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EDITORIAL

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Sealing t-tubules increases the energy cost of cardiac contraction

The work "Disruption of transverse-tubular network reduces energy efficiency in cardiac muscle contraction" by Mellor et al[1] that appears in this issue of Acta Physiologica brings together the scientific interests and technical skills of two worlds: that of fine intact cardiac muscle energetics and mechanics (eg Tran et al[2] and other studies by Loiselle and his collaborators) and the one of disease-associated cardiac t-tubule remodelling (eg Crossman et al[3] and other studies by Soeller and his collaborators). We would like to emphasize that the experimental data presented in Mellor et al[1] comes from the only laboratory in the world that, to date, is able to couple sophisticated force recordings to microcalorimetric measurements employing intact cardiac preparations of small size (ie single trabeculae) that are ideal in terms of fibre alignment, superfusate flow, etc for both mechanical and energetic assessments.[2] With their long-standing experience, the authors show here how mechanical parameters (muscle stress, muscle shortening velocity and muscle power) and energetic parameters (activation heat, peak cross-bridge heat and peak shortening heat) are altered following an acute disruption of t-tubules. To acutely detached t-tubules from the surface sarcolemma, the authors apply an osmotic shock protocol, developed in the 2000s on single cardiomyocytes,[4] and more recently adapted for use on multicellular cardiac preparations.[5] Formamide is an easily diffusible small molecule that, if added to the extracellular medium in high dosage, accumulates inside the cells without damaging them. When Formamide is removed from the extracellular environment (ie when the extracellular solution returns isoosmolar with the intracellular medium) the cells undergo a transient and rapid increase in volume (+17% approximately) which causes subsarcolemmal fragmentation of t-tubules and their seal off from the surface. This technique has been shown to be effective in isolated cardiomyocytes but was thought to be unsuitable in multicellular preparations where the spatial packing of the myocytes themselves and the diffusional delays tend to prevent rapid volume changes. Ferrantini et al[5] successfully adapted the formamide protocol to thin multicellular preparations (mainly right ventricular trabeculae with diameter <200 μm) and the procedure was subsequently adopted with no modifications and comparable results by Power et al[6] In the present work, Mellor and collaborators[1] made the osmotic shock slightly more aggressive (2 mol/L Formamide applied for 20 minutes, instead of 1.5 mol/L applied for 15 minutes) and managed to effectively detubulate larger trabeculae dissected from the left ventricle. A slightly lower efficacy of the detubulation procedure (34% vs 58% decrease in mean TT power, the index used to quantify t-tubule disruption) can be attributed to the larger preparation size and small differences in the protocol (eg the rate of superfusate flow for formamide removal, that is critical for t-tubule detachment, was higher in Ferrantini et al[5] (>5 vs 2 μL/s). What has been shown in all three studies by Mellor et al,[1] Ferrantini et al[5] and Power et al[6] using either confocal or two-photon microscopy—with the latter being more appropriate for the higher z-penetration depth—is that Formamide-shock leads to a very inhomogeneous detachment of t-tubules, resulting in a great variability in the degree of detubulation between different cells of the same trabecula, but also between different areas within the same cardiomyocyte. Far from being a limitation of the protocol, the use of formamide on multicellular preparations produces a t-tubule distribution pattern that very closely mimics that found in cardiac pathological remodelling. In heart failure and other cardiac diseases (eg HCM), in fact, the loss of t-tubules is patchy and non-homogeneous.

The formamide-induced acute detubulation model, unlike the disease-associated t-tubule remodelling, is an ideal platform to study the mechanical and energetic impact of t-tubule network disruption, as the t-tubule loss is not associated with any other type of damage. Detached tubules remain trapped inside, disconnected from the surface sarcolemma therefore not able to propagate the action potential (as confirmed with Voltage Sensitive Dyes,[5]), without leading to any structural alterations of the sarcoplasmic reticulum (SR) or surface sarcolemma. Can the acutely detached tubules reattach themselves to the surface? This possibility has been suggested by both Mellor et al[1] and Ferrantini et al[5] because some mechanical parameters that initially undergo a variation, for example, the twitch duration, tend to return to baseline values about 20-30 minutes after the osmotic shock. This may be related to the mechanical activity of the twitching muscle that favours

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t-tubule resealing, but the possible recovery of t-tubule connections to the surface was not adequately investigated by taking images at different times (10, 20 or >20 minutes) after the osmotic shock.

