Review

Structure and Composition of Tubular Aggregates of Skeletal Muscle Fibres

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Abstract. Unusual regions of densely packed membranous tubules known as tubular aggregates (TAs) have been observed in skeletal muscle fibres of mammals under numerous pathological conditions but also in health. Their causality is unclear. It is neither known whether TAs are destructive and should be treated or whether they have a compensating function in an endangered muscle. In spite of many similarities, the histochemical, immunocytochemical and ultrastructural characteristics of tubular aggregates do vary. Histochemistry provided an overall characteristic of TAs as membranous inclusions with a variety of enzymatic activities. Immunocytochemical evidence revealed that tubular aggregates contain miscellaneous proteins and that derive from membranes of sarcoplasmic reticulum and mitochondria. No evidence for the presence of contractile and cytoskeletal proteins in TAs was found. Ultrastructurally, TAs are characterized as more or less densely packed aggregates of vesicular or tubular membranes of variable forms and sizes that may contain amorphous material, filaments or inner tubules. Various reported types of tubular aggregates, namely, proliferating terminal cisterns, vesicular membrane collections, TAs with double-walled tubules, TAs with single-walled tubules, aggregates of dilated tubules with inner tubules, aggregates of tubulo-filamentous structures, filamentous tubules, riesentubuli, and related membranous structures including cylindrical spirals are summarized and analyzed here in detail.

Key words: Tubular aggregates — Skeletal muscle — Ultrastructure — Histochemistry — Immunocytochemistry

Introduction

Tubular aggregates (TAs), unusual membranous structures within skeletal muscle fibres, represent a rare but important indicator of human myopathies. Extensive research during last 33 years since their detailed description by Engel et al. (1970)

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has revealed association of TAs with numerous pathologies (Carpenter and Karpati 2001), however, their causality is yet unclear. TAs were found in biopsies of skeletal muscles affected by a variety of disorders like periodic paralysis, intoxication, inflammatory myopathies, cramps and myalgias, myotonia congenita, familial myopathies, and many other myopathies often of uncertain etiology, but also in skeletal muscles of asymptomatic probands (see for instance Engel et al. 1970, Niakan et al. 1985, Rosenberg et al. 1985; Morgan-Hughes 1998; Müller et al. 2001). It is not known, however, whether TAs are pathological structures with harmful effect on the muscle function and should be therefore treated, as could be inferred from their frequent association with myalgias, or whether they represent appreciated structures compensating muscle functions that have been compromised due to action of pathogens, as could be inferred from their ability to sequester calcium. Neither the origin nor function of TAs is completely clear. There is prevailing and widely accepted evidence on the origin of TAs from the sarcoplasmic reticulum (SR) but many data support participation of mitochondria in TAs as well (Lewis et al. 1971; Rosenberg et al. 1985; Vielhaber et al. 2001; Novotová et al. 2002). With the exception of hereditary cases, development of TAs is limited to type II skeletal muscle fibres of males (Engel et al. 1970, Rosenberg et al 1985). That is notable. How it could be that formation of TAs is so specific and so general at the same time? It seems that the answers to similar fundamental questions need a deeper insight into the structure and composition of TAs. Till now, most attention has been paid to the relation of TAs to different human diseases with a limited experimental approach. On the other hand, studies of TAs in animal skeletal muscle fibres, in most aspects similar to human TAs, have shown how instrumental animal models might be in understanding TAs (Schiaffino et al. 1977; Kuncl et al. 1989).

Principal approaches used to identify and characterize tubular aggregates include histochemistry, immunocytochemistry, and electron microscopy. Histochemistry provided overall characteristics of TAs as membranous inclusions with a variety of enzymatic activities. Immunochemistry of TAs provided specific insight into their protein composition. Electron microscopic images of TAs of various pathologies in humans and in various animal models show some degree of variability. We found these aspects of importance, as different types of TAs may have different properties and consequently also different origin and function.

General features of TAs appearance

Light microscopy

Native skeletal muscle fibres containing very large tubular aggregates may show irregular spindle-like ridges of slightly different contrast, extending for several tens of microns. Visualization is improved in fixed preparations (Figure 1) by using general stains like toluidine blue (Rosenberg et al. 1985; Klomkleaw et al. 2001) or paraphenylenediamine (Müller et al. 2001; Vielhaber et al. 2001), but attention should be paid not to confuse TAs with mitochondrial masses, which appear less homoge-

