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Ca^{2+}-Stimulated Respiration in Mitochondria Isolated From Different Fiber Types of Normal Chick Muscle and From Dystrophic Muscle[1] (38229)

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In adult mammalian muscles, fibers exhibiting a low specific activity for myofibrillar ATPase are "slow-twitch" fibers, and fibers which possess a higher myofibrillar ATPase activity are "fast-twitch" fibers (1, 2). "Slow-twitch" fibers of normal muscle always exhibit a dependence upon aerobic metabolism for energy production. "Fast-twitch" fibers include a continuum of types ranging between those showing a dependence upon aerobic metabolism and those showing a preference for anaerobic metabolism. The latter fibers depend upon metabolism of intra-fiber glycogen stores to provide most of their energy needs (3). We have referred to these fiber types as βR, and αR $\rightarrow$ αW to indicate the relative "static/dynamic" nature of the phenotypes of "slow-twitch" and "fast-twitch" fibers, respectively (4–6).

Fiber types which exhibit myofibrillar ATPase and metabolic enzyme activity patterns like those of mammalian muscles have also been described in chick muscles (4). Studies comparing respiratory performance of mitochondria isolated from each of βR, αR and αW fiber types in chick muscle pointed out that mitochondria from different fiber types have different metabolic capacities (7).

In a study of mitochondria isolated from normal and dystrophic chick muscles, it was found that dystrophic chick muscle has a significantly higher concentration of mitochondria (at 4 weeks of age) than normal chick muscle, but that qualitatively, the enzyme patterns are similar in mitochondria of both genotypes (8).

In the present report, we have compared some effects of Ca^{2+} on respiratory performance of mitochondria isolated from βR, αR, and αW fiber types of normal chick muscle, and have also examined some effects of this ion on mitochondria isolated from dystrophic chick pectoralis muscle (αW).

Materials and Methods. Chicks used in these experiments were from normal (200) and genetically dystrophic (304) lines of chicks maintained at this station, and were approximately four weeks of age. These lines have been described in detail (9).

In Expt. 1, mitochondria were isolated from the pectoralis, medial adductor, and lateral adductor muscles of ten normal chicks. These muscles vary from each other in their fiber type composition, and they have been compared cytochemically and biochemically in earlier studies (4, 6–8, 10). The mitochondria from the three muscles were compared for their capacity to oxidize succinate or glutamate in the presence of ADP or Ca^{2+}.

In Expt. 2, mitochondria were isolated from the pectoralis muscles of 25 normal and 25 dystrophic chicks and assayed as in Expt. 1. In addition, the Ca^{2+} concentration of freshly isolated mitochondria was determined, as was their ability to accumulate Ca^{2+} during the Ca^{2+}-stimulated oxidation of glutamate.

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Isolation. Mitochondria were isolated essentially as described previously (7, 8). The muscles were quickly removed from chicks that had been sacrificed by exsanguination, minced, and homogenized in 20 vol of ice-cold medium containing 0.1 *M* sucrose; 0.1 *M* Tris–HCl, pH 7.4; 0.01 *M* EDTA; 0.18 *M* KCl; 0.001 *M* ATP; 0.005 *M* $MgCl_2$; and 1% BSA (final concentrations). Homogenization was performed in a Polytron homogenizer at a setting of 5 for five sec. The homogenate was filtered through cheesecloth and centrifuged at 1900*g* for 10 min. The supernatant was filtered through cheesecloth and centrifuged at 8700*g* for 10 min. The pellet obtained was resuspended in 10 vol of isolation medium, but minus ATP and BSA, and recentrifuged. The pellet obtained was resuspended and washed for a second time, and recentrifuged at 8700*g* for 10 min. The final pellet was then used for the polarographic assays as described below.

Polarographic assays. Polarographic assays were performed at 26° in a Gilson model KM oxygraph with a Clark electrode in a final vol of 1.0 ml. The assay medium consisted of 0.3 *M* mannitol; 0.01 *M* Tris–HCl, pH 7.4; and 0.01 *M* PO_4; and 1% BSA (final concentrations). Substrate concentrations were 60 m*M* succinate, or 10 m*M* glutamate. Assays were started by the addition of approximately 2 mg mitochondrial protein.

In Expts. 1 and 2, the State 3 respiration rate (11) was measured following the second addition of 120 n*M* ADP, and the Ca^{2+}-stimulated rate was measured following the second addition of 15 μg Ca^{2+}. Ca^{2+}/O ratios were determined by dividing the μg Ca^{2+} added by the μg atoms of O consumed during the second Ca^{2+} "jump" (12)

Ca^{2+} concentration. Ca^{2+} concentration was measured by atomic absorption spectrophotometry of acid digests (13) of mitochondria in a Model 403 Perkin-Elmer spectrophotometer. Ca^{2+} concentration was determined in Expt. 2 for mitochondria freshly isolated, as described above, from normal and dystrophic chick pectoralis muscles. Mitochondrial Ca^{2+} uptake during Ca^{2+}-stimulated respiration was determined indirectly by assaying the Ca^{2+} remaining in the medium following removal of the mitochondria by centrifugation. Mitochondrial protein was determined by the method of Lowry (14).

