Superprecipitation of Hybrid Actomyosin Containing Pathologic Actin from Failing Hearts of Adults and Infants

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Abstract. Superprecipitation (SP) of artificial actomyosin, obtained by hybridization of Straub actin from the human myocardium with myosin of normal animal hearts was studied. Actin was prepared from the myocardium of persons who died of congestive heart failure and various non-cardiac diseases, as well as of infants whose death resulted from toxic pneumonia complicated or not with heart failure. It was shown that, in the control hybrid actomyosin, the substitution of normal Straub actin by that from the failing heart resulted in decrease of both the rate and extent of SP. The conclusion was made that both changes in myosin properties and Straub actin underlie the reduced contractility of the myofibrillar protein system in acute and congestive heart failure.

Key words: Heart failure — Actin — Myosin — Hybrid actomyosin — Superprecipitation

Introduction

It has been established, that in acute and chronic (congestive) heart failure in man (Kako and Bing 1958; Karsanov and Mamulashvili 1971) and experimental animals (Benson et al. 1958; Miyahara 1962; Karsanov et al. 1974) as well as infants with toxic pneumonia complicated with heart failure (Karsanov et al. 1977), the contractility of the myocardial contractile protein system is sharply reduced.

Changes have been shown to occur first in the properties of the main protein of the thin myofilament, actin, its extractibility deteriorates (Karsanov et al. 1976b), its polymerization ability decreases (Karsanov et al. 1974, 1976a), conformational alterations occur in the tertiary structure (Karsanov and Dzhinchvelashvili 1981a), the parameters of actin monomers in F-actin change (Karsanov et al. 1980); and a myosin is affected only later, at a more advanced stage of heart failure.

This conclusion was confirmed by superprecipitation (SP) studies of hybrid

actomyosins, containing either pathologic actin or pathologic myosin, in various experimental heart lesions. It was found that, under 2 h coronary occlusion, only actin was damaged (Karsanov and Eristavi 1981), while in inflammatory myocardial damages (Karsanov et al. 1981) and heart muscle athyroid dystrophy (Karsanov and Eristavi 1983) changes were observed both in Straub actin and myosin. In the first case, only the myosin hydrolytic centre was altered, while in the other one, changes occurred both in the hydrolytic centre and in sites responsible for the force generation.

With the purpose to elucidate the role of Straub actin in the development of heart failure of different origin in adults and infants, we studied SP of artificial actomyosin obtained through hybridization of Straub actin from hearts of adult patients who died of congestive heart failure and of various non-cardiac diseases (the first series of experiments) as well as those of infants (the second series of experiments), whose death resulted from toxic pneumonia complicated or not with heart failure, with normal animal heart myosin.

Materials and Methods

In the first series of experiments, we studied SP of hybrid actomyosins, containing normal myosin from animal myocardium and Straub actin from myocardium of patients who had died of acute (second myocardial infarction — 1 case), congestive heart failure due to mitral valve defect (2 cases), renal hypertension (nephrosclerosis associated with diabetes mellitus — 1 case), and of non-cardiac diseases (3 cases: cholecystectomy, bronchopneumonia, commisural ileus) — the conditionally normal control group.

In the second series of experiments, we studied SP of hybrid actomyosins, containing myosin from normal animal hearts and actin from hearts of infants who died of: 1. pneumonia with cardiovascular insufficiency ($n = 7$), 2. intestinal coli infection complicated with pneumonia and cardiovascular insufficiency ($n = 6$) and 3. pneumonia with neurotoxicosis without cardiovascular insufficiency ($n = 5$). Data of the last group were considered as conditionally normal, control, because in pneumonia proceeding with neurotoxicosis, no significant changes in contractility of myocardial glycerinated fiber bundles (MGFB) have been observed (Karsanov et al. 1977).

Heart samples to obtain actin were taken 6—24 hours after the death.

Actin was extracted from acetone dried muscle powder, which had been prepared according to the method of Straub (1942), with bidistilled water at 0 °C to decrease tropomyosin yield (Drabikowski and Gergely 1962) during two hours (because of a decreased extractibility of actin from failing hearts (Karsanov et al. 1972)).