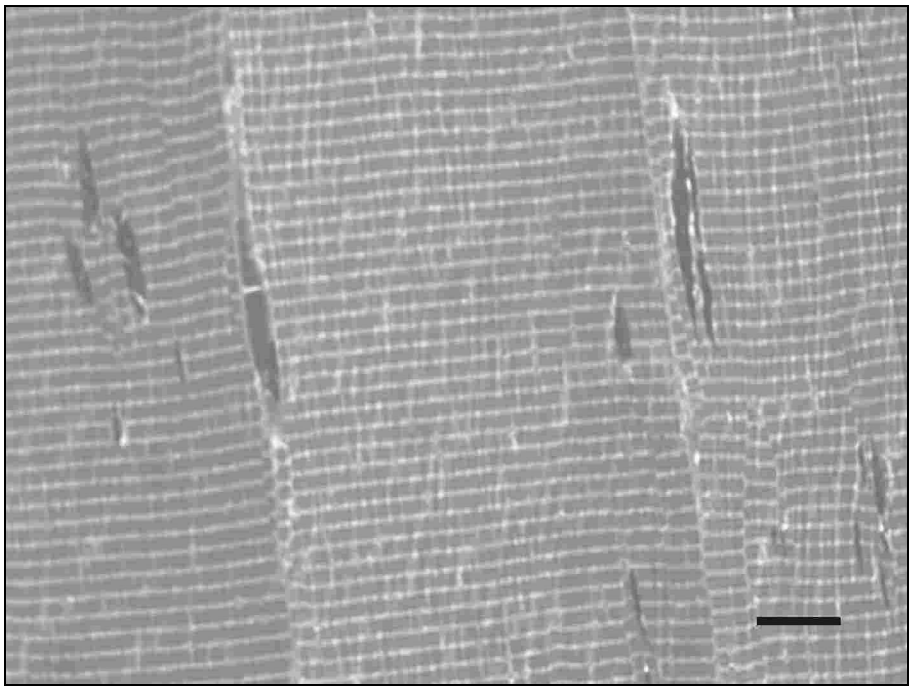

Figure 1. Tubular aggregates in semi-thick longitudinal section of *m. gastrocnemius* $CK^{-/-}$ mouse stained with toluidine blue. TAs appear as dark spindle like patches. Scale bar 10 μm.

nous (Carpenter and Karpati 2001). In thick transversal sections, histochemical reactions reveal tubular aggregates as irregular well defined sharp spots variable in number and size, often covering a substantial part of the muscle fibre cross section. TAs may distribute randomly within the fibre volume but they are often seen near sarcolemma and nuclei. Otherwise, no special association of TAs with specific muscle structures or with specific abnormality of the structure has been reported. Occurrence of TAs varies largely (Rosenberg et al. 1985). Sometimes they are seen in every section of the fibre, sometimes only occasionally. Within the same biopsy, some fascicles could be free of TAs while other showed TAs in every fibre. Affected fibres may be clustered together.

Electron microscopy

Tubular aggregates are more often seen under electron microscope than under light microscope as smaller TAs could be easily observed (Martin et al. 1997). A typical electron microscopic image of an aggregate of tubules is shown in Figure 2. In general, other muscle cell structures such as contractile myofibrils and sarcomeres, nuclei, Golgi system, sarcoplasmic reticulum, sarcolemmal transversal tubules

