Mechanosensitive ion channels in skeletal muscle from normal and dystrophic mice

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1. We examined the activity of single mechanosensitive ion channels in recordings from cell-attached patches on myoblasts, differentiated myotubes and acutely isolated skeletal muscle fibres from wild-type and *mdx* and *dy* mutant mice. The experiments were concerned with the role of these channels in the pathophysiology of muscular dystrophy.

2. The predominant form of channel activity recorded with physiological saline in the patch electrode arose from an ~25 pS mechanosensitive ion channel. Channel activity was similar in undifferentiated myoblasts isolated from all three strains of mice. By contrast, channel activity in *mdx* myotubes was ~3–4 times greater than in either wild-type or *dy* myotubes and arose from a novel mode of mechanosensitive gating.

3. Single mechanosensitive channels in acutely isolated flexor digitorum brevis fibres had properties indistinguishable from those of muscle cells grown in tissue culture. The channel open probability in *mdx* fibres was ~2 times greater than the activity recorded from wild-type fibres. The overall level of activity in fibres, however, was roughly an order of magnitude smaller than in myoblasts or myotubes.

4. Histological examination of the flexor digitorum brevis fibres from *mdx* mice showed no evidence of myonecrosis or regenerating fibres, suggesting that the elevated channel activity in dystrophin-deficient muscle precedes the onset of fibre degeneration.

5. An early step in the dystrophic process of the *mdx* mouse, which leads to pathophysiological Ca^{2+} entry, may be an alteration in the mechanisms that regulate mechanosensitive ion channel activity.

The absence of the cytoskeletal protein, dystrophin, from skeletal muscle is the primary genetic defect in Duchenne muscular dystrophy (Hoffman, Brown & Kunkel, 1987; Koenig, Monaco & Kunkel, 1988; Bonilla *et al.* 1988). However, the mechanism by which an absence of dystrophin causes muscle degeneration is not known. There are two general ideas for the role of dystrophin in the pathophysiology of Duchenne muscular dystrophy. One is that dystrophin acts as a structural protein supporting the mechanical strength of the muscle membrane. According to this view, dystrophin-deficient muscle is more fragile than normal muscle and tears when subjected to the mechanical stresses which occur during muscle activity (e.g. Menke & Jockusch, 1991; Petrof, Shrager, Stedman, Kelly & Sweeny, 1993). The resulting damage to the sarcolemma creates a pathway for ions and small molecules to diffuse into muscle cells. An alternative view is that a deficiency of dystrophin is associated with alterations in the function of specific membrane proteins. Recent studies have shown that dystrophin forms a complex with five membrane glycoproteins (Ervasti, Ohlendieck, Kahl, Gaver & Campbell, 1990; Ervasti & Campbell, 1991). These glycoproteins are expressed at low levels in dystrophin-deficient muscle (Ohlendieck & Campbell, 1991), suggesting that dystrophin may have a more general role in regulating the expression and/or function of sarcolemmal proteins.

There is strong evidence indicating that intracellular free Ca^{2+} is elevated in dystrophic skeletal muscle (reviewed in Martonosi, 1989). How the absence of dystrophin leads to the alterations in intracellular Ca^{2+} homeostasis is not understood. We showed previously (Franco & Lansman, 1990*a*) that mechanosensitive channel activity is much higher in muscle cells from the *mdx* mouse, an animal

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model for human Duchenne muscular dystrophy (Bulfield, Siller, Wight & Moore, 1984), than in muscle from normal mice. The mechanosensitive ion channels in skeletal muscle are permeable to divalent cations and may provide a pathway for resting Ca^{2+} entry (Franco & Lansman, 1990*b*). Whether the elevated channel activity in *mdx* myotubes is an early step in the dystrophic process that leads to an elevation of myoplasmic Ca^{2+} or a later step associated with the pathophysiological changes in dystrophic muscle has not been determined experimentally.

In this paper, we address several issues related to the role of mechanosensitive ion channels in the dystrophic process of muscle from the *mdx* mouse. Because undifferentiated myoblasts do not express dystrophin (Lev, Feener, Kunkel & Brown, 1987; Nudel, Robzyk & Yaffe, 1988; Oronzi-Scott *et al.* 1988), the activity of mechanosensitive channels in myoblasts would be expected to be independent of their genotype. Moreover, mechanosensitive channel activity in differentiated myotubes would be elevated only in dystrophin-deficient *mdx* muscle cells and not in those from the dystrophic mutant *dy* mouse (Meier & Southard, 1970). Although muscle cells from *dy* mice have elevated levels of intracellular Ca^{2+} and undergo extensive myonecrosis (Dangain & Vrbova, 1990; Williams, Head, Bakker & Stephenson, 1990), they possess normal levels of dystrophin (Ohlendieck & Campbell, 1991). A final concern was whether the properties of the mechanosensitive ion channels in muscle cells grown in culture differ from those in intact fibres acutely isolated from the animal. We chose to examine fibres from the flexor digitorum brevis muscle, since these small calibre muscle fibres are apparently spared from the myonecrosis characteristic of the dystrophic process (Karpati, Carpenter & Prescott, 1988; Matsumura, Ervasti, Ohlendieck, Kahl & Campbell, 1992). Our results suggest that the activity of mechanosensitive ion channels is elevated in *mdx*, but not in either wild-type or *dy* muscle cells. Furthermore, elevated channel activity is detected only after myoblasts have fused and formed differentiated myotubes.

