COMPARATIVE STUDIES OF THE MG ACTIVATED ATPASE ACTIVITY AND CA UPTAKE OF FRACTIONS OF WHITE AND RED MUSCLE HOMOGENATES*

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Since the early studies of Ranvier (1874) it has been known that the so-called white muscles, e.g. m. adductor magnus, vastus lateralis, etc., are characterized by a rapid rate of contraction resulting in a higher frequency required for tetanization, while red muscles, e.g. m. semitendinosus, soleus, etc., characterized by a higher mitochondrial and myoglobin content (Slater,1960), show a slower contraction-relaxation cycle and a lower critical frequency for tetanization.

In view of the recent implication in the relaxation process of a fraction possessing Ca-accumulating and ATPase activity (Ebashi and Lipman,1962; Hasselbach and Makinose,1961) which can be isolated from muscle homogenates by centrifugation between 8,000 and 30,000 g, so called muscle grana, it appeared of interest to examine on a comparative basis the ATPase activity of and the active Ca uptake by various muscle fractions of white and red muscles.

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Table I shows Ca uptake and ATPase activity of various muscle fractions obtained from homogenates of a typical white muscle (vastus lateralis) and red muscle (semitendinosus) of the rabbit. In all fractions - except grana III - the Ca uptake per mg of protein in red muscle is an order of magnitude lower than in the corresponding fraction of white muscle.

Table I

Ca Uptake and ATPase Activity in Various Fractions of Rabbit White and Red Muscle

Fraction		Centrifugal force, g	Ca^{++} uptake, μmole per mg of protein		Initial rate of ATPase, ΔP_i, μmole per min. per mg of protein	
			White	Red	White	Red
I	Homogenate		0.32	0.01	0.96	0.28
II	Mitochondria	8000	1.67	0.11	1.43	1.04
III	Grana I	30000	6.76	0.35	1.10	1.65
IV	Grana II	60000	1.81	0.19	0.50	1.04
V	Grana III	90000	0.13	0.09	0.04	0.11

Muscle samples were homogenized with 4 volumes of ice-cold 0.1M KCl and 5 mM histidine, pH 7.2, for two minutes in a Waring blendor. The myofibrils were sedimented by centrifugation at 1,000 x g for 20 minutes and the supernatant was fractionated by centrifugation in a Spinco model L preparative ultracentrifuge. The fractions sedimented at the forces indicated in the table were dissolved in KCl-histidine. Aliquots were added to a medium containing 0.1M KCl, 10 mM histidine, pH 7.2, 5 mM $MgCl_2$, 5 mM oxalate, 5 mM ATP and 0.2 mM $^{45}CaCl_2$, for measurements of Ca^{++} uptake (Martonosi and Feretos, 1964, a) and ATPase activity at 24°. Ca^{++} uptake values given in the table represent maximal uptake (10 min.). Protein concentrations in the assay systems were 1.5, 0.4, 0.023, 0.2 and 0.4 mg/ml, for homogenate, mitochondria, grana I, grana II, and grana III, respectively.

The figures for Ca uptake given in the table have been obtained without taking into consideration calcium present in the various fractions. The Ca content was 18 mμmoles per mg of protein for both white and red muscle homogenates. If one assumes that all the calcium present was bound, but exchangeable with added Ca^{45}, the calculated uptake by the

homogenate would be reduced by about 6%, while in the case of red muscle all the apparent uptake could be accounted for as exchange. If, as the other extreme, one assumed that all the Ca present was free, but could be bound in the presence of ATP and Mg, the white value would be increased by about 10% while that for red muscle would be increased by a factor of 1.8 under the conditions of the experiment. It is clear that in either case the large difference between the white and red homogenate remains. In all the other fractions the corrections necessitated by the presence of calcium are negligible.

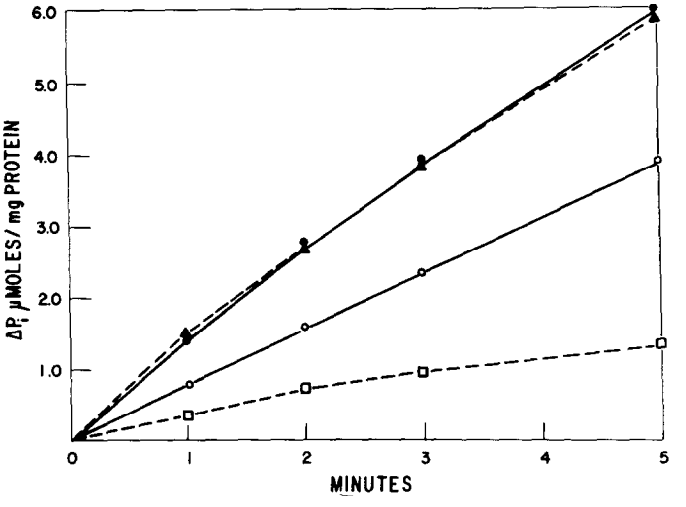

Fig. 1. Effect of EGTA on ATPase of white and red grana.

Assay medium: 0.1M KCl, 10 mM histidine, pH 7.2, 5mM $MgCl_2$ and 5 mM ATP. Protein concentration: 0.05 mg per ml. Key: ○ , white grana; □ ,white grana + 0.5 mM EGTA; ● ,red grana; ▲ ,red grana 0.5 mM EGTA.