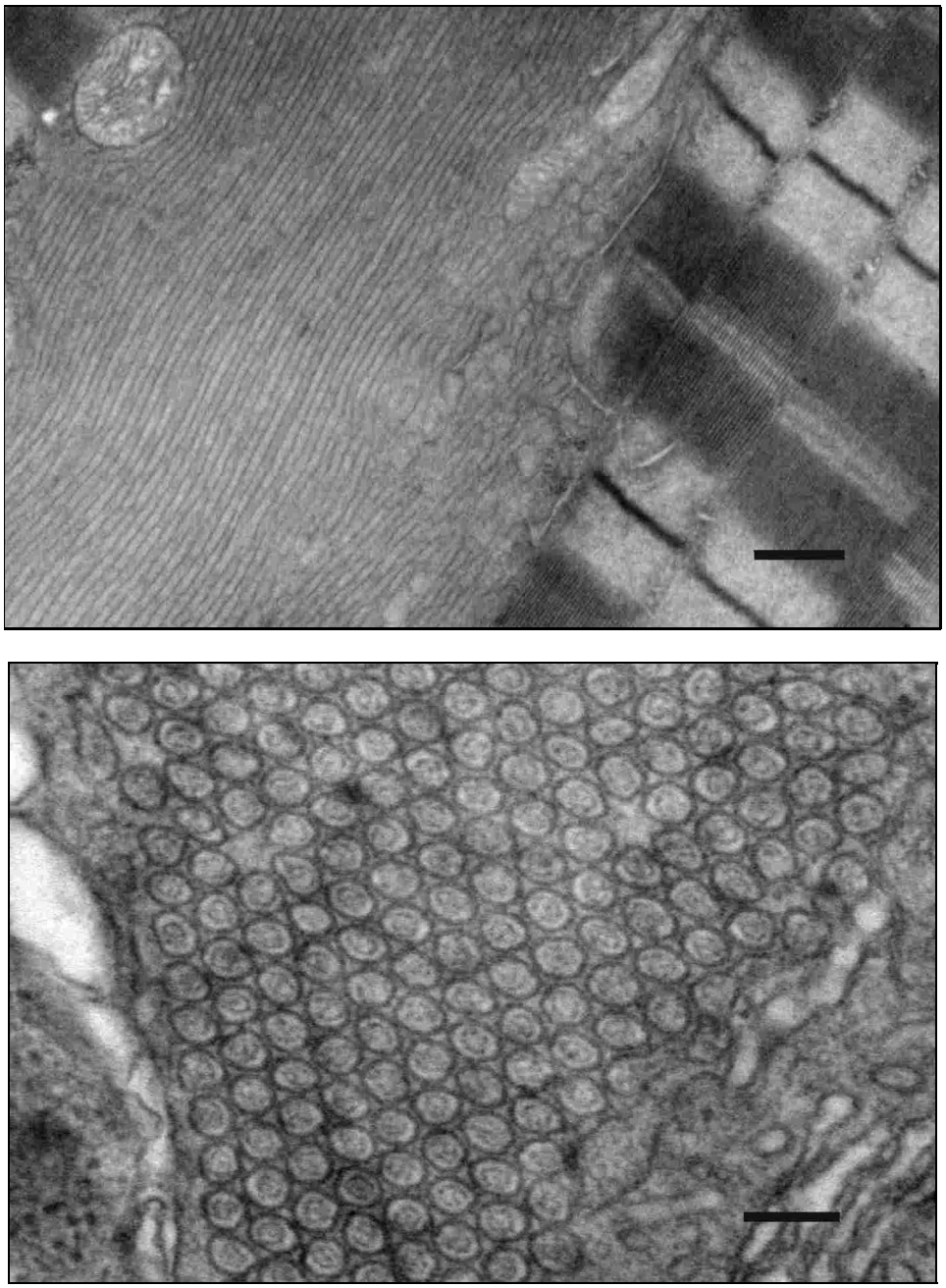

Figure 2. Electron micrograph of the typical tubular aggregate. An ultrathin section of the *m. gastrocnemius* of $CK^{-/-}$ mouse. Upper panel: longitudinal section of the muscle fibre. Lower panel: perpendicularly sectioned TA. Scale bars 500 and 100 nm for the upper and lower panels, respectively.

(T-tubules), triads, and mitochondria may appear the same as in fibres without TAs.

TAs were first ultrastructurally identified under electron microscope as masses of tightly packed long straight tubules (Engel et al. 1970). Later studies revealed variability in appearance of TAs or TA-like structures, which lead to attempts to categorize TAs (see below). The tubules in the aggregates may be either tightly packed and parallel to each other or disoriented, that is, loosely packed with more or less random orientation and with a non-tubular material, usually glycogen granules, in the free space. The tubules of tightly packed TAs are often oriented parallel to the longitudinal axis of the fibre. Nevertheless, the tubules of large aggregates may form sub-clusters with different tubule orientation relative to the fibre. Then, within a single section, tubules that are cut in the longitudinal, oblique or perpendicular direction may be observed. Occasionally, two types of the TAs were observed in the same fibre. Contractile fibrils, sarcolemma, normal or damaged mitochondria and membranous vesicles of variable electron density are usually found in the very vicinity of fully developed TAs. Nearby sarcoplasmic reticulum, terminal cisterns, T-tubules, and triads show standard placement relative to myofibrils. The sarcomeres of myofibrils at the sides of aggregates have normal appearance, but often are seen out of register. TAs stand out in the fibre as rounded defects apparently displacing myofibrils (Lewis et al. 1971). In the longitudinal sections (Fig. 1), the myofibrils to which the TA stands in the way are often seen discontinuous, such as if they were interrupted or cut out according to the contour of the TA, both before and behind the TAs. In other places, the myofibrils are bending around TAs, that is, preserve their continuity.

In spite of the variable ultrastructural appearance under the electron microscope, no related variations in light microscopic or histochemical features of TAs were reported. It should be noted, however, that a direct association between the histochemical and ultrastructural images is difficult, as histochemistry relies on frozen preparations, where the fine ultrastructure would be largely damaged.

Histochemistry

Histochemical descriptions of tubular aggregates often follow the description of Engel (1964), specifically, “focal aggregates of material rich in DPNH (now NADH) and TPNH (now NADPH) tetrazolium reductase activity”. This is a very common feature often regarded as a fingerprint of TAs in human tissue and in mouse or other animal models. Major disadvantages of these tetrazolium reductase-linked reactions are their poor discrimination among membranous organelles (Carpenter and Karpati 2001, chapter 2). Therefore, a combination of various markers should be used for convincing evidence on the presence of TAs.

In frozen sections, tubular aggregates present a large spectrum of histochemical reactions. TAs stain characteristically with NADH- and NADPH-tetrazolium

reductases (TR), Gomori's trichrome, hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Oil red O. Nevertheless, reactions of TAs cannot be considered invariable, as there are some controversies and disputes. For instance, at odds with other studies, Engel et al. (1970) reported a lack of basophilic reaction to H&E and faint dull red reaction with Oil Red O. Agbulut et al. (2000) described TAs in muscle fibres of male inbred mice that were stained by Gomori's trichrome and reacted to anti SERCA1 ATPase antibody but stained negatively with NADH-TR and succinate dehydrogenase reactions. Rosenberg et al. (1985) observed positive reactions to NADH-TR, trichrome, H&E, Verhoeff van Gieson, and non-specific esterase, but inconsistent reactions to PAS and Oil red O. Moreover, these authors clearly demonstrated positive reactions of TAs to succinate dehydrogenase (SDH) and α-glycerol phosphate dehydrogenase (GPDH), in sections serial to sections with prominent NADH-TR stain. For reasons not well understood, in most other studies these important mitochondrial marker enzymes were not reactive in TAs. Therefore, it was generally accepted that TAs couldn't be of mitochondrial origin, in spite of the fact that some other mitochondria-specific enzymes could be detected (Lewis et al. 1971).

