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Norepinephrine-Induced Membrane Responses in Normal and Dystrophic Hamster Skeletal Muscle (40872)[1]

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Abstract. Two skeletal muscle membrane responses were examined to evaluate the role of membrane alterations in dystrophic muscle function. These responses—norepinephrine-evoked changes in membrane conductance and potential—have been suggested to be associated with nonshivering metabolic pathways independent of contractile events. Norepinephrine (at concentrations ranging from 50–300 μg/kg) and/or saline was injected intravenously into anesthetized control hamsters and Bar Harbor 14.6 cardiomyopathic hamsters (2–3 months old). Glass microelectrodes were used to measure the intracellular potentials and membrane resistances of *gracilis anticus* cells. In the normal hamsters, norepinephrine administration was followed by a small, but significant membrane depolarization ($\bar{X}$ = 6.7 ± 1.4 mV) and a decrease in membrane resistance ($\bar{X}$ = 1.0 ± 0.1 mΩ). In contrast, saline had little or no effect on these parameters. The voltage and resistance changes observed in dystrophic muscle cells were in the same direction as those occurring in the normal muscle, but were significantly smaller in magnitude. The finding that these norepinephrine-induced changes were diminished in dystrophic cells supports the view that membrane properties thought not to be associated with muscle contraction are altered in dystrophic hamster muscle.

The muscular dystrophies of man and animal models are genetic diseases in which the primary biochemical defects are unknown and may in fact differ with the form of dystrophy. In general, however, one finds varying degrees of muscle weakness and progressive atrophy of muscle structure and function. Available evidence is consistent with the view that in man, the pathology involves altered plasma membranes of muscle and erythrocytes (1). Evidence suggesting plasma membrane alterations in animal dystrophies also exists. For example, in the Bio 14.6 cardiomyopathic dystrophic hamster used in the present study, decreased muscle resting potentials, increased sarcolemmal input resistances, and altered contractile properties have been reported for hindlimb muscle fibers (2, 3). In addition, the membrane bound Na^+/K^+ ATPase activity is increased in genetically dystrophic hamsters, this increase being correlated with age and progression of disease (4–6).

To further evaluate the role of membrane alterations in dystrophic hamster muscle function, we have examined two membrane responses thought to be independent of contractile events: namely, the catecholamine-induced changes in membrane potential and membrane resistance. These skeletal muscle responses to catecholamines have been suggested to be associated with nonshivering heat production (7), a function of muscle that may be altered in dystrophic hamsters (8).

Materials and methods. Male Syrian hamsters (normal controls, 100–120 g) and Bar Harbor Bio 14.6 dystrophic hamsters (2–3 months old, 80–116 g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), injected with atropine sulfate (0.02 mg per animal), and surgically prepared as follows. First, the jugular vein was cannulated for subsequent injections of sodium pentobarbital, catecholamine and saline (0.9% NaCl). The animal was then placed on a surgical board, ventral surface down, and an incision was made in the skin of the left hindlimb. The agar bridge to the

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reference electrode was positioned within this incision. A small circle of skin on the right hindlimb was removed, exposing the *gracilis anticus* muscle. The fascia covering this muscle was excised to facilitate entrance of the microelectrode. The exposed muscle tissue was bathed with mammalian Ringers solution containing (m*M* final concentration): 155 NaCl, 5.7 KCl, 2.2 $CaCl_2$, 1.2 $NaHCO_3$, and 5.5 glucose.

Intracellular potentials were measured with glass microelectrodes filled with 2.5 *M* KCl. These electrodes had an impedance of 14–40 mΩ as measured with the ramp generator of the Winston preamplifier (Model 1060). The electrodes were positioned directly above the muscle and were advanced vertically into the cells with a hydraulic microdrive (Kopf). Silver silver-chloride wires served as reference and recording electrodes. The potentials were recorded with the Winston preamplifier and displayed on a Tektronix 7403N oscilloscope and a Houston 3000 penwriter.

Resistances of the membranes of *gracilis anticus* cells were measured employing techniques as adapted from Kobayashi and Libet (9). (These techniques have been previously utilized to record the conductance and potential changes in brown fat after norepinephrine (NE) stimulation (10). Permanent records of resistance changes were collected using a Tektronix C12 oscilloscope camera. Reproduction of sample traces are seen in Figs. 1C and D. To minimize the chance that measured changes in potential reflected characteristics of membranes damaged by the microelectrode and/or preparatory manipulations, only cells with stable potentials equal to or more negative than −60 mV and located below the surface layer were used for analysis of the effects of NE on voltage and resistance. A cell was deemed nonresponsive to a drug injection if heart rate and respiration of the hamster increased with no detectable change in membrane potential.

Norepinephrine (L-arterenol bitartrate (Sigma), 40 μg/ml) was freshly prepared for each experiment by diluting a stock solution with saline containing the antioxidant, ascorbic acid (1 mg/ml saline). The stock solution, which had been stored at −10° away from light, contained 1 mg NE/ml.

Rectal temperature was measured with a YSI telethermometer. Frequency of respiration was monitored by counting breaths/minute. For every cell impaled, both parameters were recorded prior to any injection (NE or saline) and every 1 to 2 min for the 10-min period thereafter, or until termination of the resulting depolarization–repolarization sequence.