METHODS

Muscle cell preparation

Wild-type (C57BL/6J), *mdx* (C57BL/10ScSn-*mdx*) and *dy* (C57BL/6J-*dy/dy*) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice were killed by cervical dislocation according to a protocol approved by the University of California San Francisco Committee for Animal Research. Myotubes were grown in tissue culture from enriched populations of myoblasts as described previously (Franco & Lansman, 1990*a*). The hindlimbs of 3- to 7-week-old mice were removed and placed in cold (4 °C) Ca^{2+}- and Mg^{2+}-free Hanks' buffered saline. After cleaning away bone and connective tissue, pieces of muscle were incubated for 30 min at 37 °C in saline containing 1% collagenase B (Boehringer-Mannheim, Indianapolis, IN, USA) and 0·125% trypsin (UCSF Tissue Culture Facility) and constantly agitated by stirring. Satellite cells were dissociated by triturating the muscle digest repeatedly through the tip of a pipette. Cell debris was removed by filtering the suspension through a fine-mesh nylon cloth (100 μm). Fibroblasts were selectively removed by preplating the suspension onto glass for 1 h and then plating the non-adhering cells onto plastic tissue culture dishes (Falcon, USA) at a density of ~3000–5000 cells cm^{-2}. In some experiments, isolated muscle cells were plated onto tissue culture dishes that had been coated with 1 $\mu g\ cm^{-2}$ laminin (Sigma, St Louis, MO, USA). All cultures were maintained in a tissue culture incubator at 37 °C and exposed to 95·0% air–5·0% CO_2.

Myoblasts were grown in Dulbecco's modified Eagle's medium (DMEM)-H16 supplemented with 20% fetal calf serum and 0·5% chick embryo extract (Gibco, USA). After myoblasts reached ~30–40% confluency (~5–6 days in culture), they were induced to fuse and form myotubes by changing the medium to one which contained 5% fetal calf serum, 5% horse serum (UCSF Tissue Culture Facility) and no chick embryo extract. We observed that wild-type myoblasts divided and grew more quickly than either *mdx* or *dy* myoblasts grown under identical conditions. Myoblasts grown on laminin fused more quickly and formed more extensive myotube networks than myoblasts of the same genotype grown on plastic alone (Ocalan, Goodman, Kuhl, Hauschka & Von Der Mark, 1988). Single-channel recordings were made from myoblasts in culture after they reached ~10–20% confluency (~2–4 days). Recordings were made from myotubes ~2–5 days after the first multinucleated myotubes appeared in culture (~7–8 days after plating the myoblasts).

Intact skeletal muscle fibres were dissociated from the flexor digitorum brevis (FDB) muscle from 2-week-old wild-type and *mdx* mice following the procedure described by Bekoff & Betz (1977). The FDB muscle was dissected from the planar surface of the hindlimb of the mice. The muscle was suspended in 0·25% collagenase B in DMEM-H16 medium for 30 min and stirred. The muscle was then rinsed in DMEM-H16 medium containing 0·5% horse serum and single fibres were dissociated mechanically by trituration through a fire-polished Pasteur pipette. Dissociated fibres were plated onto plastic tissue culture dishes that had been coated with Matrigel® (Collaborative Research Inc., Bedford, MA, USA) and allowed to adhere to the bottom of the dish for 15 min at room temperature (~21–25 °C). Heterozygous *dy* mice cannot be distinguished from homozygous animals prior to 4–6 weeks of age and so we were unable to compare age-matched *dy* FDB fibres with wild-type or *mdx* fibres.

Solutions

Physiological saline solution contained (mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2·5 $CaCl_2$ and 10 Hepes. Isotonic barium and calcium solutions contained 110 mM of their respective chloride salts with 10 mM Hepes. The bathing solution was an isotonic potassium aspartate solution containing (mM): 150 KOH, 150 aspartic acid, 5 $MgCl_2$, 10 K-EGTA and 10 Hepes. The pH of each solution was adjusted to 7·5 by adding NaOH. The osmolarity of each solution was adjusted to 320–330 mosmol l^{-1} by adding glucose.

The potassium aspartate bathing solution was used to zero the cell's resting potential so that the patch potential would

be equal to the applied voltage command. Measuring the single-channel current–voltage relationship before and after patch excision indicated a maximum voltage error of ~5 mV. The potassium aspartate bathing solution produced no detectable signs of cell deterioration.

Electrophysiological methods

Single-channel activity was recorded from cell-attached patches using the technique described by Hamill, Marty, Neher, Sakmann & Sigworth (1981). Patch electrodes were pulled in two steps from Boralex haematocrit pipettes (Drummond Scientific Co., Broomall, PA, USA), coated with Sylgard (Dow Corning Chemical Co., Midland, MI, USA), and the tips heat polished with a microforge. Patch electrodes had resistances of 2–4 MΩ when filled with physiological saline solution and immersed in the potassium aspartate bathing solution. Membrane currents were recorded with a List EPC-7 patch-clamp amplifier. Current records were stored on videotape and replayed onto the hard disk of a laboratory computer (LSI 11/73) for analysis. Current records were filtered with an eight-pole Bessel filter (−3 dB at 1 kHz) and digitized at 5–10 kHz. Unless otherwise noted, all recordings were made from cell-attached patches at a constant holding potential of −60 mV. All experiments were done at room temperature (~21–25 °C).

Data analysis

Channel open probability was measured by integrating idealized records of channel opening and closing transitions and dividing this by the time integral of the single-channel current. The idealized records were obtained by setting a threshold at one-half of the amplitude of the open channel current and considering an opening event to occur when at least two consecutive sample points crossed this threshold (Colquhoun & Sigworth, 1983). Since the number of channels varied from patch to patch, the measured open probability is the open probability of each individual channel (p_o) multiplied by the number of channels (n).

Resting activity was measured during a roughly 1 min period of continuous recording at the beginning of the experiment. The resting activity measured from a large number of patches did not follow a normal distribution. We chose to represent the distribution of channel open probability (np_o), measured in different recordings under the same experimental conditions, in the form of box plots (Chatfield, 1983). The median value is shown as the line through the centre of the box and the two ends of the box enclose 50% of the data points. The outlying points represent the three highest values and the single lowest value of the high and low quartiles.

To quantify the response of channel activity to mechanical deformation of the sarcolemma, activity recorded in the absence of any applied pressure was subtracted from the channel activity elicited in response to pressure applied to the electrode. The measurements were normalized to the number of channels in the patch, which was estimated as the maximum number of superimposed openings during maximal mechanical activation. Measurements of channel open probability are shown as a function of pressure applied to the patch electrode. The relationship between the amount of pressure applied to the patch electrode and channel open probability was fitted with a Boltzmann relation of the form:

$$p_o = p_{max}/[1 + \exp(P - P_{½})/\pi],$$

where p_o is the mean channel open probability, p_{max} is the maximum channel open probability, P is the pressure applied to the electrode (in mmHg), $P_{½}$ the amount of pressure required to give a p_o of 0·5, and π is the steepness of the relation (in mmHg). The data points were fitted using a non-linear, least-squares algorithm.