Unambiguous consent was reached, however, on lack of contractile and cytoskeletal proteins in TAs. There were some expectations, as many types of TAs show conspicuous fibrillar components and therefore were assumed to contain this type of proteins. In classical staining experiments for myofibrillar ATPase, muscle fibres with TAs show empty or unstained patches co-localizing with regions positive in histochemical reactions for TAs. A similar picture was observed with any other cytoskeletal protein immunocytochemical reaction (Rapuzzi et al. 1995; Vielhaber et al. 2001).

Lipid composition of tubular aggregates was studied in detail by Lewis et al. (1971). These authors reported that TAs identified as variable masses of weakly basophilic material (H&E) stained bright red with modified Gomori's trichrome method and positive to PAS were also positive to Sudan Black, Acid haematin, Solochrome cyanine, Plasmal reaction, and UV-Schiff, but negative to Mallory's phosphotungstic acid haematoxylin and alcoholic Luxol Fast Blue. Lewis and co-authors (1971) concluded that the predominant lipid of the aggregates is an acetone- and alcohol-soluble acidic phospholipid containing a high proportion of plasmalogens (derived phosphoglycerids) and unsaturated fatty acids, a pattern compatible with mitochondrial lipid composition in mouse skeletal muscle.

Taken together, histochemistry of TAs seems to be complex. Part of the problem could be in unstable association of soluble enzymes with cellular structures (Meijer 1988). In this systematic histochemical study, Meijer concluded: "in view of the histochemical findings it is not possible to reach a sound conclusion about nature of these pathological structures". Therefore attention of investigators interested in understanding the composition and origin of TAs turned to electron microscopy, immunochemical identification of its protein constituents, or a combination of the two.

Immunochemistry

Protein composition of tubular aggregates was first assessed by Salviati et al. (1985) who provided evidence for the Ca-pump and calsequestrin, marker proteins of the sarcoplasmic reticulum, in human TAs by means of immunofluorescent staining. These findings provided strong support for Engel's et al. (1970) hypothesis on SR origin of TAs. Later studies confirmed the presence of both, SERCA2 (Martin et al. 1997 and Vielhaber et al. 2001, in humans) and SERCA1 (Agbulut et al. 2000 and Novotová et al. 2002, in mouse) isoenzymes of Ca-pump ATPase, and of calsequestrin (Vielhaber et al. 2001, in humans). The ryanodine receptor of the SR cistern membranes, namely the skeletal muscle-specific RyR1 isoform of the calcium release channel, was also identified in tubular aggregates (Vielhaber et al. 2001). Documentation of the four major proteins of the sarcoplasmic reticulum and its terminal cisterns provided indisputable evidence for participation of SR in formation of tubular aggregates.

Very recently immunochemical evidence for the presence of a mitochondrial protein in TAs has been obtained. Novotová et al. (2002), using a double immunogold labelling approach, showed that the bc 1 complex, the integral inner mitochondrial membrane protein of the complex III of the respiratory chain, occurs in TAs of $CK^{-/-}$ mice together with SERCA1 protein. This new finding gave strong support to the old hypothesis (Engel 1964; Lewis et al. 1971) on participation of mitochondria in formation of TAs, based on histochemical reactions, and led to formulation of a new hypothesis on the joint participation of both the SR and the mitochondrial membranes in formation of TAs (Novotová et al. 2002).

Other findings of the mainstream TAs research have also disclosed that the protein composition of TAs is not just that of the SR. TAs were shown to contain epitopes of the 72 kD heat shock protein (Martin et al. 1991), which is known to undergo increased synthesis following different cellular insults including physical trauma or chemical injury. It should be noted that heat shock proteins are known to reversibly change the conformation of proteins and that they participate in transport of proteins to mitochondria. Martin et al. (1997) in their extensive immunohistochemical study report indication of association of the tau protein with TAs.

Robertson et al. (2000) showed that TAs in muscles of apolipoprotein E (ApoE) deficient mice provided a positive reaction to antibodies against Alzheimer's amyloid peptide and the beta amyloid protein, both of which diminished after supplying the animals with the lipid transport protein ApoE. These authors claim that in their ApoE deficient mouse accumulation of TAs may result from the five-fold accumulation of lipids/cholesterol due to the disturbed lipid metabolism. The presence of amyloid peptides in TAs could be explained by the role of TAs in degradative mechanisms, which, when disturbed, may result in accumulation of metabolites in their lumens.