Results and discussion. The average resting potential of normal muscle cells (74.9 ± 0.6 (SE); 252 cells from 14 animals) was significantly greater ($P < 0.001$) than that of dystrophic cells (71.6 ± 0.8 (SE); 215 cells from 15 animals), a finding in agreement with other investigations of animal dystrophies (2, 11, 12). This difference is attributed to the altered ionic composition of dystrophic muscle which appears to contain less potassium and more sodium than does normal muscle (13–16). The etiology of these differences is unknown, but it is postulated to involve either a defect in the sodium pump and/or an aberrant leakiness of the muscle membrane (5, 12).

Mean values of the magnitude of the depolarization following saline and NE injections are given in Table I. In both normal and dystrophic hamster muscle cells, saline had little effect on membrane potential. On the other hand, depolarization was consistently observed following intravenous administration of NE. The voltage changes in the dystrophic cells were in the same direction but of lesser magnitude than those of normal muscle ($P < 0.05$). That this decreased magnitude did not simply reflect the fact that fewer dystrophic cells responded to the catecholamine signal is indicated by the finding that comparison of only the responsive cells yielded the same result as comparing all cells examined: namely, that the magnitude of the dystrophic cell depolarization was less than that of normal cells after NE administration ($P < 0.05$; Table I).

Concurrent with membrane depolarization, there occurred a fall in membrane resistance in both normal (an average decrease of 1.03 ± 0.11 (SE) mΩ; $N = 7$) and

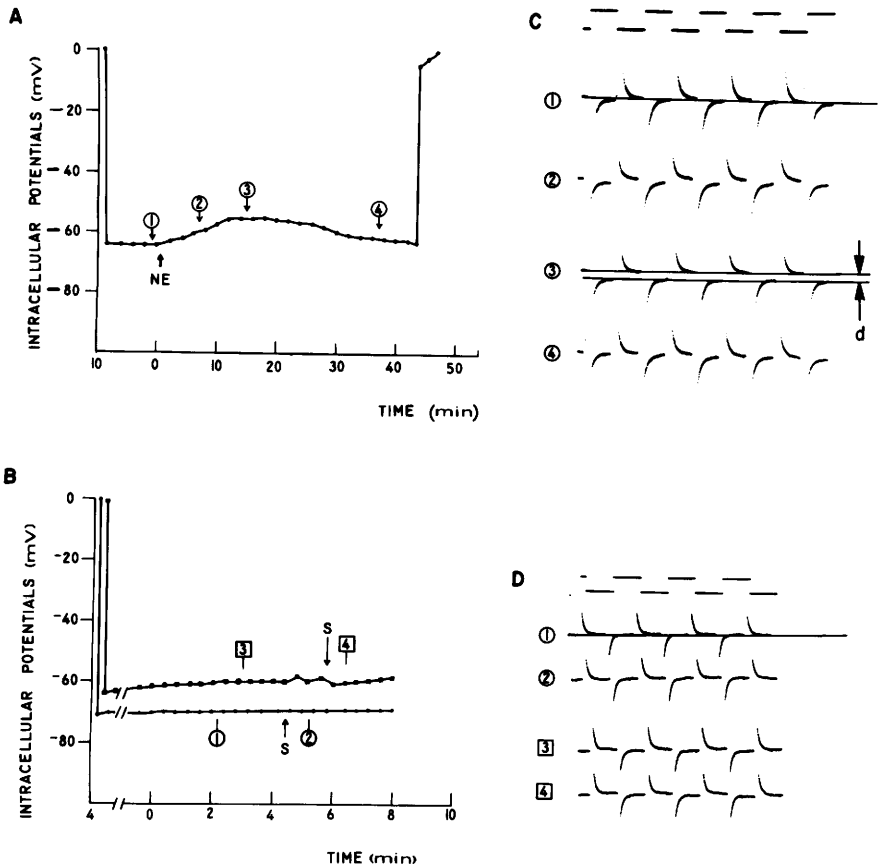

FIG. 1. Transmembrane potentials (A, B) and resistances (C, D) in *gracilis anticus* muscle cells before and after administration of norepinephrine NE (A, C) or saline (B, D) to anesthetized control hamsters. (A) Effect of 10 μg NE (administered at arrow) on the transmembrane potential of a single muscle cell. The circled numbers correspond to the times for which membrane resistance was measured concomitantly (C). (B) Transmembrane potentials of two different muscle cells before and after saline administration (at arrows marked S). As in A, the enclosed numbers correspond to times of concurrent measurement of membrane resistances (D). (C) Plasma membrane resistance measurements for the same cell as in A. Traces 1–4 represent measurements at the times indicated by the corresponding numbers in A. The top square wave trace in C has a period of 20 msec and is the voltage applied to the bridge circuit for measuring resistance. In trace 1, the line drawn through the waveform indicates the balanced bridge configuration prior to norepinephrine application. In trace 3, the two lines drawn through the waveform (separated by distance d) emphasize that the bridge is unbalanced. The direction of change relative to the reference waveform is the same as that seen when the value of a test resistor in the bridge circuit is decreased. (D) Membrane resistances of cells before and after saline administration. Traces 1 and 2 are the resistances for one of the cells in B (corresponding to times 1 and 2); while traces 3 and 4 represent resistances for the second cell in B. For both cells, the bridge circuit remained balanced after saline administration.