RESULTS

Determination of the mechanical strength of normal and dystrophic muscle cells

The first set of experiments examined the mechanical strength of membrane patches on dystrophin-containing and dystrophin-deficient muscle cells. The amount of pressure required to rupture the patch of muscle membrane was measured by slowly applying increasing amounts of suction to the patch electrode after it had sealed to the cell surface. Rupture of the cell membrane was observed as the rapid increase in the capacitive current that occurs when the patch-electrode filling solution becomes electrically continuous with the cytoplasm. Figure 1 shows the results of experiments on myoblasts, myotubes and acutely isolated FDB fibres from wild-type, *mdx* and *dy* mice. We could detect no difference in the amount of pressure required to rupture the membrane of dystrophin-containing

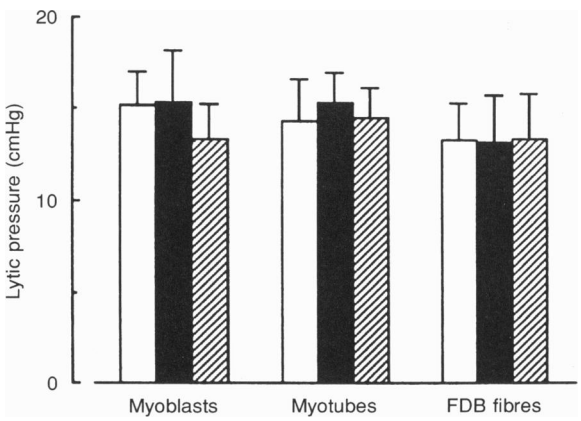

Figure 1. Determination of pressure required to rupture the cell membrane
The amount of pressure applied to the patch electrode required to rupture the cell membrane of myoblasts, myotubes and FDB fibres from wild-type (□), *mdx* (■), and *dy* (▨) mice was determined. After a gigaohm seal formed between the patch electrode and the cell membrane, suction was gradually applied to the electrode until the membrane ruptured and the whole-cell recording configuration was established as indicated by an increase in the capacitive current.

Table 1. Single-channel conductances of mechanosensitive channels in wild-type and *mdx* muscle cells

	Physiological saline			110 mM $BaCl_2$			110 mM $CaCl_2$		
	g	E_{rev}	n	g	E_{rev}	n	g	E_{rev}	n
Myotubes									
mdx; low activity	25·0 ± 2·0	14·0 ± 7·0	6	18·5 ± 2·0	18·0 ± 8·0	5	8·0 ± 3·0	38·0 ± 11·0	3
mdx; high activity	24·0 ± 2·0	16·0 ± 8·0	6	19·0 ± 3·0	18·0 ± 8·0	9	7·0 ± 2·0	40·0 ± 0·5	4
Wild type	26·0 ± 1·0	10·0 ± 2·0	2	19·0 ± 2·0	20·5 ± 6·0	4	10·0 ± 1·0	32·0 ± 11·0	3
FDB fibres									
mdx	25·0 ± 2·0	9·0 ± 2·0	7	17·0 ± 0·5	15·0 ± 2·0	3	—	—	—
Wild type	—	—	—	16·0 ± 0·5	20·0 ± 2·5	8	8·0 ± 1·0	27·0 ± 3·0	3

g, single-channel conductance (pS); E_{rev}, reversal potential (mV); n, number of experiments; values given as means ± S.D.

or dystrophin-deficient muscle. We also found no differences in the mechanical strength of patches of membrane on *dy/dy* muscle cells compared with either wild-type or *mdx* muscle cells, even though muscle degeneration is extensive in the *dy* mouse (Williams *et al.* 1990). Essentially the same values of pressure were found by Hutter, Burton & Bovell (1991) to be required to rupture patches of membrane on wild-type and *mdx* FDB fibres. However, these results do not rule out the possibility that the mechanical stability of dystrophin-deficient muscle is weakened at strategic regions of the sarcolemma.

Ion channel activity in normal and dystrophic muscle cells

Recordings were made from cell-attached patches on wild-type and dystrophic muscle cells at membrane potentials near the resting potential. In these experiments, we detected differences between wild-type and dystrophic muscle cells in the activity of two distinct types of ion channel. Figure 2 shows that the predominant form of channel activity in muscle cells from wild-type and *mdx* mice arose from mechanosensitive ion channels (Guharay & Sachs, 1984; Franco & Lansman, 1990*a*, *b*). Figure 2 shows that, in addition to the activity of mechanosensitve ion channels, recordings from *dy/dy* myoblasts and *dy/dy* myotubes show activity of the nicotinic acetylcholine-receptor (nAChR) channel in virtually all recordings, even though the patch electrode did not contain acetylcholine. Spontaneous openings of nAChR channels were observed much less frequently in wild-type or *mdx* muscle cells (e.g. Jackson, 1984). The behaviour of the spontaneous nAChR channel openings is not considered further here. In this