Tubular aggregates do not contain immunochemically detectable amounts of the major proteins of the contractile myofibrils and of cytoskeleton. Vielhaber et al.

(2001) and Martin et al. (1997) in their extensive studies found TAs negative to a spectrum of antibodies against muscle cell cytoskeletal and membrane proteins involved in different forms of muscle dystrophies and sarcoglycanopathies: dystrophin 1, 2, 3; β-dystroglycan; desmin; α2-laminin; merosin; developmental myosin heavy chain; α-, β-, γ-, δ-sarcoglycan; β-spectrin; ubiquitin; utrophin; and vimentin.

The protein composition of tubular aggregates suggests that formation of TAs results from abnormal adaptation or pathological processes that involve membranous organelles rather than the cytoskeletal system. Nevertheless, the origin of the filamentous structures in tubular aggregates remains unknown.

Morphology and ultrastructure

Engel et al. (1970) showed continuity of some tubules with the sarcoplasmic reticulum and pointed to a similar content of amorphous material in TAs as that present in normal lateral sacs of the sarcoplasmic reticulum. As they did not found continuity of TAs with mitochondria, they thought TAs to be massive proliferations of the SR. This conclusion was affirmatively reverberated in many studies but only occasionally documented. In fact, observation of the continuity of the SR with any TA type is rather rare (Van Engelen and Ter Laak 1999). With more rigorous criteria electron microscopic evidence can be even questioned, as the thickness of tissue sections is usually larger than the diameter of the SR or TA tubules and spatial dynamics of these structures within the section volume is very high. In effect, membranes are often sectioned oblique, providing images with a relatively low resolution of the membrane limits, which mostly prohibits identification of membrane continuity beyond doubts. Likewise, the similarity of the TA content with the content of the SR terminal cisterns may be questioned. Firstly, the appearance of the TA content, i.e., granularity, electron density and contrast, under electron microscope is variable, and additionally, the content of the mitochondrial matrix has similar appearance as that of the terminal cisterns or tubular aggregates. Other membranous structures that in principle might contribute to TA formation were excluded. Engel et al. (1970) excluded mitochondria according to the lack of their continuity with membranes of TAs, and Pierobon-Bormioli et al. (1985) excluded the T-tubule system because TAs were not loaded by lanthanum from the extracellular space.

Already in the study of Engel et al. (1970), variability in morphology of tubular aggregates and occurrence of other types of membranous aggregates were reported. These included variability in the number of inner tubules, hexagonal or random packing of tubules in the aggregates, and the occasional presence of quadrilaminar structures formed by two adjacent dilated sacs. Soon after that seminal report, Schröder and Becker (1972) described six types of TAs in biopsies from human muscles and provided the first classification according to their ultrastructure: Ia, proliferated terminal cisterns; Ib, tubular aggregates with a central tubule- or a microtubule-like structure; Ic, tubular aggregates with empty or moderately dense flocculent material; Id, dilated parallel tubules; IIa, tubules with filaments; and IIb, giant tubules with filaments.

A different ultrastructural classification was suggested by Maron and Ferrans (1974). They classified any aggregates of tubular structure in any tissue into two basic groups, single-walled tubules and double-walled tubules. The single-walled tubules were subclassified according to branching within the aggregates. The branching single-walled tubules were further classified according to their continuity with the T-tubule system or with the sarcoplasmic reticulum. The aggregates of non-branching single-walled tubules and aggregates of double-walled tubules were not further subclassified. On the other hand, Cameron et al. (1992) categorized TAs into three types according to their own and Morgan-Hughes et al. (1981) electron microscopic observations: I, closely packed parallel tubules with a smaller central tubule; II, tubular structures containing a moderately dense flocculent material; III, dilated parallel tubules containing a number of smaller microtubule-like structures. Müller et al. (2001) related Schröder's and Becker's types Ib, Ic, and Id to types I, II, and III of Cameron and coworkers, respectively, and added a new, previously not described type Ie – tubular aggregates with tubulofilamentous structures, to the list of Schröder and Becker (1972).

Comparison of these classifications and including other reports led us to the opinion that verbal description and illustration of individual observations led to some divergence in classification of TAs. Therefore, instead of following the more or less arbitrary classification criteria, we attempted to summarize the published ultrastructures of tubular aggregates either in humans or in animal models. Figure 3 illustrates typical features of different types of aggregates which we characterize as: proliferating terminal cisterns (PTC); vesicular membrane collections (VMC); tubular aggregates with double walled tubules (DWTA); tubular aggregates with single walled tubules (SWTA); aggregates of dilated tubules with inner tubules (DTTA); aggregates of tubulo-filamentous structures (TFTA); filamentous tubules (FTTA); riesentubuli (RTTA); and other membranous structures.

