A LABYRINTHINE STRUCTURE FORMED FROM A TRANSVERSE TUBULE OF MOUSE VENTRICULAR MYOCARDIUM

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INTRODUCTION

The transverse (T) tubules of mammalian myocardial cells and vertebrate skeletal muscle fibers both are invaginations into the cell interior whose membranes are continuous with the sarcolemma. Some pronounced differences exist between tubules from these two sources, however. One is the obvious disparity in size; the diameter of myocardial tubules may reach over 3,000 Å in the guinea pig (Sperelakis and Rubio, 1971), but T tubules of frog sartorius are only 260-280 Å in diameter (Freygang et al., 1964; Sperelakis and Schneider, 1968). Basal lamina lines the myocardial T tubules, but no evidence for its existence has been found along the walls of skeletal T tubules. The relationships between T tubules and sarcoplasmic reticulum (SR) appear morphologically similar in both muscle types, but on the basis of studies employing osmotic response (Birks and Davey, 1969) and electron-opaque tracer material (Rubio and Sperelakis, 1972), it has been concluded that the T tubule and SR terminal cisternae of frog sartorius are open to one another. Thus far, it appears that myocardial SR is a system closed to the intercellular space and T tubules (Forssmann and Girardier, 1970; Forbes et al., 1972). T tubules are absent in both muscle types in early embryonic stages. In chick skeletal muscle, SR is present in such early embryonic stages (Ezerman and Ishikawa, 1967; Shimada et al., 1967). Therefore, SR-T tubule contacts in the chick must arise after initial stages of T tubule formation. However, in embryonic rat skeletal muscle, the SR and T system form couplings during the early development of T tubules (Kelly, 1969, 1971). In the course of an ultrastructural study of mouse heart, a complex formation was found which supports the concept, already advanced by Jewett and Sommer (1970), that cardiac T tubule formation may come about in a manner similar to that proposed by Ishikawa (1968) for skeletal T tubules.

MATERIALS AND METHODS

Right ventricular wall from a white mouse (ICR strain, Flow Research Animals, Dublin, Va.) was
prepared for electron microscope examination by perfusion fixation with 3% glutaraldehyde in an aqueous solution (pH 7.2) of 3% dextran (mol wt 40,000-90,000) and 3% dextrose (Rostgaard and Behnke, 1965). After 5 min of perfusion, small blocks of tissue were fixed an additional 3 h, postfixed 1 h in 1% phosphate-buffered osmium tetroxide (Mil- long, 1962), and stained en bloc for 30 min in a saturated aqueous solution of uranyl acetate. After dehydration in alcohols, the tissue was passed through propylene oxide, embedded in Epon 812 (Luft, 1961), in 1% phosphate-buffered osmium tetroxide (Mil- long, 1962), and stained en bloc for 30 min in a saturated aqueous solution of uranyl acetate. After dehydration in alcohols, the tissue was passed through propylene oxide, embedded in Epon 812 (Luft, 1961), and sectioned with a diamond knife. Thin sections were affixed to a 150-mesh copper grid; they were stained for 2 rain with a saturated uranyl acetate solution in 50% acetone, and for 45 sec with 0.4% alkaline lead citrate (Venable and Coggeshall, 1965). The grid was examined in a Hitachi HU-11E-1 electron microscope, which was calibrated periodically against a replica of an optical grating.

RESULTS AND DISCUSSION

An unusual structure, apparently composed of a system of anastomosing tubules (500-800 Å in diameter) and vesicles (800-1,200 Å in diameter), was encountered in a thin section of one cell (Fig. 1). The SR forms several junctional complexes ("couplings") with this system; one of these complexes is shown at high magnification in the inset of Fig. 1. Three additional (nonconsecutive) sections were found which show details of the structure at successive levels of the cell (Figs. 2-4). The sections reveal that this labyrinthine mass is connected to a T tubule. This structure therefore appears homologous with those formed in cultured skeletal muscle (Ishikawa, 1968; Pappas et al., 1971). None of our sections reveals clear examples of either the ordered hexagonal or tetragonal patterns of tubule anastomosis found by Ishikawa. However, the overall appearance of the cardiac structure is quite similar to the less organized T tubular networks formed in young cultures of skeletal cells (Ezerman and Ishikawa, 1967); this suggests that its formation proceeds in a manner similar to that in cultured skeletal muscle. A possible difference in structural organization is that some of the 500-600 Å diameter "inter-
tubular spaces" of the cardiac network may not constitute open cavities extending completely through the structure. Instead, they appear to end in depressions or "dimples" in the limiting membrane of the structure (double-headed arrows, Fig. 1). Therefore, much of the true three-di-

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Figure 1  Elaborate structure found in a mouse ventricular myocardial cell. Three regions (short filled arrowheads) appear similar to couplings formed between SR and T tubules. The uppermost of these couplings is given at high magnification in the inset to show the junctional granules (JG) in the lumen of the SR junctional cisterna and the junctional processes (single arrows) which extend between the apposed SR and T tubule membranes. In the tubular mass, structures representing either depressions in the limiting membrane or intratubular dense granules are indicated by double-headed arrows. Other structures (short open arrowheads) may represent true intertubular spaces. Fig. 1, × 72,000; scale bar = 0.5 μm. Inset, × 180,000; scale bar = 0.1 μm.
Figures 2–4 Sections of the region shown in Fig. 1, taken at successive levels in the cell. In Figs. 2 and 3, a mass of tubules, apparently constituting part of the same structure seen in Fig. 1, is continuous with a normal-appearing T tubule (TT). The same coupling formed by a junctional SR cisterna (J-SR) with the T tubule is visible in all three figures. Fig. 4, scale bar = 0.5 μm. Figs. 2–4, × 72,000.
develop in the maturing heart cell, followed by axial tubules, which then may develop from the T tubules by a process of "buddling-off" of vesicles at approximate right angles to the T tubule. In order to test the validity of such an hypothesis, it would be necessary to document the development of the T-axial tubular system in newborn mammals by use of electron-opaque tracers. Therefore, it appears that the structure described here may represent an aberrant example of axial tubule formation in a myocardial cell.

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