Following the detachment of t-tubules, active stress is reduced, more pronouncedly at high pacing rates, and contraction kinetics are prolonged. These mechanical changes are robust findings because of the intrinsic experimental modality (coupled comparison between data obtained from the same muscle before and after detubulation) and are further strengthened by qualitatively and quantitatively concordant results in Mellor et al,[1] Power et al[6] and Ferrantini et al.[5]

Mellor and collaborators[1] are the first to perform mechanical measurements on detubulated trabeculae during work-loop contractions. In addition to reduced stress at any afterload, they show that following detubulation, trabeculae shorten less, at a reduced velocity and with reduced power. The reduced shortening at low afterloads, coupled with the reduced stress, results in reduced mechanical work output. Mellor and collaborators[1] also report that detubulation reduces activation heat by 20% (consistent with 21% reduction in the Ca^{2+} transient amplitude reported by Ferrantini et al[5]) and reduced cross-bridge heat by 34% (in line with the approximately 30% reduction in active stress reported by Mellor et al,[1] Power et al,[6] and Ferrantini et al[5]).

As the authors nicely explain, cross-bridge heat can be partitioned into an isometric component and a shortening component, the proportions of which depend on afterload. Under isometric conditions, cross-bridge heat consists entirely of isometric heat, and is quantified as the peak cross-bridge heat, that is indeed reduced after t-tubule detachment. Peak shortening heat at virtually zero afterload, instead, is not significantly changed, in line with no significant changes of peak shortening. In the detubulated myocardium, given an essentially unchanged maximal shortening velocity and a relative force-velocity relationship that looks like the same as before acute detubulation, the reduction in power is much likely due to a reduction in the number of motors (while the properties of the motors are maintained the same); this reduction in the number of the motors is consistent with a reduction in the calcium activation following detubulation.

In fact, in simple terms, Mellor et al[1] show that changes occurring in the detubulated myocardium create a discrepancy between the marked decrease in force production by the myofilaments and the pretty modest decrease in their energy consumption. The authors did not investigate the mechanisms underlying this discrepancy that increases the energy cost of tension generation. One possible explanation is that the loss of tubules introduces a great inhomogeneity during calcium activation. Areas without tubules connected to the surface are activated with a delay, through a cascade-like mechanism based on calcium diffusion/propagation from neighbouring areas (see for instance Ferrantini et al[7] or other reviews on this topic). This *propagated* E-C-coupling mode, associated with t-tubule disruption, causes the coexistence at the same time of in-series sarcomeres with different levels of calcium activation (higher levels of activation where the tubule is maintained, lower levels where the tubule is disconnected), thus establishing a mechanism of inter-sarcomere dynamics and inhomogeneity of sarcomere lengths *during* contraction. Of note, as Mellor et al[1] and Ferrantini et al[5] observed, resting sarcomere length is not made inhomogeneous by the detubulation process per se (eg in Ferrantini et al[5] sarcomere length fluctuations and its range of variability slightly increased during Formamide exposure but were normalized upon Formamide removal). During contraction, instead, the inhomogeneity of calcium activation levels and consequently sarcomere lengths—that particularly occurs during the twitch rising phase—leads to a reduction in the developed tension, while the consumption of ATP at any given tension level may increase (because of the non-isometric sarcomere working conditions), creating the energetic discrepancies described by Mellor et al[1] This mechanism could be tested experimentally by making calcium activation within the detubulated preparations more homogeneous through positive inotropic interventions (eg β-adrenergic stimulation, high extracellular calcium levels) or through the administration of pharmacological agents that resynchronize the calcium transient by favouring calcium propagation in the regions in which the tubules are disconnected (eg low doses of caffeine as in Ferrantini et al[5]). It would be of interest to see whether, under these conditions of increased/resynchronized calcium activation, the detubulated myocardium still shows a reduction in energy efficiency of about 30%, or if the energy efficiency is restored to values that are closer to those of the healthy myocardium.

We believe that the work of Mellor et al[1] that exploits the detubulated myocardium as an experimental tool, is the first to demonstrate that t-tubular disruption represents one structural pathophysiological pathway that can contribute to reduction in cardiac mechanical efficiency in disease conditions. The energy impairment introduced by t-tubule disruption can be relevant in the pathophysiology of many cardiac conditions associated with t-tubular structural remodelling and consequent loss of synchrony in calcium activation (eg Ferrantini et al[7]). First is Heart Failure, with an increased energy cost of contraction that notoriously derives from reduced myocardial force due to structural alterations (eg reduction of myofilaments density, increased presence of collagen) besides the increase in energy consumption required for calcium cycling and, likely, at myofilament level. Of high relevance are also Hypertrophic Cardiomyopathy, with cardiomyocyte energy depletion often due to altered cross-bridge kinetics (because of the presence of a mutant sarcomeric protein or its lack of expression) (eg Piroddi et al[8]) and Dilated Cardiomyopathy, with reduced mechanical efficiency due to impaired tension transduction to the sarcolemma and the extracellular matrix.

In all these conditions, a loss of t-tubules and a desynchronization of the calcium transient have been reported and the pathophysiological mechanism described by Mellor et al,[1] that is, a "t-tubule depletion"-associated increase in the energy cost of tension generation, can represent an additional source of energy mismatch on top of others.

CONFLICT OF INTEREST

There is no conflict of interest.

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