Proliferating terminal cisterns (PTC)

Figure 3A. Abundant membranes arising from proliferating terminal cisterns of the sarcoplasmic reticulum are easily recognized according a characteristic electron density of their luminal content and their continuity with terminal cisterns (TC) near the I-band and the T-tubules. Membrane proliferations of this type frequently protrude along the myofibrils, replacing the network of the longitudinal SR over a few sarcomeres. Then, in longitudinal sections of the muscle fibre, the PTCs are cut longitudinally and display single or double membranes arranged regularly with 80–90 nm spacing (Schröder and Becker 1972). The double membranes with a structure similar to that of triads are separated by 20 nm (Schröder and Becker 1972, Fig. 4c). An occasional single-membrane form of the sectioned aggregate (Engel et al. 1970, Fig. 3) is difficult to reconcile with aggregated tubules, rather it suggests a bag-like structure folded into meanderings as in bellows. Unfortunately, the appearance of these structures in transverse sections was not described. Terminal cisterns may eventually proliferate irregularly, leaving the I-band area and T-tubules and develop into various formations (Schröder and Becker 1972, Fig. 5c;

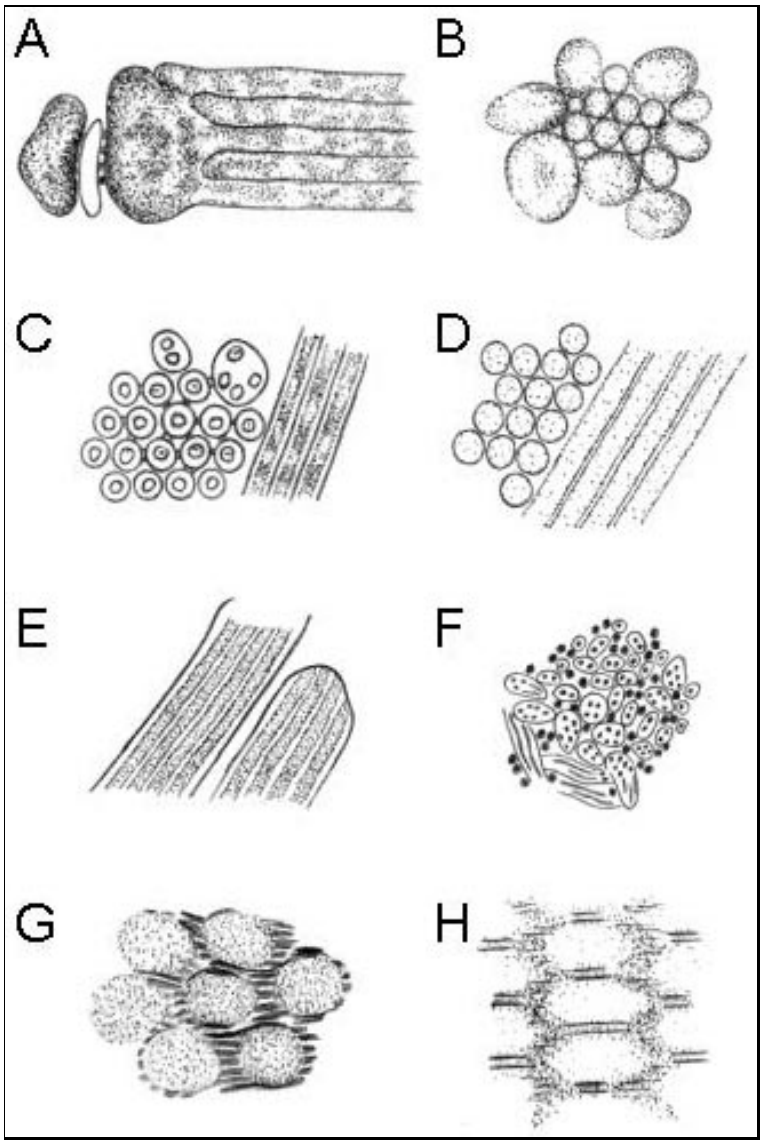

Figure 3. A schematic representation of tubular aggregates of various types. The schemes are drawn in relative proportions.

Müller et al. 2001, Fig. 3e,g, and 4b,c,d). In contrast to normal TC, both the regular and irregular types of TC proliferations lost the budding branches (outgrowth) of the longitudinal SR tubules. Electron micrographs of the proliferation of the SR cisterns are documented in Engel et al. (1970; Fig. 4); Schröder and Becker (1972;

type Ia; Figs. 4c, 5a–c); Van Engelen and Ter Laak (1999); and Müller et al. (2001; Figs. 3e, 4b–d).

Vesicular membrane collections (VMC)

Figure 3B. Dilated tubules or vesicles, or oval bodies bounded by a single membrane, containing unrecognized non-fibrillar material were shown by Engel et al. (1970) and Lewis et al. (1971) in the vicinity of the classical TAs. Similar rounded structures of variable diameter, filled with a moderately dense flocculent material instead of the central tubule, were identified as a new entity by Cameron et al. (1992, type II, Fig. 4) in subsarcolemmal aggregates. Although tightly packed, the vesicular collections do not show hexagonal arrangement and vary widely in size (70–400 nm) within the same cluster. These membrane aggregates were not observed in the longitudinal direction, suggesting that in fact they may represent vesicular and not tubular collections. Müller et al. (2001) assigned this type of aggregates (the Cameron's and co-workers type II aggregates) to the type Ic of the Schröder and Becker (1972) classification, that is, the densely packed and hexagonally arranged tubular structures of 40–50 nm in diameter that are empty or filled with a moderately dense flocculent material. We see Cameron's type II and Schröder and Becker type I_C as different structures (see also SWTA below).