Results. Data presented in Table I show that mitochondria isolated from muscles containing predominantly αW, αR, or βR fibers performed in a similar manner in the presence of ADP whether glutamate or succinate was the substrate. Mitochondria from the "red" fiber types (αR and βR) had significantly higher rates than those from the αW fibers. The addition of Ca^{2+} to the reaction vessel stimulated respiration in mitochondria from each fiber type, but the relative rates of respiration for each substrate were roughly in proportion to the rates obtained after addition of ADP. Ca^{2+}/O ratios were not significantly different for mitochondria from the different fiber types whether glutamate or succinate was the substrate, but as expected the Ca^{2+}/O ratios were higher for glutamate than for succinate.

Data presented in Table II show that State 3 respiration rates were higher for mitochondria from dystrophic muscle than

TABLE I. Respiratory Performance of Mitochondria Isolated from βR, αR and αW Fiber Types of 5-Week-Old Normal Chickens.

	Glutamate			Succinate		
	QO_2[a]	Ca^{2+}[b]	Ca^{2+}/O	QO_2	Ca^{2+}	Ca^{2+}/O
Pectoralis (αW)	3.0	2.2	6.4	3.1	2.7	3.4
Lateral adductor (βR)	4.0[c]	3.6[c]	6.6	4.8[c]	4.8[c]	3.1
Medial adductor (αR)	4.2[c]	3.6[c]	6.6	4.7[c]	5.2[c]	3.8

[a] Rate of respiration following second addition of 120 n*M* ADP (μg atoms/O/mg protein/hr.).

[b] Rate of respiration following second addition of 15 μg Ca^{2+} (μg protein/hr.).

[c] $P < 0.05$ compared to pectoralis (αW).

TABLE II. Comparison of Respiratory Performance of Mitochondria Isolated from the Pectoralis Muscle of 5-Week-Old Normal and Dystrophic Chickens.

	Glutamate		Succinate		μg Ca^{2+}	μg Ca^{2+} uptake
	QO_2[a]	Ca^{2+}[b]	QO_2	Ca^{2+}	mg protein[c]	mg protein
Normal	3.0	2.4	3.1	2.8	0.5	3.1
Dystrophic	4.0[d]	3.3[d]	5.1[d]	4.3[d]	0.8[d]	4.4[d]

[a] Rate of respiration following second addition of 120 n*M* ADP (μg atoms O/mg protein/hr.).
[b] Rate of respiration following second addition of 15 μg Ca^{2+} (μg atoms O/mg protein/hr.).
[c] Ca^{2+} concentration of freshly isolated mitochondria.
[d] $P < 0.05$ compared to normal.

for normal muscle mitochondria for both substrates. Ca^{2+}-stimulated rates were also higher, but as was found for mitochondria from the different fiber types of normal muscle (Table I) the rates after addition of Ca^{2+} were roughly proportional to the rates obtained after ADP stimulation. The Ca^{2+} concentration of freshly isolated mitochondria and the Ca^{2+} accumulated during Ca^{2+}-stimulated glutamate oxidation were both significantly higher for dystrophic chick muscle mitochondria.

Discussion. Mitochondria isolated from a variety of vertebrate tissues exhibit a marked stimulation of State 4 respiration upon the addition of small amounts of Ca^{2+} (12). In each case the stimulated rate is followed by a return to State 4 and this pattern has been referred to as the "Ca^{2+} jump." Results presented here show that chick skeletal muscle mitochondria behave qualitatively in a similar fashion.

The stimulation of respiration by Ca^{2+} is accompanied by Ca^{2+} uptake and suggests that mitochondria may play an important role in the regulation of free Ca^{2+} in the cytoplasm (12, 15). Small additions of Ca^{2+} to *in vitro* systems have been shown to modify adenine nucleotide translocation (16), ATP-stimulated succinate oxidation (17), and pyruvate kinase activity (18). It has been suggested that mitochondrial uptake of Ca^{2+} may play a role in regulation of Ca^{2+}-stimulated contraction–relaxation of skeletal and heart muscle (19, 20).

