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Studies on Actomyosin of Heart Muscle

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Superprecipitation, ATPase activity of actomyosin, and isometric tension of glycerol-extracted muscle fibers, and also the action of ouabain on these actomyosin preparations were investigated on the skeletal and cardiac, both normal and experimentally failing, muscles of dogs. There were no qualitative differences between skeletal and cardiac muscle actomyosin. Superprecipitation and ATPase activity were depressed in actomyosin prepared from right ventricles compared with that from left ventricles of the dogs with surgically induced right-sided heart failure. Isometric tension-development, on the other hand, was reduced in both ventricles. Low concentrations of ouabain improved superprecipitation, both did not affect ATPase activity. Tension-development was unaffected in a Ca free medium, but in a Ca containing medium, the inhibitory action of $10^{-2} \sim 10^{-3}$ M of Ca was eliminated by this concentrations of ouabain. High concentrations, on the other hand, depressed superprecipitation, ATPase activity, and also tension-development. In contrast to low concentrations, high concentrations of ouabain enhanced the inhibitory action of Ca on tension-development. These actions of ouabain in various concentrations were similarly observed in all of skeletal, and both normal and failing cardiac muscles.

THE CONCEPT that the common type of congestive heart failure are due to defects in energy utilization has been almost established. Based on this concept, some workers have studied to detect the primary changes in the properties of actomyosin (AM) extracted from failing heart muscles. Another problem related to the cause of congestive heart failure is the action of cardiac glycoside, of which effective mechanism on heart has been yet completely unknown, despite the numerous and extensive studies including those on the interaction of AM and glycosides. The purpose of this paper was (1) to study the precise comparisons of AM extracted from cardiac muscles with that from skeletal muscles (2) to find out any change in the properties of AM prepared from experimentally failing hearts, and (3) to observe effect of ouabain on AM from skeletal and cardiac, both nor-

mal and failing muscles.

METHODS

AM solution used in the present study was extracted with modified Bing's method.¹⁾ Prior to the observation of superprecipitation in the water bath maintained at 20°C, AM solution was incubated for five minutes at 5°C after the addition of ATP solution. ATPase activity was determined by means of the measurement of inorganic phosphate²⁾ liberated during 5 minutes at 25°C after the addition of ATP solution.

Isometric tension measurements: Strips of trabecular muscles of left and/or right ventricle were stored in fifty per cent glycerol at 0°C for three days, then transferred to fresh fifty percent glycerol, and preserved at -20°C for at least two weeks. Under binocular microscope, the glycerol-extracted strips were separated into small fibers with diameter of about 0.2 mm and length of about 20 mm. Isometric tension of these fibers was measured by the tension meter, modified from that described by Weber.³⁾

RESULTS

(1) The property of AM extracted from cardiac and skeletal muscles of the normal dogs. A) Superprecipitation. The property of superprecipitation of AM extracted from cardiac muscle was previously reported from our laboratory.⁴⁾ In that report, we showed that cardiac muscle had no qualitative differences from skeletal muscle, in regard to the optimal conditions for the formation of the plug, such as pH and the concentrations of AM, ATP, and KCl, and that the inhibition or acceleration of superprecipitation by Ca and Mg was also observed in almost similar manner with that of AM from skeletal muscle. B) ATPase activity of AM. The optimal pH, temperature and also the potassium dependency of ATPase activity of heart muscles was the same as those of skeletal muscles. The activity was enhanced by Ca and inhibited by Mg

about one-tenth of that of skeletal muscle. The optimal pH, and the optimal concentrations of ATP and K was 8.0, 5 mM and 0.06 M, respectively. The temperature was maintained at 20°C in the present experiments. Mg enhanced Ca and inhibited the tension-development (Fig. 1). In higher concentrations of K, Mg and ATP, but not of Ca, the tension was reduced gradually after it reached to its maximum.

(2) The properties of AM extracted from right-sided failing heart of the dogs. Tricuspidal insufficiency and pulmonary stenosis were surgically produced by the method described by Barger et al.⁵⁾ Chronic right-sided heart failure was demonstrated by weakness, reduced tolerance to exercise and pathological findings of congestion in liver and spleen in all cases, and ascites in one case. The physicochemical properties of AM extracted from right ventricles (failing)

Fig. 1. Effect of Mg and Ca on tension-development.

except at lower concentrations of K (0.5 M). Under the optimal conditions the activity was about a half of that of skeletal muscle in the present experiment. C) Isometric tension of glycerol-extracted fibers. Diameter of the fiber for the development of the optimal tension was about 200 μ . The maximal tension was developed within a few minutes, and its average value under the optimal conditions (pH, and the concentrations of ATP, K and Mg) was 200 g/cm², being

were compared with those of AM from left ventricles (normal) in six cases. A) Superprecipitation (Fig. 2-A). It was obvious that the superprecipitation of AM from the right ventricles was significantly retarded, while that of left ventricles showed normal superprecipitation. B) ATPase activity (Fig. 2-B, and Fig. 3). Statistically significant reduction of ATPase activity was observed in right ventricles compared with that in left ventricles, whereas there were no sta-

Fig. 2. Change in the properties of AM extracted from the right-sided failing dog hearts.

