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A COMPARISON OF THE RELAXING-FACTOR SYSTEMS FROM HEART AND SKELETAL MUSCLES*

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SUMMARY

The relaxing-factor systems from heart and skeletal muscle are interchangeable. Both systems inhibit the Mg-activated ATPase (EC 3.6.1.4) activity of either cardiac or skeletal myofibrils. The inhibition of cardiac-myofibrillar ATPase activity by either relaxing-factor granules, non-granular relaxing factor or EDTA, however, is not reversed by Ca^{2+}. This contrasts with the effect of Ca^{2+} on the inhibition of skeletal-muscle ATPase by these various relaxing factors. The results indicate that cardiac- and skeletal-muscle relaxing-factor systems operate by the same mechanism but that there is a difference between cardiac and skeletal myofibrils.

INTRODUCTION

The properties of the Marsh–Bendall relaxing-factor system of skeletal muscle have been reviewed elsewhere[1]. The factor inhibits the Mg-activated ATPase (EC 3.6.1.4) activity of skeletal-muscle myofibrils as well as ATP-induced tension development in single glycerinated muscle fibers. The activity of the factor is associated with the microsome-like granules of muscle. These granules produce a soluble or non-granular factor in the presence of ATP and Mg^{2+} which accounts for the inhibitory activity of the granules[2].

Recently an active relaxing-factor system has been isolated from cardiac muscle[3] which is similar to the relaxing factor of skeletal muscle. Cardiac relaxing-factor granules produce a non-granular relaxing factor in the presence of ATP and Mg^{2+} which inhibits tension development in single muscle fibers[3] and the ATPase activity of cardiac myofibrils[4]. The interchangeability of cardiac and skeletal relaxing factor, however, has not been established conclusively, since FINKEL AND GERGELY[5] were unable to demonstrate an inhibition of cardiac-myofibrillar ATPase activity using skeletal-muscle relaxing-factor granules. The results presented below show that skeletal relaxing factor will, in fact, inhibit cardiac myofibrillar ATPase; they offer a possible explanation for the observations of FINKEL AND GERGELY.

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During the course of this study it was observed that Ca^{2+} had no effect on the inhibition of cardiac-myofibrillar ATPase activity by skeletal relaxing-factor granules. This contrasts sharply with the effect of Ca^{2+} on the ATPase activity of skeletal myofibrils inhibited by skeletal relaxing factor. In the latter case, Ca^{2+} is a potent antagonist of relaxing-factor activity. The present report shows that the inhibition of cardiac-myofibrillar ATPase activity by cardiac and skeletal relaxing-factor granules, the skeletal non-granular relaxing factor and the non-physiological relaxing factor EDTA, is unaffected by calcium ions.

METHODS

Myofibrils, relaxing-factor granules and non-granular relaxing factor were prepared from rabbit muscle as described elsewhere[2]. Granules were stored frozen in 0.32 M sucrose until used. Myofibrils were stored in 50% glycerol at — 16°. Prior to use the glycerol was removed by washing in 0.065 M succinate buffer (pH 7). Relaxing-factor activity was assayed by measuring the extent to which it inhibited myofibrillar ATPase activity. The results are expressed as per cent inhibition. Myofibrillar ATPase activity (cardiac and skeletal) was determined at 25° in a pH 7.0 buffer containing 50 mM KCl, 20 mM Tris, 2.5 mM oxalate, 5 mM ATP and 5 mM Mg^{2+}. The final volume was 2.0 ml. The reaction was started by the addition of myofibrils (final concentration 1.0 mg/ml). After 5 min the reaction was stopped by the addition of 2.0 ml of 10% trichloroacetic acid and the inorganic phosphate liberated was determined in the protein-free supernatant by the method of FISKE AND SUBBAROW[6]. Protein was determined by the biuret method according to LAYNE[7]. Radioactivity was measured by drying 0.05-ml samples on filter-paper discs affixed to aluminum planchets and counting in a gas-flow counter.