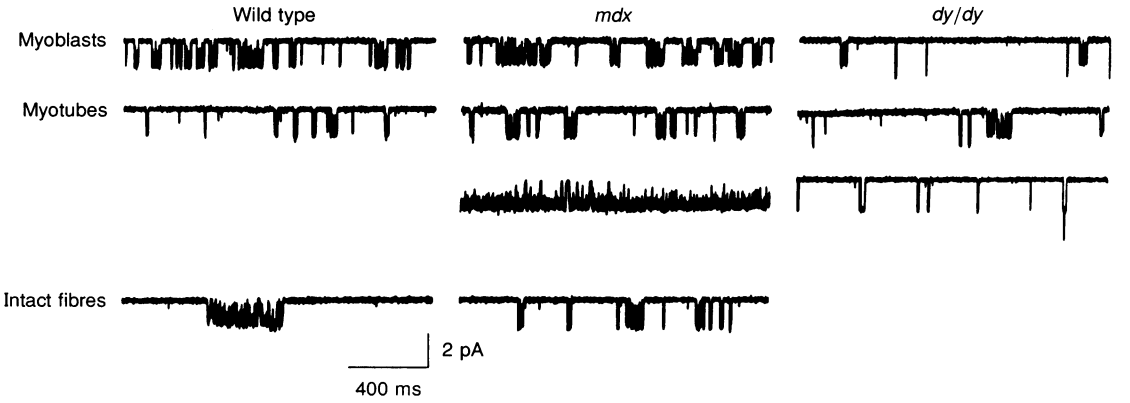

Figure 2. Representative records of recordings from cell-attached patches on myoblasts, myotubes and acutely isolated FDB fibres from wild-type, *mdx* and *dy* mice
The patch electrode contained physiological saline solution. No recordings were made from *dy/dy* FDB fibres. All recordings were made at a constant holding potential of −60 mV. Single-channel records were filtered at 2 kHz and sampled at 5 kHz. Note that in *mdx* myotubes there are two distinctly different forms of channel activity: one composed of short bursts of openings (top trace) and one in which the channel was open virtually continuously (bottom trace; see also Franco & Lansman, 1990*b*). In *dy/dy* myoblasts and myotubes, the larger single-channel events arise from spontaneous opening of the nicotinic acetylcholine-receptor channel.

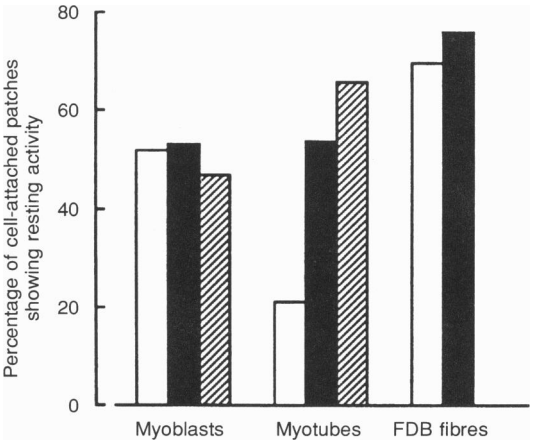

Figure 3. Percentage of recordings from cell-attached patches in which the activity of mechanosensitive channels was detected
Resting activity was detected in: ~52% (wild type, □), ~53% (*mdx*, ■) and ~47% (*dy/dy*, ▨) of cell-attached patches from the surface of myoblasts; ~21% (wild type), ~54% (*mdx*) and ~66% (*dy/dy*) of cell-attached patches from myotubes; and ~70% (wild type) and ~76% (*mdx*) of patches on FDB fibres.

paper, we have restricted the analysis to the behaviour of mechanosensitive channels in wild-type and dystrophic muscle.

Mechanosensitive ion channel activity can be detected in undifferentiated myoblasts (Franco & Lansman, 1990*a*) as well as differentiated myotubes grown in tissue culture (Guharay & Sachs, 1984; Franco & Lansman, 1990*a*). Mechanosensitive channel activity can also be recorded from acutely isolated skeletal muscle fibres. The properties of single mechanosensitive ion channels recorded from acutely isolated fibres are indistinguishable from those in myoblasts and myotubes (see also Figs 6 and 7). A striking exception was in recordings from *mdx* myotubes, which frequently showed a form of activity where channel open probability approached unity under resting conditions (Fig. 2, third trace in the second column; see also Franco & Lansman, 1990*a*). This type of gating behaviour was not observed in myoblasts or FDB fibres. The results in Table 1 show that the high open-probability events have the same single-channel conductance as the lower open-probability events with either physiological saline solution or an isotonic Ba^{2+}- or Ca^{2+}-filling solution in the patch electrode. The high open-probability channel events arise from a second mode of mechanosensitive gating which appears in dystrophin-deficient myotubes.

Mechanosensitive channel activity during myogenesis

We first asked whether the density of mechanosensitve channels differs in wild-type and dystrophic muscle cells at different stages of myogenesis. Channel density was estimated as the fraction of all of the recordings from cell-attached patches in which channel activity was detected. Figure 3 shows that channel density was similar in myoblasts regardless of the genotype of the mouse from which they were isolated. After myoblasts had fused and formed differentiated myotubes, channel density was lower in wild-type compared with *mdx* or *dy* myotubes. On the other hand, FDB fibres acutely isolated from either wild-type or *mdx* mice had comparable numbers of channels. We did not compare *dy/dy* fibres with wild-type or *mdx* fibres because homozygous *dy* mice cannot be identified until 4–6 weeks of age and there are age-dependent changes in channel properties (Haws & Lansman, 1991). Although we could not detect any differences between normal and *mdx* FDB fibres in channel density, it is possible that there are differences in the spatial localization of channels not easily detected by recordings from cell-attached patches.

Figure 4*A* shows that, as expected for undifferentiated cells that do not express dystrophin, channel activity was roughly the same in myoblasts from all three strains of mice. Figure 4*B* shows, on the other hand, that the activity of mechanosensitive ion channels in *mdx* myotubes was roughly 3–4 times higher than in either wild-type or *dy/dy* myotubes. We were concerned that the high level of channel activity recorded from *mdx* myotubes might have reflected some aspect of the conditions under which cells were grown in tissue culture. To test the possibility that an interaction between the cell surface and the substrate influences channel activity, myotubes were grown on either uncoated plastic tissue culture dishes or dishes that were coated with laminin, a component of the basement membrane. Figure 5 shows that myotubes grown on laminin-treated dishes generally had higher levels of resting activity regardless of their genotype, but channel activity was still substantially higher in *mdx* than in wild-type myotubes. In addition, the proportion of low and high open-probability channel events was not altered when cells were grown on laminin rather than on plastic alone (data not shown). These results provide further support for the conclusion that the differences between normal and dystrophic myotubes in the level of channel activity depend on the presence or absence of dystrophin. Evidently, cell–substrate interactions can modulate the overall levels of channel activity.