Tubular aggregates with double-walled tubules (DWTA)

Figure 3C. Very large assemblies of tightly packed long membranous tubules arranged in a hexagonal pattern, described originally by Engel et al. (1970). A hallmark of these classical TAs is a double wall appearance, that is, within a larger outer tubule of 50 to 80 nm in diameter, a smaller inner tubule or an electron dense material of tubular form of 20–30 nm in diameter is contained (Engel et al. 1970, Lewis et al. 1971). Although the space between the inner and outer tubules is empty, they are still fairly concentric. Some of the outer tubules that contain two or even more inner tubules (up to 10) were of larger-than-proportional cross-sectional area, with inner tubules often arranged near their circumference. Within a single section of very large TA clusters, sub-clusters of tubules sectioned perpendicularly, obliquely and also longitudinally were seen (Cameron et al. 1992, Fig. 2; Morgan-Hughes 1998; Martin et al. 1997, Fig. 3B; Vissing et al. 1999, Fig. 1), suggesting a lack of particular DWTA orientation within the myofibres. The endings of the outer tubules are closed at the periphery of the aggregate but sometimes also in the centre of the cluster, as can be inferred from a missing single tubule or even four tubules in a particular perpendicular section that is present in the nearby slices, as the tubules still keep a regular hexagonal arrangement. The endings are slightly dilated, with the inner tubules terminating there as a bulge of amorphous granular material, sometimes resembling a drumstick (Engel et al., 1970) in a bag. Although tightly packed, the outer tubules do not touch each other, probably due to small bridging structures frequently seen in pairs between outer surfaces of their membranes.

Tubular aggregates with single-walled tubules (SWTA)

Figure 3D. These structures strongly resemble classical DWTA in many aspects. Similar to DWTA, the SWTA also lack a particular orientation within the muscle fibres and are well defined in both the longitudinal and transversal directions (Agbulut et al. 2000, Fig. 3; Robertson et al. 2000, Fig. 3; Craig and Allen 1980, Figs. 6 and 8). Few tubules may be missing in the middle of the cluster while the surrounding tubules still keep the regular hexagonal arrangement, as do the DWTA. Bridging structures, or spacers, also can be seen between the tubules in pairs (unpublished data). However, there are substantial differences from the ultrastructural point of view. In SWTA, the individual tubules are either empty, or contain pale to moderately dense material, but not inner tubules or other structured material. Diameter of the SWTA tubules is fairly constant both along the long axis and among aggregates. The tubules of SWTA are slightly thinner than the outer tubules of DWTA, about 40 to 50 nm in diameter (Schröder and Becker 1972). SWTA do not contain large diameter tubules, as do DWTA with multiple inner tubules. Schröder and Becker (1972) denoted these TAs as type Ic. Müller et al. (2001) related the Schröder's and Becker's type Ic to the type II described by Cameron et al. (1992). Nevertheless there are substantial differences regarding the size. While type Ic aggregates of Schröder and Becker varied only slightly in diameter, around 40–50 nm, the size of Cameron's et al. type II TAs varied substantially between 70–400 nm. See also VMC described above.

Aggregates of dilated tubules with inner tubules (DTTA)

Figure 3E. Aggregates of double wall tubules consisting of much wider (130–400 nm) and much shorter "dilated" tubules than classical TAs, and containing a number of smaller inner tubules of 25–40 nm in diameter were described by Cameron et al. (1992) and classified as a new type III aggregates. Both the outer and inner tubules ended as expected for membranous tubular structures. The inner tubules, in contrast to inner tubules of DWTA, are slack, often slightly undulated, and sometimes even bent, as if being longer than could be accommodated freely by the outer tubule. In contrast to DWTA and SWTA, the DTTA are less compact and less organized structures. While the inner tubules lie always in parallel and equidistant inside the outer tubule, the outer tubules form mostly irregular clusters. Consequently, individual tubules within a single section appear cut in any direction.

Aggregates of tubulo-filamentous structures (TFTA)

Figure 3F. A new type of TAs discovered recently by Müller et al. (2001) in dominantly inherited familial myopathy may be characterized as large accumulations of relatively short membranous tubules of a variable diameter (30–200 nm) proportional to the number of inner filaments (1–21). The inner filaments of 14–18 nm in diameter sometimes show a light centre, suggesting that they may represent hollow fibres or tubules. Individual filaments were separated from each other or

from the wall of the tubule by 10 nm with no spacing bridges reported. The aggregates are mostly formed of single- or double-filament tubules packed at a moderate density, arranged at varying directions without a special symmetry pattern or regularity. The free space between tubules is loosely filled with glycogen granules. When cut longitudinally, on the first sight they do not look much different from DTTA, namely, the outer tubules seem to be relatively short and not arranged in any particular direction. Nevertheless, both quantitative and qualitative differences are clear. To this type could be assigned also TAs described by Maron and Ferrans (1974) in cardiac myocytes.