RESULTS

The results in Table I show that cardiac- and skeletal-muscle relaxing-factor granules are interchangeable. They will inhibit the ATPase activity of either cardiac or skeletal

TABLE I

THE EFFECT OF Ca^{2+} ON THE INHIBITION OF MYOFIBRILLAR ATPASE ACTIVITY BY RELAXING-FACTOR GRANULES

The conditions are described under METHODS. The concentrations of relaxing-factor granules and Ca^{2+} are given in the table.

Source of		*Concentration of*		*Per cent inhibition of myofibrillar ATPase*	
myofibrils	*relaxing-factor granules*	*granules (µg/ml)*	*Ca^{2+} (mM)*	*without Ca^{2+}*	*with Ca^{2+}*
Skeletal	Skeletal	885	1.0	73.4	19.3
	Skeletal	885	1.0	73.5	23.5
	Skeletal	150	—	85.9	—
	Heart	200	0.5	37.3	11.9
Heart	Heart	200	0.5	45.0	45.0
	Skeletal	885	1.0	27.0	21.5
	Skeletal	815	2.0	37.4	34.9

myofibrils. It is evident that skeletal relaxing factor is more active than its heart counterpart. Skeletal relaxing-factor granules (150 μg protein/ml) causes an 86% inhibition of skeletal-myofibrillar ATPase activity while a comparable concentration of heart relaxing-factor granules causes only 37% inhibition.

During the course of these experiments it was observed that heart myofibrils stored in 50% glycerol at — 16° or in 65 mM succinate at 5° for periods of 1–2 weeks lose their ability to respond to the action of either skeletal or cardiac relaxing factor. A similar aging effect has never been observed in this laboratory with 24 batches of skeletal myofibrils prepared and stored under similar conditions for periods of 4–5 months. This aging effect may account in part for the finding that a given concentration of skeletal relaxing-factor granules (885 μg protein/ml) is about 63% less active with cardiac myofibrils than with skeletal myofibrils. In addition, the aging effect may explain the failure of FINKEL AND GERGELY[5] to demonstrate inhibition of cardiac-myofibrillar ATPase by either heart or skeletal relaxing factor.

The interesting feature in Table I is the observation that calcium ions have little or no effect on the inhibition of cardiac-myofibrillar ATPase by skeletal or cardiac relaxing-factor granules. This contrasts sharply with the effect of Ca^{2+} on the inhibition of skeletal-myofibrillar ATPase by both types of relaxing-factor granules. The granule and Ca^{2+} concentrations are comparable in both cases. In the latter system the Ca^{2+} concentrations used cause a 70% inactivation of relaxing-factor activity. Similar results are obtained if either EDTA or the soluble or non-granular relaxing factor prepared from skeletal relaxing-factor granules are used as a myofibrillar ATPase inhibitor (see Table II). Calcium, at the concentrations used, causes a marked reduction in the inhibition of skeletal-myofibrillar ATPase by both EDTA and the non-granular relaxing factor. Comparable concentrations of Ca^{2+}, however, have no effect on the inhibition of cardiac-myofibrillar ATPase by these agents. STAM AND HONIG[4] have shown in similar experiments that Ca^{2+} has no effect on the inhibition of cardiac-myofibrillar ATPase by cardiac non-granular relaxing factor.

It is possible that added Ca^{2+} is bound to heart myofibrils. Such binding could result in a decrease in the Ca^{2+} concentration to a level insufficient to overcome the inhibition of heart-myofibrillar ATPase by the various inhibitors used. The results in

TABLE II

THE EFFECT OF Ca^{2+} ON THE INHIBITION OF MYOFIBRILLAR ATPASE ACTIVITY BY EDTA AND THE NON-GRANULAR RELAXING FACTOR

The conditions are described under METHODS. The soluble or non-granular relaxing factor was prepared from skeletal-muscle relaxing-factor granules and is equivalent to 940 μg of granule protein per ml of reaction mixture.

