Mechanical properties of normal and mdx mouse sarcolemma: bearing on function of dystrophin

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Summary

The tensile strength of the muscle fibre surface membrane was estimated (1) from the suction required to burst membrane patches and (2) by aspiration of sarcolemmal vesicles into micropipettes of uniform bore. Each method gave an average value close to 60 μN cm⁻¹ for the maximum tension sustainable by normal mouse sarcolemma and only slightly lower values for sarcolemma from mdx mice which lack dystrophin. The elastic modulus of area expansion, as measurable by pipette aspiration of sarcolemmal vesicles, was found to have an average value of 3160 μN cm⁻¹ for normal and 2770 μN cm⁻¹ for mdx mouse sarcolemma.

The tensile strength of the sarcolemma is much too small for any differences in it to be the basis for the different osmotic behaviour of normal and mdx muscle fibres reported recently (Menke & Jockusch, 1991). By analogy with the better understood origin of the osmotic fragility of different types of red blood cells, the higher osmotic fragility of mdx muscle fibres is suggested to be of morphological origin. We postulate that dystrophin functions as an element of the submembrane cytoskeleton so as to maintain the normal folding which safeguards the sarcolemma against mechanical damage.

Introduction

In Duchenne muscular dystrophy (DMD), skeletal muscle fibres undergo segmental necrosis which has been ascribed to localized damage of the surface membrane (i.e. sarcolemma) of the fibre (Mokri & Engel, 1975; Carpenter & Karpati, 1979). This view has been strengthened by the discovery that in DMD the protein dystrophin is missing or malformed (Hoffman & Kunkel, 1989). Dystrophin is closely associated with the sarcolemma and its structure suggests that it is an element of the submembrane cytoskeleton (Zubrzycka-Gaarn et al., 1988). The mechanical properties of the sarcolemma and how far these depend on the presence of dystrophin are therefore of interest.

Here we describe methods for determining several mechanical parameters of the sarcolemma, and we compare sarcolemma from normal murine muscle with sarcolemma from a strain of mice (mdx) which lacks dystrophin. We find only a small difference in tensile strength between normal and dystrophic sarcolemma and we are therefore led to the view that the propensity to membrane damage of muscle fibres lacking dystrophin may result from a lesser degree of membrane folding, a feature which in normal muscle ensures that the total membrane area can accommodate large extension in fibre length (Dulhunty & Franzini-Armstrong, 1975). This hypothesis can account for the recently reported increased osmotic fragility of mdx fibres (Menke & Jockusch, 1991) without the necessity to postulate differences in the intrinsic mechanical properties of the muscle membrane.

Materials, methods and results

In a first attempt to estimate the maximum tension $T^*$ (μN cm⁻¹) which the sarcolemma can sustain, we adapted the patch-clamp technique to measure the suction pressure at which membrane patches rupture, $P^*$ (μN cm⁻¹). Using fibres isolated from muscle flexor digitorum brevis (FDB; Bekoff & Betz, 1977) in a solution whose chief component was 140 mM K-methylsulphate, gigaseals were formed with pipettes containing 140 mM KCl. Progressively greater suctions, monitored by a pressure transducer, were then applied to the pipette until the patches broke, as attested by a sudden increase in the holding current flowing in response to an imposed potential usually of $-40$ mV. For 107 fibres from 16 normal mice 3–7 weeks old, the maximum sustainable suction was $18.7 \pm 0.3$ cm Hg as compared to $17.2 \pm 0.4$ cm Hg for 85 fibres from 10 similarly aged mdx mice. This small difference in $P^*$ was statistically significant ($p < 0.01$), but as $P^*$ and $T^*$ are related by $T^* = P^* (r/2)$, where $r$ is the unknown radius of curvature of the patches, an element of uncertainty enters as to whether the significant difference applies also to $T^*$. From the measured resistance of the hard-glass patch-pipettes used and data provided by Sakmann and Neher (1983), tip diameter was estimated as approximately...
Fig. 1. Three stills from a video record of a typical vesicle aspiration experiment. Top: a sarcolemmal vesicle (diameter 85 μm) was aspirated into a pipette (diameter 15 μm) with less than 1 cm H₂O suction. Middle: when suction was increased progressively to 10 cm H₂O, the projection moved further into the pipette. Bottom: further suction caused membrane rupture and vesicle collapse. In the Top and Middle, part of the spherical vesicle is beyond the left margin of the video frame. Images slightly defocussed to improve visibility.

1 μm, with no obvious systematic difference between the pipettes used on normal and mdx fibres. If it is assumed that all patches were hemispherical within the tip, the above results are equivalent to values for $T_*$ of 62 and 57 μN cm⁻¹ for normal and mdx sarcolemma respectively.

To avoid the uncertainties inherent in the patch–rupture method, we turned to study the mechanical properties of sarcolemmal vesicles from normal and mdx mice. When such vesicles (40–90 μm diameter) are aspirated with pipettes of uniform bore (10–20 μm) the membrane is stressed and the vesicles are deformed so as to project into the pipette. With progressively greater aspiration the projection lengthens until the vesicle ruptures (Fig. 1). From such experiments the mechanical properties of the sarcolemma can be determined in a manner originally applied to red blood cells (Evans et al., 1976) and to artificial vesicles of diverse lipid composition (Kwok & Evans, 1981; Needham & Nunn, 1990).

Sarcolemmal vesicles were produced as previously described (Burton et al., 1988) by exposing excised muscles to 140 mM KCl containing 100 units ml⁻¹ collagenase. Applied to muscles, most suitably semi-membranous, from normal mice this procedure yielded vesicles aplenty. However, with mdx muscle only scant vesiculation occurred, and it was necessary to increase the concentration of collagenase to 500 units ml⁻¹ and to enhance the activity of the enzyme by adding 0.1 mM Ca²⁺ in order to obtain a good yield of vesicles. Vesicles from both normal and mdx muscle vary in refractivity, probably owing to unequal dilution of sarcoplasm by inflowing KCl solution. For the present experiments highly refractive vesicles giving good video images were selected.

Most sarcolemmal vesicles are initially slightly flaccid and weak suction is sufficient to aspirate a short projection into the pipette. As soon as the major portion of the vesicle outside the pipette acquires a tight spherical membrane, the projection into the pipette can grow only on dilation of the membrane area of the structure in response to increased membrane stress produced by stronger suction pressures.

Figure 2 illustrates the membrane stress–strain relation for the sarcolemma as determined by vesicle aspiration. Typically it is linear right up to the last measurement of membrane tension and areal strain before the vesicle bursts, i.e. sarcolemmal vesicles behave as perfectly elastic structures. Accordingly, the slope of the plot gives the elastic modulus of area expansion. The values for these parameters as found for vesicles from normal and mdx mice are summarized in Table 1. Surprisingly, the value for the rupture tension of normal vesicles turns out to be very similar to the patch rupture tension as estimated above. So our scepticism of the relatively uncontrolled patch rupture method was probably unjustified. The tensile strength of mdx vesicles was found to be slightly lower, again in accordance with the patch rupture results.

Also given in Table 1 are the mechanical properties of sarcolemmal vesicles from normal dog muscle (sterno-