Fig. 3. The difference of ATPase activity of left and right heart AM in both normal and failing dogs.

tistic differences of the activities between left and right ventricles of six normal dogs. C) Tension-development (Fig. 2-C). The maxi-

mal tension was reduced in both sides of ventricles of four out of five dogs. The exceptional one showed the slightest congestion among five dogs. No differences of maximal tension-development were observed between left and right ventricles in all cases.

3) Effect of ouabain on the properties of AM extracted from both skeletal and cardiac muscles of the normal dogs. A) Superprecipitation (Fig. 4-C) of AM from cardiac muscles was accelerated by low concentrations ($10^{-7}M$) of ouabain. In contrast, it was inhibited by high concentration (10^{-5} or $2 \times 10^{-5}M$) of ouabain. No effect was observed by the addition of Ca in concentrations of $10^{-2} \sim 10^{-5}M$. Ouabain had the same effect on superprecipitation of AM from skeletal muscles in both higher and lower concentration. B) ATPase activity (Fig. 4-A) of AM from cardiac muscles was inhibited by 10^{-5} or $2 \times 10^{-5}M$ ouabain, while it was unaffected by 10^{-6} and $10^{-7}M$, in either Ca free or Ca

Fig. 4. Effect of various concentrations of ouabain on AM extracted from cardiac and skeletal muscles.

TABLE I COMBINED EFFECTS OF OUABAIN AND CALCIUM ON MAXIMAL TENSION

Conc. of Ouabain	Conc. of Ca				
	(—)	10^{-5} M	10^{-4} M	10^{-3} M	10^{-2} M
(—)	100%	99%	92%	73%	45%
10^{-7} M	98%	93%	91%	91%	73%
10^{-5} M	97%	84%	87%	83%	60%
2×10^{-5} M	69%	64%	63%	50%	36%

pH: 8.0. KCl: 0.06M. ATP: 5×10^{-3} M.
Temp.: 20°C.

containing medium. This ouabain-effect was similarly observed in ATPase activity of AM from skeletal-muscles. C) Tension-development (Fig. 4-B and Table I). Ouabain showed the similar effect on the tension-development of both cardiac and skeletal AM fibers with that on ATPase activity, both in Ca-free and in $10^{-4} \sim 10^{-5}$ M Ca containing medium. In

contrast, in the medium containing higher concentrations of Ca (10^{-2} , 10^{-3} M,) ouabain affected in a different manner. Lower concentrations of ouabain eliminated, and higher concentrations (2×10^{-5} M) of ouabain enhanced the depressant effect of Ca of 10^{-2} , 10^{-3} M on the tension-development.

(4) Effect of ouabain on the properties of AM extracted from failing heart muscles of the dogs. (Fig. 5) Various concentrations of ouabain had exactly the same effect on superprecipitation, ATPase activity and tension-development of AM prepared from the failing (right) ventricle with that from the normal (left) ventricle, either in Ca-free or Ca containing medium.

DISCUSSION

In the present experiments, the physicochemical properties of AM extracted from normal heart muscles were precisely compared with that of AM from normal skeletal muscles and it was confirmed that superprecipitation, ATPase activity and isometric tension of fibers of AM from heart did not show any qualitative difference from that of skeletal muscles.

With regard to the changes in the properties of contractile proteins from failing hearts, Benson and coworkers reported diminished concentrations of AM⁶⁾ and also diminished contractility of glycerol-extracted muscle fibers⁷⁾ prepared from the hearts of the dogs with surgical pulmonary stenosis and tricuspidal insufficiency. Kako and Bing¹⁾ reported a similar decrease of contractility of AM bands prepared from the hearts of patients with congestive heart failure. The opinion which supported these results was presented by Olson,⁸⁾ who observed the changes in the physicochemical properties of myosin extracted from the heart of the dogs subjected to the same valvular surgery with that of Benson's experiments. However, as Hajdu and Leonard⁹⁾ correctly pointed out, it is unreasonable that the change in contractile protein was observed in both ventricles despite of the right-sided heart failure. Considering this particular point, the differences of the properties between