Small calibre fibres in the *mdx* mouse do not suffer the degeneration that is typical of the larger muscle groups (Karpati *et al.* 1988). Up to 4–6 weeks of age, *mdx* FDB

fibres are histologically indistinguishable from wild-type fibres (A. Franco-Obregón Jr & J. B. Lansman, unpublished observation). Since FDB fibres from *mdx* mice at this stage do not undergo myonecrosis, our electrophysiological recordings were not made from a mixed population of degenerating and regenerating fibres. Figure 4*C* shows that FDB fibres isolated from young *mdx* mice before the appearance of any histopathological abnormalities had higher levels of mechanosensitive channel activity than age-matched wild-type fibres. The levels of channel activity were roughly an order of magnitude lower in wild-type and *mdx* fibres than in myotubes. The box-plot analysis (see Methods) showed, however, that the median value of channel open probability in *mdx* fibres is greater than in wild-type fibres and the distribution of open probabilities falls towards higher values (see also Haws & Lansman, 1991). The results in Fig. 4 suggest that mechanosensitive ion channel activity is elevated in *mdx*, but not in either wild-type or *dy* muscle cells, only after myoblasts have fused and formed differentiated muscle fibres.

Mechanosensitive gating in wild-type and *mdx* FDB fibres

We compared the channels in wild-type and *mdx* FDB fibres to determine whether the mechanosensitive gating mechanism is similar to that of the channels in tissue-cultured myoblasts and myotubes, particularly since the membrane properties of tissue-cultured cells might be expected to differ from intact fibres. Figure 6 shows that applying either positive (Fig. 6*A*) or negative (Fig. 6*B*) pressure to the patch electrode increased channel activity. This gating behaviour is characteristic of the mechanosensitive channels studied previously in wild-type skeletal muscle cells in culture (Guharay & Sachs, 1984; Franco & Lansman, 1990*a*). Figure 6*C* shows the relationship between the amount of pressure (either positive or negative) applied to the patch electrode and channel open probability. The increase in channel open probability with either positive or negative pressure was well fitted by a Boltzmann equation with similar steepness and half-activation pressures (details in legend to Fig. 6). In this experiment, the relationship between pressure and channel open probability reached a minimum at ~2 mmHg, suggesting that only a small fraction of the resting activity can be attributed to the residual tension on the membrane that may remain as a result of the process of seal formation.

We compared the response of channels in wild-type and *mdx* fibres to suction applied to the patch electrode to determine whether there might be differences in the

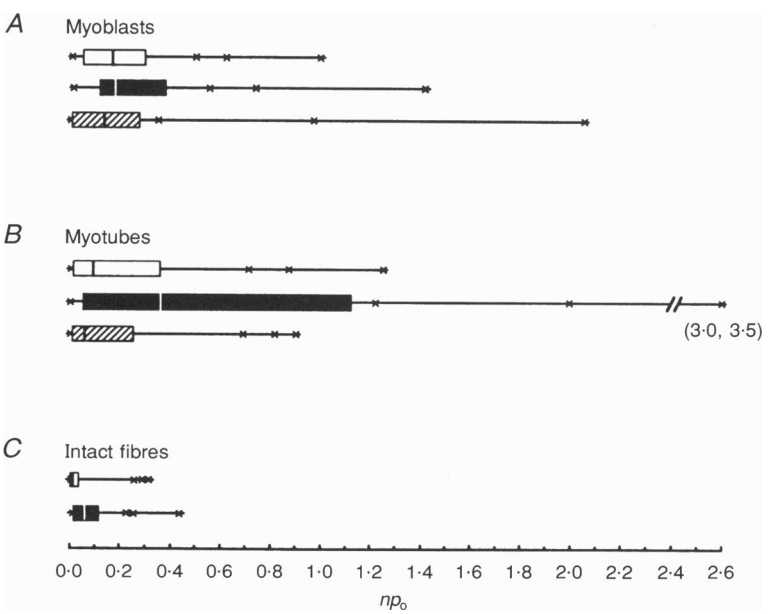

Figure 4. Comparison of the open probability of mechanosensitive channels in myoblasts (*A*), myotubes (*B*) and FDB fibres (*C*) from wild-type, *mdx* and *dy* mice

The distribution of resting open probabilities (np_o) is shown in the form of box plots. The line in the centre of the box indicates the median value of the distribution, while the outer margin of the box encloses 50 % of the observations. The outlying points represent the three highest values and the single lowest value of each data set. The calculated mean open probabilities were: 0·24 ± 0·06 (wild type, □; $n = 18$), 0·30 ± 0·09 (*mdx*, ■; $n = 16$) and 0·28 ± 0·13 (*dy/dy*, ▨; $n = 15$) for recordings from myoblasts; 0·25 ± 0·07 (wild type; $n = 24$), 0·73 ± 0·21 (*mdx*; $n = 29$) and 0·17 ± 0·04 (*dy/dy*; $n = 44$) for recordings from myotubes; and 0·045 ± 0·02 (wild type; $n = 35$) and 0·075 ± 0·02 (*mdx*; $n = 38$) for recordings from intact fibres. Deviations from the mean are given as ± S.E.M.

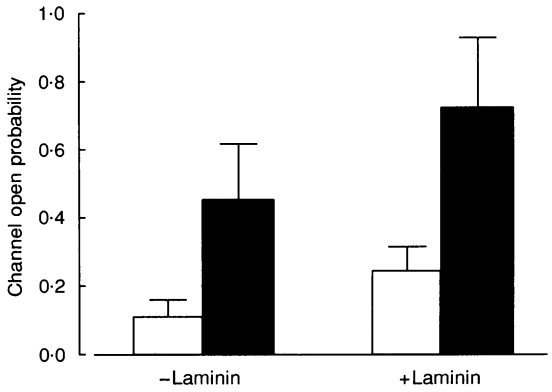

Figure 5. Effect of laminin on the mechanosensitive channel activity recorded from wild-type and *mdx* myotubes
Channel open probability was 0·11 ± 0·05 (□; $n = 20$) in wild-type and 0·41 ± 0·17 (■; $n = 35$) in *mdx* myotubes grown on plastic tissue culture dishes and 0·25 ± 0·07 ($n = 24$) in wild-type and 0·73 ± 0·21 ($n = 29$) in *mdx* myotubes grown on laminin-coated tissue culture dishes. Values are given as means ± S.E.M.

mechanical properties of the membrane of dystrophin-deficient *mdx* fibres and wild-type fibres. Figure 7*C* shows that channel activity recorded from either wild-type (open circles) and *mdx* (filled circles) fibres increased with suction; however, the same amount of pressure evoked somewhat less channel activity in *mdx* fibres than in wild-type fibres.