The most active fraction is that which is obtained by centrifuging the white muscle homogenate between 8,000 and 30,000 x g, with a value of 6 μmoles of Ca taken up per mg of protein. The corresponding Ca uptake in similar fractions, obtained from mixed (red and white) muscle

homogenates has been usually of the order of 2 μmole per mg (Martonosi and Feretos,1964,a). Recently, Hasselbach and Makinose (1964) have reported Ca uptakes of the order of 6 μmoles per mg of protein after fractionation of mixed grana by centrifugation in various concentrations of sucrose. In the light of our results this would presumably mean that they obtained the fraction which is derived from white muscle.

Martonosi and Feretos (1964,b) have shown that EGTA inhibits the ATPase activity of mixed muscle grana preparations by complexing with Ca^{++}. It appears that this Ca^{++} requirement is characteristic of white grana since EGTA depresses their ATPase activity, but not that of red grana.

Oxalate, by stimulating Ca uptake, under certain conditions leads to lower ATPase activity of mixed grana (Martonosi and Feretos,1964,b). This depression can also be demonstrated with white grana, but not with red. Depression of ATPase activity occurs if essentially all the Ca present is removed. A detailed study of the effect of oxalate of grana-ATPase will be published later.

The difference between white and red grana is also brought out by ageing at 0^{o}. Fig. 2 shows that 3 week-old white grana have a considerably increased ATPase activity while that of the red grana is significantly decreased.

A clue to the differences between white and red muscle grana can be found in the different behavior of the ATPase activity with respect to azide. Azide is known to inhibit ATPase of mitochondrial origin (Siekevitz et al., 1958) and it has been shown that the so-called microsomal fraction from various organs is inhibited to varying extents by azide (Schwartz,1963). Comparison of the 8 to 30,000 g fraction from white and red muscle is given in Fig. 3. The grana fraction from white muscle is essentially uninhibited while the red fraction shows substantial inhibition. Azide had no effect on the Ca uptake by white grana.

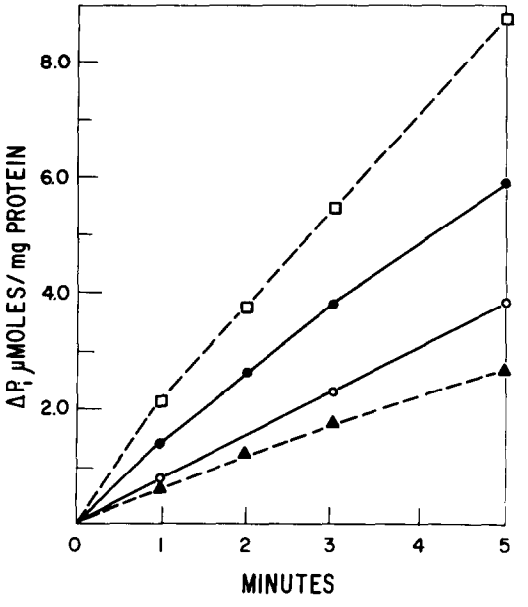

Fig. 2. Comparison of ATPase activity of fresh and aged white and red muscle grana.

Experimental conditions are described in the legend to Fig. 1. ○ , fresh white grana; □ ,white grana; ● ,fresh red grana; ▲ ,aged red grana.

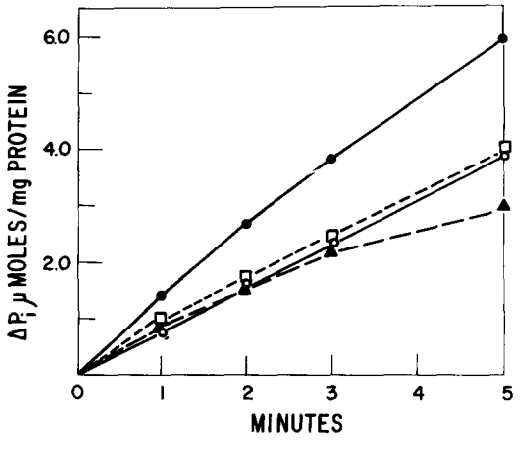

Fig. 3. Effect of azide on the ATPase activity of white and red muscle grana.

Experimental conditions are the same as those described in the legend to Fig. 1, except that azide (5 mM) was added as indicated. ○ ,white grana; □ ,white grana + 5 mM azide; ● ,red grana; ▲ , red grana + 5 mM azide.

Preliminary electron microscopic studies carried out in collaboration with Dr. H. Webster have shown striking morphological differences between white and red grana preparations. Electron micrographs of red grana preparations are very similar to electron micrographs published on mixed grana preparations consisting of round vesicles interspersed with narrower tubular elements, whereas white grana preparations consist of narrow tubular elements with very few vesicles. Two possibilities may have to be considered: the fraction most active in Ca uptake may consist of the narrow tubular elements of the T system(Andersson-Cedergren,1959) of the triads, while the red muscle grana consist of the wider elements of the sarcotubular system. Or, the narrow elements visible in white muscle may be typical of sarcoplasmic reticulum, including the T system, and the vesicular elements may reflect the presence of mitochondrial fragments. The resolution of this question must be elucidated by further research.

The fact that no fraction could be obtained from red muscle homogenates that shows Ca uptake comparable to that in the grana fraction of white muscle raises the question whether, if one accepts the view that the Ca uptake of white muscle grana is connected with the mechanism of relaxation in vivo - the relaxation of red muscles was regulated by a mechanism different from that in white muscle. If, in vivo, red muscle relaxation too is based on the uptake of Ca by the sarcoplasmic reticulum, the present result would show that this structural element sufficiently differs from its counterpart in white muscle so as to lead to loss of Ca uptake activity on isolation.

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