In a previous report (7) we demonstrated that mitochondria isolated from different fiber types of chick muscle differed from each other qualitatively. Mitochondria from αW fibers, but not those from the βR or αR fibers, were able to oxidize α-glycerol phosphate. On the other hand, mitochondria from βR fibers, but not those from αR or αW fibers, exhibited controlled respiration with β-hydroxybutyrate as substrate. In this report it is shown that Ca^{2+}-stimulated State 4 respiration in mitochondria from each of the fiber types is approximately proportional to the rates at which the mitochondria from the fibers oxidize various substrates in the presence of ADP. It would seem therefore that the potential of mitochondria in "white" fibers to regulate intracellular Ca^{2+} concentrations would be significantly less than that of "red" muscle fiber mitochondria. Not only are Ca^{2+}-stimulated rates of respiration lower in αW fibers, (and therefore respiration dependent Ca^{2+} uptake), but αW fibers have significantly lower total mitochondrial protein than either αR or βR fibers (8).

Earlier, we reported that mitochondria isolated from dystrophic chicken pectoralis muscle were qualitatively like mitochondria isolated from normal chicken pectoralis muscle since organelles from both sources actively oxidized α-glycerol phosphate, a characteristic of αW muscle fiber mitochondria (8). However, it was also found that dystrophic muscle of chickens of this age contained higher concentrations of mitochondria. Further, the State 3 rate of respiration of dystrophic muscle mitochondria was higher than that of normal muscle mitochondria when succinate was the substrate. The latter finding is confirmed here (Table II). It can also be seen that the State 3 rate of respiration using glutamate

as substrate is also higher for the dystrophic organelles, and as was found in the comparison of mitochondria from the three fiber types (Table I), the Ca^{2+}-stimulated rates with either glutamate or succinate appear to be in proportion to the ADP-stimulated rates for both genotypes. Qualitatively then, dystrophic chick muscle mitochondria appear to respond *in vitro* in a normal manner when Ca^{2+} is added to the medium. The higher rates of Ca^{2+}-stimulated respiration are reflected in higher amounts of Ca^{2+} removed from the medium by the mitochondria (Table II).

Freshly isolated mitochondria from dystrophic muscle also exhibit significantly higher Ca^{2+} concentrations (Table II). It is not likely that these differences result from Ca^{2+} uptake during isolation since very high (10 m*M*) concentrations of EDTA are used throughout the procedure. This is further suggested by the very low levels of Ca^{2+} that are present in the organelles after isolation under these conditions. It would seem more likely that the Ca^{2+} present in the freshly prepared mitochondria represents tightly bound Ca^{2+} that was resistant to removal during preparation. If this be the case, then the higher Ca^{2+} levels in dystrophic mitochondria may be indicative of a greater capacity of dystrophic muscle mitochondria to bind Ca^{2+} *in vivo*.

Wrogemann, Blanchear and Jacobson (21) recently reported a Ca^{2+}-associated defect of respiration by skeletal muscle mitochondria isolated from genetically dystrophic hamsters. They observed, as we have for dystrophic chickens, that mitochondria freshly isolated from the myopathic muscle of hamsters contain significantly larger amounts of Ca^{2+}. They also observed uncoupled respiration by dystrophic hamster mitochondria, but were able to restore their function to normal by removing the Ca^{2+} with high levels of EDTA during isolation. In addition they could induce a similar disturbance in respiration of normal hamster muscle mitochondria by addition of Ca^{2+} in amounts found in the dystrophic organelles. Our inability to observe uncoupled respiration in dystrophic chick muscle mitochondria likely results from the high levels of EDTA used in our preparations (8). Howland and Chollberg have recently reported altered respiration and proton premeability in liver mitochondria from genetically dystrophic mice (22). Since liver tissue in these animals does not appear to be affected in a functional or morphological way by the genetic defect, it would appear that an alteration of cation movements across mitochondria membranes is by itself not sufficient for cell death. As these latter authors suggest, however, due to the central role of Ca^{2+} flux across membranes, such an alteration may have more significant consequences for muscle tissue.

Summary. Mitochondria were isolated from normal chick muscles composed predominantly of αW, αR, or βR fibers and compared polarographically. Rates of respiration after addition of ADP were higher for mitochondria from αR and βR fibers than for mitochondria from αW fibers. Respiration of mitochondria from all three fiber types was enhanced by the addition of Ca^{2+} to the medium but at rates similar to those observed after addition of ADP. Ca^{2+}/O ratios were not different for the mitochondria from the different fiber types.

Mitochondria were isolated from the pectoralis muscle (αW) of normal chicks and chicks with hereditary muscular dystrophy. Mitochondria from dystrophic chick muscle exhibited higher rates of respiration after addition of ADP than mitochondria from normal chick muscle. Respiration in mitochondria from both genotypes was enhanced proportionately by the addition of Ca^{2+}. Dystrophic organelles accumulated more Ca^{2+} than normal mitochondria during Ca^{2+}-stimulated respiration, but the increased uptake was proportional to this higher rate of respiration. Freshly isolated mitochondria from dystrophic muscle contained higher levels of Ca^{2+} than those from normal muscle. Since very high levels of EDTA (10 m*M*) were used throughout the isolation procedure, it is suggested that dystrophic muscle mitochondria may have a higher binding capacity for Ca^{2+}.

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