Figure 8*A* shows recordings of single-channel activity from wild-type and *mdx* fibres before, during the application of suction (–10 mmHg) to the electrode, and after releasing the suction applied to the electrode. Although suction increased channel activity in both types of fibre during its application, channel activity recorded from *mdx* fibres did not return to control levels. Figure 8*B* shows the results from a number of similar experiments which show the suppression of channel activity by the prior application of suction during recordings from *mdx* FDB fibres. This experiment indicates that measurements of resting activity in *mdx* FDB fibres may give low values because of the deformation of the patch membrane during seal formation.

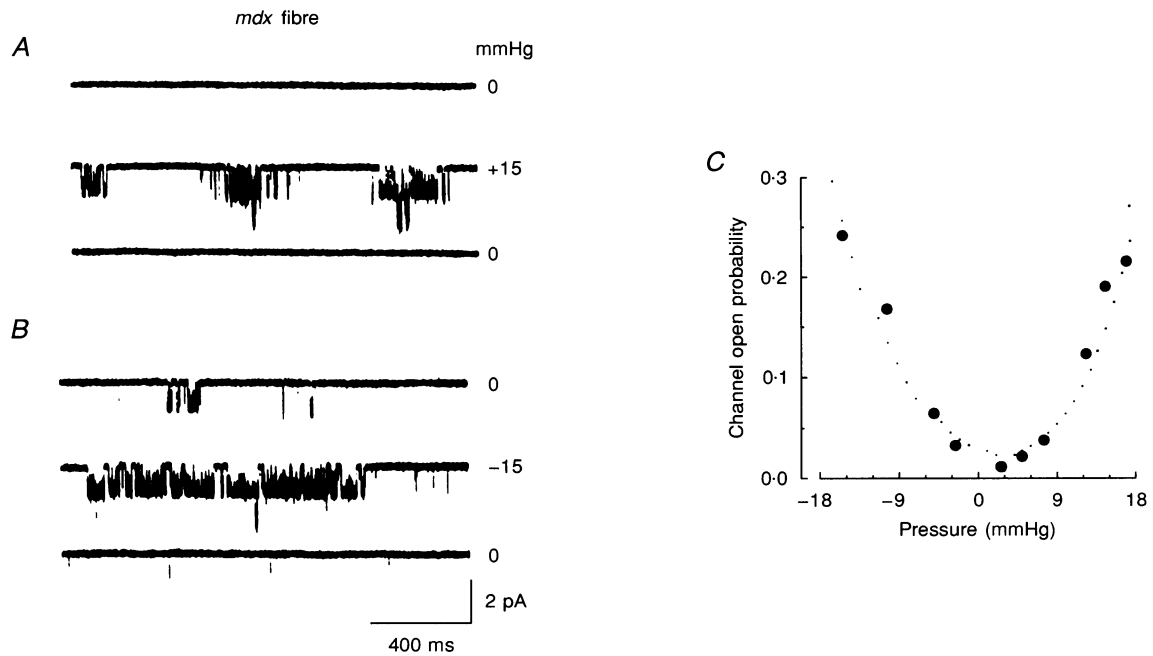

Figure 6. Effect of positive and negative pressure on channel activity
The effect of positive and negative pressure applied to the patch electrode on channel activity in a recording from an *mdx* FDB fibre is shown in *A* and *B*, respectively. The increase in channel activity with positive or negative pressures was fitted with a Boltzmann relationship (*C*) as described in Methods. For channel activity elicited with negative pressure, $P_{½} = -21·0$ mmHg and $\pi = -6·0$ mmHg. For channel activity elicited with positive pressure, $P_{½} = 24·0$ mmHg and $\pi = 5·0$ mmHg.