Fig. 5. Effect of ouabain on the properties of AM extracted from the right-sided failing dog hearts.

right (failing) and left (normal) heart were investigated in the present study. In agreement with the results of others mentioned above,¹⁷⁾ the isometric tension of glycerol-extracted fibers was diminished in both ventricles. The outstanding findings, however, was the depression of ATPase activity, in accordance with that of superprecipitation, of AM from the failing right ventricles. The discrepancy between the results on the properties of AM solution and that of AM-fibers may be due to the structural difference of these models. In spite of these changes in AM, there still remains a question whether these changes in AM are the cause or result of congestive heart failure. Nevertheless, it is probable that at least some part of the mechanism of congestive heart failure is related to some changes in the properties of

AM of cardiac muscles.

Conflicting reports¹⁰⁻²¹⁾ or an effect of cardiac glycoside on contractile protein have appeared. Several factors might account for this conflict, such as the variety of methods for the preparation of AM, the difference in the concentrations of cardiac glycoside, and the kind of the prepared muscles, skeletal, normal or pathologic heart muscles. Particular difficulty exists in obtaining pure materials from cardiac, especially from pathologic, muscles. Our experiments were undertaken to observe the effects of various concentrations of ouabain on AM, which was carefully extracted from both skeletal and cardiac muscles. Higher concentrations (toxic dosis) of ouabain suppressed all of superprecipitation, ATPase activity and isometric contraction on glycerinated fibers. One

particular finding was that the inhibition of tension development by Ca was further enhanced by ouabain ($2 \times 10^{-5}M$). The enhancement or appearance of toxic effect of cardiac glycoside by Ca has been observed by numerous workers.⁸⁾ Our findings suggest that membrane-free protein, AM, is one of the locuses of this combined action. The action of lower concentrations (therapeutic doses) of ouabain was somewhat different.

Superprecipitation was enhanced, while ATPase was unaffected. Especially careful experiments were performed on the isometric tension of AM fibers, in Ca free and also in various concentrations of Ca containing medium. In contrast to the results of others,^{1,17,18)} however, the increase of contractility by ouabain could not be detected. This result coincides with the recent opinion of Bing²¹⁾ who previously reported the positive effect of glycoside on AM bands prepared from the failing human hearts. Our experiments showed that the effect of ouabain on the properties of AM was essentially the same in both cardiac and skeletal muscle. Moreover, AM extracted from experimentally failing hearts did not show any difference from that of normal hearts. As was described by Moran & Cotten,²²⁾ recent data indicate that glycosides have similar qualitative effects on both failing and non-failing hearts. However, non-specificity of ouabain-action on AM from failing hearts observed in the present experiments, may not be associated with above concept. From our results, it could be concluded that the toxic effect of glycosides has possibility to relate to the action on contractile protein, but therapeutic effect has no or little relation directly with that on contractile protein.

SUMMARY

Superprecipitation, ATPase activity of actomyosin (AM) solution, and isometric tension of glycerol-extracted fibers were studied on skeletal and cardiac, both normal and experimentally failing, muscles of the dogs. The effect of ouabain in various concentrations was also studied on these AM materials. The results were as follows.

1) The properties did not show any

qualitative difference between skeletal and normal cardiac muscles. ATPase activity and maximal tension of cardiac muscles was about a half and one-tenth, respectively, of those of skeletal muscles.

2) In the right-sided failing heart of the dogs subjected to cardiac valvular surgery, the retardation of superprecipitation and significant decrease of ATPase activity was observed in the right (failing) heart compared with that of the left (normal) heart muscles. Isometric tension of glycerol-extracted fibers was lower in both right and left ventricles than control groups.

3) Lower concentrations of ouabain improved superprecipitation, and did not affect ATPase activity. Tension development was unaffected either in Ca free or lower concentrations of Ca containing medium. In higher concentrations of Ca ($10^{-2} \sim 10^{-3}M$) containing medium, however, ouabain suppressed the inhibitory action of Ca, but the increase of tension above control levels was never observed. Higher concentrations of ouabain depressed superprecipitation, ATPase activity, and tension-development. In contrast to the lower concentrations, the higher concentrations of ouabain enhanced the inhibitory action of 10^{-2} and $10^{-3}M$ of Ca on tension development. These action of ouabain in various concentrations were similarly observed in skeletal and both normal and failing cardiac muscles. Thus, there was no specific action of ouabain on AM extracted from the failing heart.

4) From the results described above, the mechanism of congestive heart failure and that of the action of cardiac glycosides were discussed.

Co-workers

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