ELEVATED POTASSIUM EFFLUX FROM DYSTROPHIC DIAPHRAGM: INFLUENCE OF DIPHENYLHYDANTOIN AND LITHIUM

Gene R. Herzberg,* Mark D. Challberg, Barbara C. Hess and John L. Howland

Committee on Biochemistry
Bowdoin College, Brunswick, Maine 04011

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Summary: Potassium efflux from diaphragm of dystrophic mice is elevated when compared to efflux observed with normal tissue. When dystrophic mice are treated with both lithium chloride and diphenylhydantoin, the efflux returns to within normal limits, neither agent having such an effect when administered alone.

Dystrophic diseases of muscle are characterized by extensive degeneration of the sarcolemma leading to substantial loss of various muscle constituents, ranging in size from inorganic ions to cytoplasmic proteins (1). We have recently reported increased leak of univalent cations from erythrocytes obtained from dystrophic humans and have suggested that systemic changes in transmembrane ion conductance may underlie some dystrophic diseases, leading to muscle dysfunction by interfering with excitation-coupling events at the plasma membrane (2). This point of view is in conformity with evidence that membrane defects exist in genetic muscle disease (3-6) and, moreover, suggests that inhibition of the relevant ion conductance might lead to a diminution of muscle pathology. We report here that potassium efflux is substantially elevated in diaphragm from dystrophic mice. The present communication also reports that a combination of two drugs, LiCl and diphenylhydantoin (DPH), known individually to

*Present address: Department of Biological and Physical Sciences, Lowell State College, Lowell, Massachusetts 01850.

exhibit a stabilizing influence upon aspects of membrane function (7,8) leads to diminished K^+ efflux from diaphragm of dystrophic mice. If the increased K^+ efflux provides a valid measure of the dystrophic state in mice, then this observation may yield useful insight into possible approaches to therapy.

METHODS: Adult mice of strains 129/ReJ (normal), 129/ReJ-dy (dystrophic) and C57Bl/6J-dy^{2J} (dystrophic) were obtained from The Jackson Laboratory, Bar Harbor, Maine 04609. The animals were maintained on a diet of Purina Mouse Chow and water supplemented as follows. Groups of normal and dystrophic animals were given either tap water, water containing 0.4 mg/ml diphenylhydantoin, water containing 4.0 mg/ml LiCl, or water containing both diphenylhydantoin and LiCl. A diminution of drinking by animals receiving diphenylhydantoin was noted initially but, after the first week, water uptake was normal at approximately 5 ml per animal per day. Animals were maintained under these conditions for 21 days, after which they were killed by decapitation, the diaphragm removed and rinsed in K^+-free buffer containing 120 mM NaCl, 2.0 mM $CaCl_2$ and 1.0 mM Tris, pH 7.35. The tissue was then preincubated for 5 minutes at 20° in Na^+ and K^+-free medium containing 86 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM ouabain, and 1 mM Tris, pH 7.35. The purpose of the preincubation was removal of extracellular potassium. The tissue was removed from the medium, lightly blotted, and placed in potassium-free buffer whose composition was given above but also containing 10^{-4} M ouabain Potassium efflux was estimated using a potassium ion electrode (Corning No. 476132) which exhibited about an 80-fold selectivity for K^+ as compared with Na^+. The diaphragm was prevented from contacting the electrode by a perforated plastic disc, upon which was placed a magnetic stirring bar. All procedures were

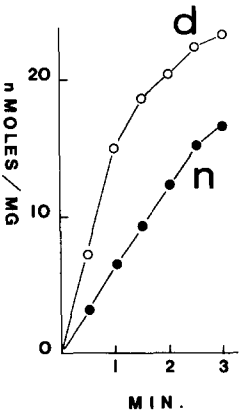

Figure 1. Efflux of K^+ from mouse diaphragm. Conditions are as described in the Methods section. Data are corrected for electrode drift and expressed on the basis of milligrams of tissue. "n" denotes diaphragm for a normal animal, "d" from a dystrophic animal of strain 129/ReJ-dy.

carried out at 20° C. and in a chamber volume of 5 ml. At the end of efflux measurements, the tissue was removed from the chamber, blotted and weighed.

RESULTS AND DISCUSSION: Figure 1 illustrates the characteristic loss of potassium from a normal and dystrophic diaphragm, the increased efflux in the dystrophic case being evident. Efflux is linear for about one minute in the dystrophic case and somewhat longer in the normal, a difference probably reflecting greater loss from dystrophic diaphragm during the preincubation. A number of such experiments are summarized in Table I where significantly increased efflux (measured as initial rates) is seen in two strains of dystrophic mice. It is noteworthy that K^+ efflux is greater in the severely dystrophic 129/ReJ-dy strain than is the case of C57Bl/6J-dy^{2J} strain where the dystrophy is relatively benign.

Treatment of dystrophic animals with LiCl + DPH lowered the rate of K^+ efflux to a value indistinguishable from normal ($p>0.05$), while treatment with either LiCl or DPH alone was

TABLE I - Efflux of K^+ from normal and dystrophic diaphragm

Strain	Phenotype	Treatment	n	Efflux nmoles/mg/min
129/ReJ	normal	none	12	8.34 ± 1.18
129/ReJ-dy	dystrophic	none	11	18.48 ± 1.94
C57Bl/6J	normal	none	7	7.36 ± 0.47
C57Bl/6J	normal	LiCl + DPH	4	8.88 ± 1.65
C57Bl/6J-dy^{2J}	dystrophic	none	8	12.28 ± 0.71
C57Bl/6J-dy^{2J}	dystrophic	LiCl + DPH	6	8.22 ± 0.73
C57Bl/6J-dy^{2J}	dystrophic	LiCl	3	12.10 ± 1.40
C57Bl/6J-dy^{2J}	dystrophic	DPH	4	12.50 ± 0.92

*Values are expressed ± the standard error.

without effect. Moreover, treatment of normal animals with LiCl + DPH did not produce significant inhibition of efflux so that the treatment appears specific in retarding that component of efflux associated with the dystrophic state.

Since increased efflux of potassium from diaphragm appears to be useful measure of the dystrophic state in mice, it is of interest that treatment with LiCl and DPH lowers that efflux to normal values. If it is true that the cation leak is an aspect of the etiology of the disease, it will be of considerable interest to examine the influence of these agents upon features of muscle function and experiments are being conducted to this end. Since either LiCl or DPH, alone, is known to inhibit K^+ movement through membranes (7,9) (presumably by different mechanisms), the requirement for both in the present instance requires comment. It is possible that the requirement for both agents is absolute in the sense that they are performing quite different functions with, for instance, DPH acting as a general membrane stabilizer with Li^+ inhibiting K^+ transport directly. On the other hand, it is also possible that the requirement for both agents merely reflects the impossibility of using either, alone, at a concentration high enough to be effective owing to the attainment of toxic levels.

Finally, these results should be viewed in the context of the recent report describing decreased loss of creatine phosphokinase from human dystrophic muscle upon administration of lithium gluconate (10), a finding which indicates a measure of restored integrity of the dystrophic sarcolemma. Similarly, the influence of DPH reported in this communication should be considered in light of a recent report of the influence of that compound in reversing membrane damage in the case of steroid-induced myopathy (11).

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