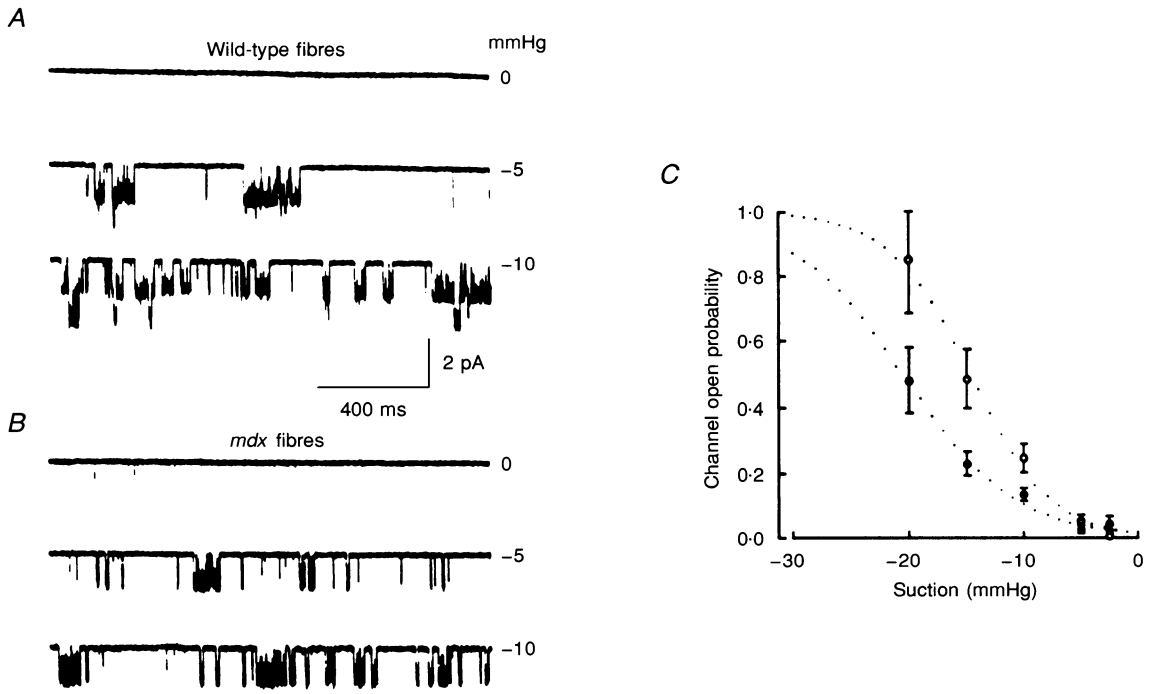

Figure 7. Mechanosensitive gating in wild-type and *mdx* FDB fibres
Channel activity recorded from wild-type (*A*) and *mdx* (*B*) FDB fibres with the indicated pressures applied to the patch electrode; 17% of recordings from wild-type and 22% of the recordings from *mdx* fibres failed to respond to suction applied to the patch electrode. *C* shows the effects of the amount of suction applied to the patch electrode on channel open probability in recordings from wild-type (○; $n = 22$) and *mdx* (●; $n = 25$) FDB fibres. Error bars represent ± S.E.M. The data were fitted with a Boltzmann relationship (dotted lines) with $P_{½}$ of −14·0 and −20·0 mmHg and π of −3·0 and −5·0 mmHg, for wild-type and *mdx* fibres, respectively.

Voltage-sensitive gating of mechanosensitive channels in wild-type and *mdx* fibres

Mechanosensitive channels possess an intrinsic voltage-sensitive gating mechanism (Guharay & Sachs, 1985; Franco & Lansman, 1990*a*). One explanation for the elevated mechanosensitive channel activity in *mdx* fibres is that changes in the density of fixed charges at the membrane surface alter the electrical potential that falls across the channel voltage sensor. We examined the voltage dependence of channel open probability in wild-type and *mdx* fibres to test this possibility. Figure 9*A* shows representative records of single-channel activity recorded from an *mdx* fibre at the patch holding potentials indicated. As shown previously, channel activity increases with depolarization as a result of an increase in channel burst duration (Franco & Lansman, 1990*a*). Figure 9*B* shows the mean channel open probability plotted as a function of the patch potential. Mechanosensitive channel activity increased ~e-fold for 56 mV (open circles, wild-type fibres) and ~53 mV (filled circles, *mdx* fibres) of depolarization. Evidently, the voltage dependence of channel opening in wild-type and *mdx* fibres does not differ sufficiently to explain the increased open probability of *mdx* fibres.

DISCUSSION

We have suggested that dystrophin may have a role in regulating the activity of mechanosensitive ion channels in skeletal muscle (Lansman & Franco, 1991). Attempts to explain the mechanism by which dystrophin regulates channel activity must be considered to be only speculative at present. Nonetheless, the results allow some inferences to be made. We have shown that the resting activity of mechanosensitive ion channels is elevated in dystrophin-deficient skeletal muscle cells from the *mdx* mouse and not in those from either normal or *dy* mice, which possess normal amounts of dystrophin. Furthermore, channel activity is elevated only after *mdx* myoblasts have fused to form differentiated muscle cells. That channel activity is virtually the same in myoblasts isolated from all three strains of mice is consistent with the time course of the developmental expression of dystrophin, which is absent in undifferentiated myoblasts but appears in myotubes and differentiated muscle (Lev *et al.* 1987; Nudel *et al.* 1988; Oronzi-Scott *et al.* 1988). Evidently, there is a correlation between the alterations in channel activity and dystrophin expression during myogenesis.

Mechanosensitive ion channels in myotubes grown in tissue culture are indistinguishable from those in intact

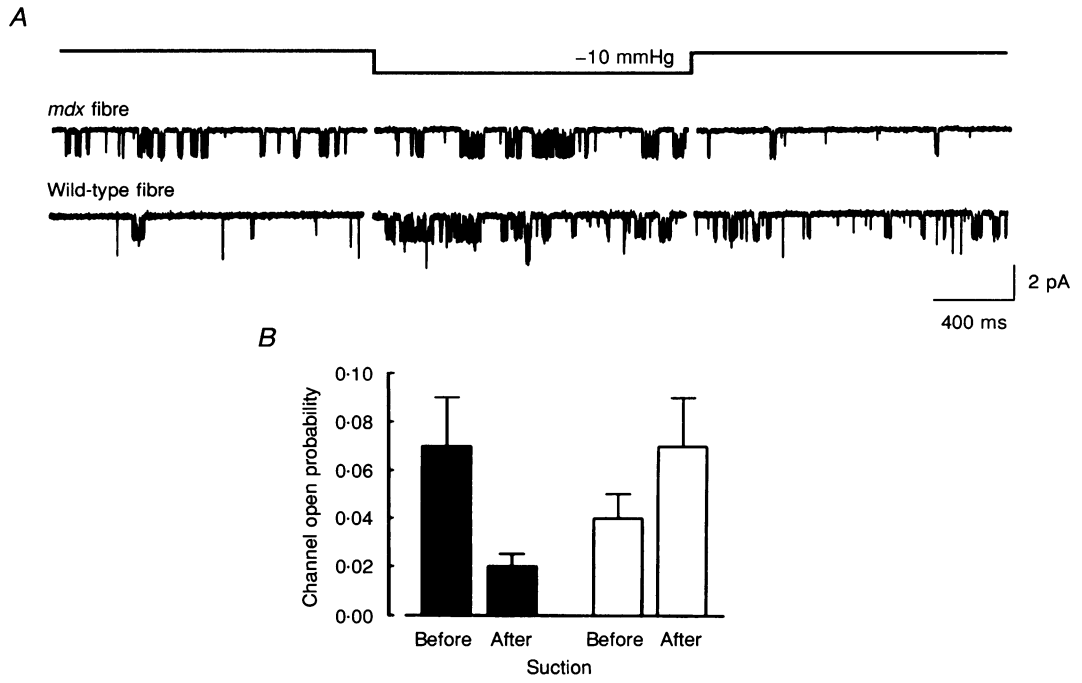

Figure 8. Changes in resting activity following the application of suction in FDB fibres
A, representative records of channel activity before, during and after suction was applied to the patch electrode for the first time. The value of −10 mmHg suction was used because it is comparable in magnitude to that used in forming a seal between the electrode and the membrane. Channel activity was reduced after suction in ~73% of patches from *mdx* fibres ($n = 30$). On the other hand, channel activity increased following suction in ~65% of patches ($n = 31$) from wild-type fibres. *B*, mean channel activity before and after suction. The mean values (± S.E.M.) of channel open probability were 0·04 ± 0·01 in wild-type (□; $n = 31$) and 0·07 ± 0·02 in *mdx* (■; $n = 30$) fibres before applying suction to the electrode, and 0·07 ± 0·02 in wild-type and 0·02 ± 0·005 in *mdx* fibres after applying suction.