Myofibrils	*Inhibitor*	*Ca^{2+} concentration (mM)*	*Per cent inhibition*	
			without Ca^{2+}	*with Ca^{2+}*
Skeletal	1.0 mM EDTA	1.0	80.9	6.0
	2.0 mM EDTA	1.0	86.0	24.2
	Non-granular factor	0.05	46.4	0.0
Heart	1.0 mM EDTA	2.0	25.0	25.0
	2.0 mM EDTA	2.0	38.7	38.7
	Non-granular factor	0.05	25.6	23.3

Table III show, however, that this is not the case. Neither heart nor skeletal myofibrils remove any measurable amounts of Ca^{2+} from solution under conditions similar to those used in the experiments reported in Tables I and II. There is no doubt that some Ca^{2+} is bound to relaxing-factor granules (see ref. 2). At the Ca^{2+} concentrations used, however, there is sufficient non-granule-bound Ca^{2+} to overcome the action of the relaxing-factor granules on skeletal-myofibrillar ATPase. The concentration of non-granule-bound Ca^{2+} is the same in both the skeletal- and cardiac-myofibril systems since the granule concentrations used in both cases are the same. The same argument applies in those experiments where EDTA was used as an inhibitor.

TABLE III

THE BINDING OF CALCIUM IONS TO HEART AND SKELETAL MYOFIBRILS

Myofibrils (1.1 mg protein/ml) were suspended in buffer at pH 7.0 containing 20 mM Tris, 50 mM KCl, 2.5 mM oxalate, 5 mM ATP, 5 mM $MgCl_2$ and 0.075 mM $^{45}Ca^{2+}$ ($4.26 \cdot 10^8$ counts/min/mmole). Carrier Ca^{2+}: 1.0 mM. The mixture was allowed to stand 2 min, centrifuged, and the activity remaining in the supernatant measured. The activity added was determined on a sample taken before myofibrils were added to the system.

	Activity (counts/min/ml)	
	Skeletal myofibrils	*Heart myofibrils*
Activity added	32 400	31 000
Activity after protein removed	32 800	30 900

DISCUSSION

The results reported here and elsewhere furnish a clear indication that cardiac and skeletal relaxing-factor granules and the non-granular relaxing factor produced by these granules are interchangeable. Both cardiac and skeletal relaxing factors (granular and non-granular) will inhibit the ATPase activity of either cardiac or skeletal myofibrils. It is, therefore, reasonable to conclude that heart and skeletal relaxing factor are similar and act by similar mechanisms. The fact that the ATPase activity of both types of myofibrils is inhibited by relaxing factor but only the ATPase activity of inhibited skeletal myofibrils is activated by Ca^{2+} indicates that cardiac and skeletal myofibrils differ in one or more respects. This is not unreasonable in view of the differences in the contractile proteins from these two types of muscle[8].

It has been proposed by WEBER[9] and by EBASHI[10] that both relaxing factor and EDTA act by removing Ca^{2+} bound to actomyosin which is essential for maximal ATPase activity and contraction. This is supported by the fact that relaxing-factor granules as well as EDTA bind Ca^{2+} (see refs. 2 and 11). Some doubts about this mechanism have been raised by PARKER AND GERGELY[12] who were unable to demonstrate a Ca^{2+} requirement for skeletal-myofibrillar ATPase. These authors concluded that the removal of Ca^{2+} from actomyosin does not, in itself, account for the action of either the muscle relaxing factor or EDTA. The findings presently reported above are consistent with this latter view. Since cardiac and skeletal relaxing factor appear to have the same mechanism of action, it is difficult to reconcile the fact that

Ca^{2+} has no effect on the inhibition of cardiac-myofibrillar ATPase activity by the various relaxing factors with the assumption that relaxing factor acts by binding Ca^{2+} which is essential for ATPase activity. It cannot be argued that there is insufficient Ca^{2+} in the experiments with heart myofibrils since under comparable conditions the inhibition of skeletal-myofibrillar ATPase activity is reversed. It is more reasonable to suppose that the non-granular factor interacts with the ATPase of the myofibril (actomyosin) causing inhibition and that Ca^{2+} interferes with this interaction only in the case of skeletal myofibrils. A similar proposal for the role of Ca^{2+} in relaxation has been made recently by WEBER AND WINICUR[13].

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