dystrophic cells (an average decrease of 0.60 ± 0.18 (SE) mΩ; N = 5), thereby suggesting an increase in membrane permeability. Once again the responses of the dystrophic muscle were identical in direction but smaller in magnitude than those of the normal muscle ($P < 0.05$). In both groups of cells, however, the depolarization–repolarization sequence was correlated with conductance changes as can be seen in Fig. 1. The decrease in resistance following NE was slight, but in keeping

TABLE I. EFFECT OF NOREPINEPHRINE AND SALINE ON MEMBRANE POTENTIALS OF NORMAL AND DYSTROPHIC MUSCLE CELLS[a]

Animal group (*N*)	Drug/dose (μg/kg)	No. of cells	Depolarization (mV)	% change
Normal (11)	Saline	41	0.37 ± 0.23	0.46 ± 0.29
Dystrophic (11)	Saline	41	0.63 ± 0.31	0.77 ± 0.37
Normal (11)	NE/50–300	22	6.68 ± 1.39	8.06 ± 1.57
Dystrophic (6)	NE/100–200	22	3.49 ± 0.73[b]	4.41 ± 0.91[b]
Normal[c] (11)	NE/50–300	19	8.17 ± 1.49	9.85 ± 1.76
Dystrophic[c] (6)	NE/100–200	14	5.48 ± 0.70[b]	6.94 ± 0.86[b]

[a] Values represent mean ± SE with the number of hamsters from which the cells were obtained being given in parentheses. Data were subjected to a one-way analysis of variance, and the responses of dystrophic cells were compared to those of the normal cells.

[b] Dystrophic values less than normal at $P < 0.05$.

[c] Refers to responsive cells only.

with the relatively small ion flux that would be needed to alter the potential 3 to 8 mV. The lack of change in membrane potential and resistance following administration of saline (Fig. 1) was typical of most cells, although in one dystrophic hamster, saline did evoke a small voltage change four times—each time after an injection of NE. (These data were all included in the statistical analysis of Table I.)

These observations are consistent with the view that the sarcolemmal depolarization seen following *in vivo* administration of NE reflects, at least in part, an increase in membrane permeability (manifested experimentally as a decrease in membrane resistance). In addition, the smaller magnitude of the NE-induced responses seen in the dystrophic hamster muscle suggests the presence of functional changes in the membranes of these muscles. Although altered responses to catecholamines have been observed in other forms of muscular dystrophy, these vary depending on the system being examined. Moreover, the degree to which these alterations are related to the findings in the present study is difficult to ascertain. (As an example of the types of responses noted, Takamori *et al.* (17) found enhanced effects of isoproterenol on contractile properties of some human dystrophic muscles. Similarly, Brust (18) reported supersensitivity of dystrophic mouse muscle to caffeine. On the other hand, decreased responsiveness to caffeine has been observed in intact (19) as well as skinned (20) muscle fibers from patients with Duchenne dystrophy.)

The basis of the catecholamine-induced alterations observed in the present study is not yet clear. Among the possible explanations is that the reduced magnitude of the NE-evoked changes in membrane potential and resistance may reflect modification of one or more component(s) directly involved in the NE–membrane interaction (e.g., changes in adrenergic receptor properties and/or changes in the receptor–response transduction mechanism (whatever it may be)). Unfortunately, no data are as yet available regarding adrenergic receptor characteristics in dystrophic hamster muscle (i.e., numbers of receptors per cell; relative distribution of α- and β-receptors; affinity of receptors for adrenergic agents).

An alternative explanation for the decreased NE-evoked changes in membrane potential and resistance of dystrophic muscle may lie in the permeability properties of the sarcolemma. For example, a smaller NE-induced change in membrane permeability might be expected if the dystrophic membrane were already leakier than normal. That the membrane of the dystrophic hamster muscle may in fact be abnormally leaky is suggested by the lower than normal resting potentials of the cells which indicate an altered ionic distribution across the membrane. (The higher input resistances measured in some dystrophic muscle fibers do not necessarily imply greater membrane permeabilities. Rather they may reflect the

fact that these muscle cells have smaller diameters (3). To determine if the permeability of these cells is altered, one needs to know their surface area so that resistance per unit area of membrane can be calculated.)

Regardless of the mechanism involved, it is clear that NE elicits less pronounced changes in the membrane resistance and potential of dystrophic hamster muscles, a finding consistent with the view that altered membrane properties may underlie at least some of the cellular dysfunctions seen in the dystrophic hamster. In addition, these diminished effects on the sarcolemma may be related to the reduced amount of nonshivering thermogenesis evoked when these hamsters are administered catecholamines (8). Evaluation of this possibility awaits determination of the quantitative contribution of skeletal muscle to the thermogenic responses of these animals, a contribution that may, in fact, be small (21, 22).

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