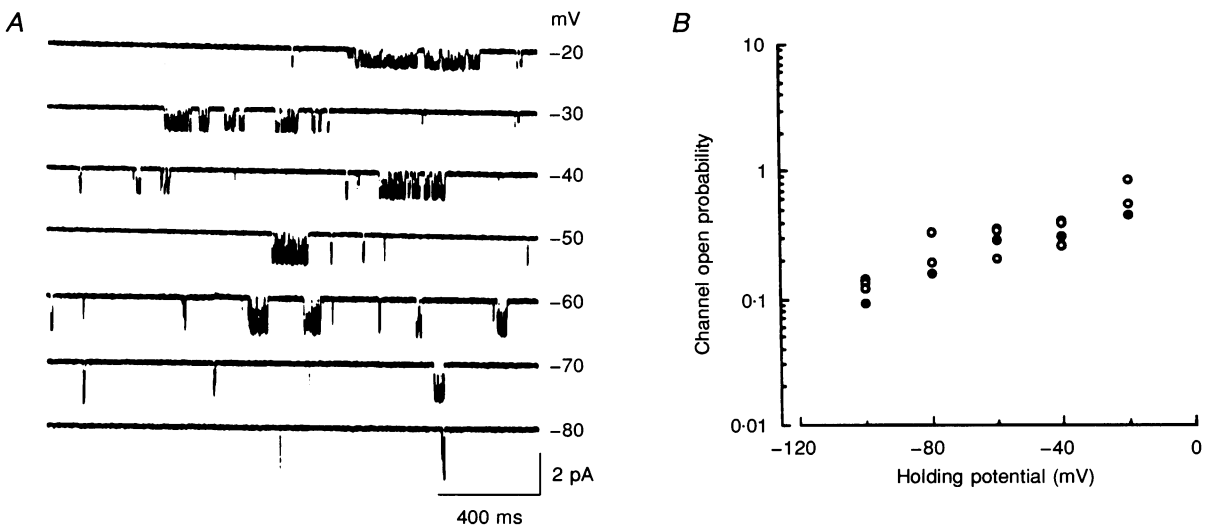

Figure 9. Effect of membrane potential on mechanosensitive channel activity from FDB fibres
A, representative records of channel activity from an *mdx* FDB fibre at the indicated patch potentials. *B*, effect of holding potential on channel activity in recordings from wild-type (○) and *mdx* (●) FDB fibres. Channel activity increased e-fold for ~56 ± 5 mV (mean ± S.D.; $n = 3$) and ~53 mV ($n = 1$) of depolarization in wild-type and *mdx* fibres, respectively.

fibres acutely isolated from the animal. This finding supports the interpretation that the properties of these channels cannot be attributed to artifacts associated with alterations in structural membrane properties which might occur in tissue-cultured cells. On the other hand, the substrate on which the cells were grown had an effect on the overall level of channel activity. It has become increasingly apparent that cell surface molecules which associate with the extracellular surface are associated with cytoskeletal structures and that this coupling is part of a dynamic signalling pathway (reviewed by Ervasti & Campbell, 1993; Gumbiner, 1993). It is not surprising, therefore, that interactions between the sarcolemma and the extracellular surface could lead to alterations in channel activity, perhaps via the phosphorylation of the channel or channel-associated proteins.

We were surprised to find that the level of mechanosensitive channel activity in acutely isolated fibres from either normal or *mdx* FDB muscle was roughly an order of magnitude smaller than that recorded from either myoblasts or myotubes in tissue culture. In a previous study of mechanosensitive channel activity in *mdx* fibres (Haws & Lansman, 1991), we suggested that the small fraction of patches showing high levels of activity in *mdx* fibres may represent sites at which there is a localized influx of extracellular Ca^{2+} which could be sufficient to produce focal damage. If such focal lesions accumulated, they could produce a much larger disruption of sarcolemmal integrity. Histological examination of FDB fibres from 4- to 6-week-old *mdx* mice showed no evidence of fibre necrosis or regeneration. Thus, any differences between normal and *mdx* fibres in channel activity cannot be attributed to muscle damage. Nonetheless, it is interesting to speculate that focal lesions produced by highly localized Ca^{2+} entry may be the cause, rather than the result, of sarcolemmal damage by the stresses that occur during contraction (Petrof *et al.* 1993).

Tinsley *et al.* (1992) recently identified a protein that is an autosomal homologue of dystrophin. Matsumura *et al.* (1992) suggested that this protein, termed utrophin, may compensate for the absence of dystrophin in small calibre fibres from *mdx* mice. The relatively small differences between normal and *mdx* fibres in the mechanosensitive gating mechanism may represent the ability of utrophin to compensate for the lack of dystrophin. It remains to be determined if fibres from muscle groups which lack utrophin as well as dystrophin have much larger alterations in mechanosensitive channel activity.

Comparison with other work

Our results differ from those of Fong, Turner, Denetclaw & Steinhardt, 1990 who described a class of Ca^{2+} leak channel whose activity was higher in *mdx* myotubes than in wild-type myotubes. The channels described by Fong *et al.* (1990) had a smaller single-channel conductance and were voltage insensitive, unlike those described here. Other than spontaneous openings of the nAChR, we have never observed the activity of a small conductance leak channel in the hundreds of recordings we have made from cell-attached patches on cells in tissue culture or on intact fibres. The leak channels described by Fong *et al.* (1990), however, may represent channel activity that appears as *mdx* cells degenerate in tissue culture. Examination of age-related changes in channel activity during the development in tissue culture of normal and dystrophic muscle cells will help resolve the role of Ca^{2+} leak channels in the pathogenesis of the dystrophic process in dystrophin-deficient muscle.

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