult unless a stereomicroscope is used. The macroscopic dis-

Fig. 11. Dog papillary muscle. Detail of an intercalated disc (ID) of two working myocardial fibers, showing the typical zig-zag dispositions of the apposed cell membranes. × 23,000.

Fig. 12. Dog false tendon. Intercalated disc (ID) between two Purkinje fibers. Notice the less wavy appearance compared with the preceding figure. × 15,900.
tinction is supported by the electrophysiological data which showed that “reddish muscle strands” have potentials typical of those generated by working myocardial fibers (Fig. 1D) whereas “whitish muscle strands” gave potentials similar to those of Purkinje cells (Fig. 1C). On the basis of these observations it seems to us that the term “strand” applied to trabeculae carneae is misleading, since unless qualified, either electrophysiologically and/or by its colour, it is not possible to know whether one is dealing with a strand composed of working myocardial cells or of Purkinje fibers. We believe that the strand of cardiac muscle described by Johnson and Sommer\textsuperscript{19} is, in fact, a segment of the extramural Purkinje network.

The value of morphological and electrophysiological correlation when dealing with the myocardium is exemplified by the results obtained in rabbits. In these animals a clear separation be-

Fig. 13. Rabbit extramural Purkinje network with slender mitochondria ($M_s$). Intercalated disc ($ID$). Tight junction (arrow heads). $\times 21,000$.

Fig. 14. Rabbit extramural Purkinje network. Portion of a Purkinje fiber showing the presence of dilatations of the sarcotubular elements containing a fibrogranular material (arrows), and slender mitochondria ($M_s$). $\times 21,000$. 
between the “true trabeculae carneae” and the extramural Purkinje network was possible when a stereomicroscope was used. The former appears reddish, whereas the latter appears whitish like the false tendon. The electrophysiological explorations confirmed the similar nature of myocardial fibers from the extramural Purkinje network and that of the false tendon. As one might expect, the reddish-appearing myocardial tissues, such as the trabeculae carneae, papillary muscle and ventricular wall, gave potentials typical of working myocardial fibers (Fig. 1B, D, F).

Consistent morphological differences between Purkinje and working myocardial fibers have been obtained during this correlative study. The main structural differences are found in the T system, the mitochondria and the intercalated discs. In dogs, rabbits and cats Purkinje fibers are devoid of triads but peripheral couplings like those described by others in these fibers were frequently observed. Our findings are thus similar to those reported in the rabbit cardiac strand by Johnson and Sommer but are at variance with previous descriptions. It is of interest that in figure 15 of Johnson and Sommer, one can also appreciate that the intercalated discs of myocardial cells of the strand (Purkinje cells) are similar to those described by us. It seems, therefore, that the less wavy appearance of the intercalated discs may also be used to differentiate Purkinje and working myocardial fibers. According to the prevailing hypothesis on the role played by the tight junctions in the propagation of impulses, the Purkinje system should have more tight junctions since Purkinje cells possess a higher conduction velocity than other cardiac fibers.

Purkinje fibers lack triads such as the ones found in working myocardial cells (Fig. 15) we are inclined to consider, in agreement with Johnson and Sommer, that the peripheral couplings found in Purkinje cells represent the morphological substratum for the excitation-contraction coupling mechanisms in Purkinje cells.

With respect to intercalated discs in Purkinje fibers we found in contrast with previous descriptions that these discs have a less marked zig-zag appearance than those of working myocardial fibers. It is of interest that in figure 15 of Johnson and Sommer, one can also appreciate that the intercalated discs of myocardial cells of the strand (Purkinje cells) are similar to those described by us. It seems, therefore, that the less wavy appearance of the intercalated discs may also be used to differentiate Purkinje and working myocardial fibers. According to the prevailing hypothesis on the role played by the tight junctions in the propagation of impulses, the Purkinje system should have more tight junctions since Purkinje cells possess a higher conduction velocity than other cardiac fibers.

Purkinje and working myocardial fibers can also be differentiated by the presence in the former of two types of mitochondria. The slender mitochondria are found only in Purkinje cells. Small mitochondria have been described in Purkinje cells but it was Johnson and Sommer who stressed that in these cells there was a mixed popu-
ulation of mitochondria. Without further work it is not profitable to elaborate further on the morphological similarity between the slender mitochondria found in Purkinje fibers, and those described in nerve axons and in some neurons of the central nervous system.

Membrane-bound granules, referred to by several authors as specific granules, have been described in the bundle of His and in the atrial myocardial cells of all vertebrates studied. In agreement with Bompiani et al., we were unable to find specific granules in Purkinje fibers.

The significance of the fibrogranular material found in the dilated cisternae of the sarcoplasmic reticulum is at present unclear. The greater prominence in Purkinje cells of the fibrogranular material present in the sarcoplasmic reticulum in contrast with its discrete occurrence in working myocardial cells could be used to distinguish between these two types of myocardial fibers.

Addendum:—Shortly before the present manuscript was sent to the Editor, Sommer and Johnson published a paper (J. Cell. Biol., 36: 497, March, 1968) stating, in agreement with our results, that Purkinje fibers can be clearly distinguished ultrastructurally from the ventricular fibers of several species.

REFERENCES