Filamentous tubules (FTTA) and riesentubuli (RTTA)

Figure 3G and 3H. Schröder and Becker (1972, Fig. 7b–d) observed a distinct type of round-oval filamentous bodies of up to 6.5 μm long and 2.5 μm in diameter within muscle fibres and classified them as type IIa tubules – tubules with filaments. In cross section, the profiles of tubules 90–140 nm in diameter, were blended or connected with about 10 nm thick filaments. Müller et al. (2001, Fig. 2a) have recently described similar structures with tubule profiles of only about 40–50 nm in diameter, which were also classified as type IIa. Cross sections of these structures were organized in a hexagonal pattern, although not as regularly as classical TAs. In a single section, the fibres were seen to connect either to the nearest or to the next-to-nearest tubule profiles. Schröder and Becker (1972, Fig. 7b–d) also described a single observation of "riesentubuli" or giant tubules of otherwise similar construction to their type IIa but with tubule profiles of about 200–250 nm in diameter and classified them as type IIb giant tubules with filaments. Longitudinal sections of FTTA and RTTA (type II tubules) were not reported and therefore the term tubular does not seem to be fully substantiated. Alternatively, the images might be suggestive of a sponge-like structure as well.

Other membranous structures

Of interest should be also cylindrical spirals (CS), membranous structures described first by Carpenter et al. (1979) that provide similar cytochemical reactions as TAs but, according to Carpenter and Karpati (2001), have characteristics that allow to distinguish them from tubular aggregates. Likewise TAs, however, the cylindrical spirals occur in clusters in type IIb fibres of males, are not disease specific, and often accompany tubular aggregates (Rapuzzi et al. 1995). Rarely, small spirals or concentrically circular structures can be found within tubular aggregates, namely within SWTA (Carpenter and Karpati 2001, Fig. 9. 116), or may merge into tubular vesicular structures strongly resembling tubular aggregates (Danon et al. 1989; Taratuto et al. 1991). The TAs accompanying CS could be classified as the SWTA type (Carpenter and Karpati 2001). It would not be surprising if the CS and TAs were shown to be of the same origin.

Many investigators report abnormal membrane structures in muscles with histological reactions indicating presence of TAs. Engel et al. (1970; Figs. 9, 10) described stacks of quadrilaminar-saccular complexes formed by large dilated sacs in

some regions of classical TAs. At contact areas between sacs, covering most of the sac surface, the membranes were separated by two laminae, thus forming the 27 nm wide quadrilaminar membranes with centers of laminae separated by 8.7 nm. The laminae between membranes showed wire-screen like crystalline structure when cut *en face*. Schröder and Becker (1972; Fig. 4e,f) described system of large flattened laminar cisterns arranged in stacks, with intermembrane separation by 20–25 nm, and classified them as type III. Müller et al. (2001) observed annulate lamellae between their type Ie TAs (their Fig. 3f; see TFTA). Craig and Allen (1980, Fig. 7) demonstrate very large dilated sacs connected to the sarcoplasmic reticulum.

Descriptions of proliferated terminal cisterns adopting bizarre shapes and size (Müller et al. 2001, Figs. 3d,e,g, 4b–d, 5b,c; Schröder and Becker 1972, Fig. 5c) or of mitochondria in different stages of deterioration (Craig and Allen 1980, Klomkleaw et al. 2001, Fig. 3b; Müller et al. 2001, Fig. 5) are reported occasionally.

Conclusions

Taken together, it seems that tubular aggregates, as referred to in the literature, are anything but well-defined invariable structures. Rather, TAs should be seen as dynamic structures, which commence and cease, progress and retreat, change their structure, functionality, and composition under multi-factorial yet not well defined influences. Factors affecting formation of TAs in skeletal muscle fibres may, however, have different structural and/or functional influences on other cell types. For instance, Robertson et al. (2000) in addition to TAs in type II muscle fibres found large mitochondria tightly packed with cristae in type I muscle fibres and fibrillo-granular inclusions in hippocampal protoplasmic astrocytes in ApoE deficient mice. Vielhaber et al. (2001) have found decreased respiratory chain enzyme activities in 5 out of 7 patients with TAs and pointed to a possible functional link between mitochondrial dysfunction and presence of TAs, as proposed by others before (Pierobon-Bormioli et al. 1985; Bendahan et al. 1996; Nishikawa et al. 2000) and as supported by growing evidence on participation of mitochondria in the development of TAs (Novotová et al. 2002). Comparative studies, especially on transgenic animals, might be instrumental in this field.

From the point of muscle physiology it is of interest why type II fibres are so much more prone to develop TAs than type I fibres. Yet there seems to be no clue how to solve this puzzle. The structural and functional development of tubular aggregates remains also unknown. Nevertheless, recent methodological approaches show directions that may move the field forward.

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