Actin was not exposed to additional purification by cycles of polymerization, sedimentation and depolymerization, since in this case, pathologic, poorly polymerizing actin would have been lost (Karsanov and Dzhinchvelashvili 1981a).

The removed G-actin was polymerized by addition of KCl to the final concentration of 0.1 mol/l.

According to data of sodium dodecyl sulfate (SDS) gel electrophoresis, Straub actin contained about 80% of actin and about 20% of regulatory proteins, mainly of tropomyosin.

For the first series of experiments, myosin was obtained according to the method of Szent-Györgyi (1951) by means of KI extraction from bovine and porcine hearts (delivered on ice from a slaughter-house), and in the second series, it was extracted from normal canine hearts by the method of Shiverick et al. (1975).

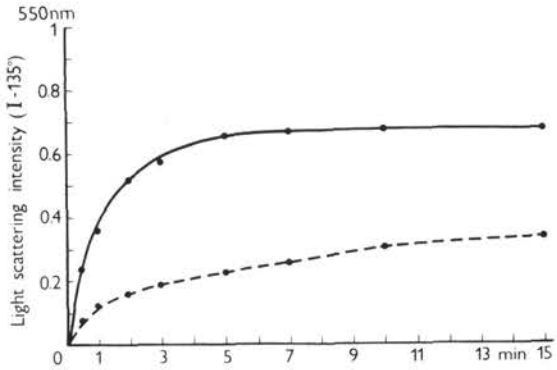

Fig. 1. SP of artificial actomyosin obtained through hybridization of actin from hearts of adult patients: ——— death of non-cardiac disease (conditionally normal, control group) – – – death of heart failure with normal myosin. Experimental conditions: 3×10^{-1} mg/ml of hybrid actomyosin. Medium: 2×10^{-3} mol . l^{-1} Tris-maleate buffer, pH 6.8; 3×10^{-2} mol . l^{-1} KCl; 1×10^{-3} mol . l^{-1} $MgCl_2$; 1×10^{-4} mol . l^{-1} $CaCl_2$; 1×10^{-3} mol . l^{-1} ATP; $t = 20$ °C.

Hybrid actomyosin was prepared by mixing F-actin with myosin in a w/w ratio of 2:5. SP of artificial actomyosin was recorded either as changes in light scattering intensity at 135° (in the first series) or as changes in optical density (in the second series) of actomyosin suspension after the addition of ATP. The Straub actin polymerization ability was assayed by viscosimetry as viscosity changes of actin solution after the addition of KCl to a final concentration of 0.1 mol . l^{-1}. The Fox viscosimeter was used. Mg-activated ATPase activity of hybrid actomyosin was determined by P_i-liberation according to the method of Turakulov et al. (1967). All experiments were carried out at 20 °C.

Data obtained were statistically processed according to the formulae, proposed for independent and nonequivalent groups on a few number of experiments (Bailey 1959).

Results

The first series of experiments: The polymerization ability of myocardial Straub actin from patients, who had died of acute and congestive heart failure was by 67% lower than that of actin from the conditionally normal, control group (patients who had died of non-cardiac diseases): the viscosity was 3.9 ± 0.27 dl/g in the latter and 1.3 ± 0.27 dl/g in the heart failure group ($P < 0.001$).

The extent of SP of hybrid actomyosin formed from failing actin and normal myosin was also by 52% lower than that from the control one ($P < 0.01$). The rate of SP was also significantly diminished; however, due to a large scatter in results, this decrease was statistically insignificant (Fig. 1). However, mathematical analysis of these data has shown that increasing of the total number of cases in experimental and control groups from 7 to 14 would make the difference in SP rates significant ($P < 0.05$).

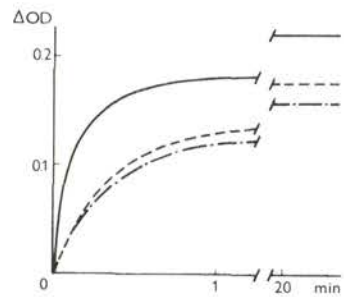

Fig. 2. SP of artificial actomyosin obtained through hybridization of actin from hearts of infants: ——— death of pneumonia with neurotoxicosis (conditionally normal, control group); – – – death of toxic pneumonia with cardiovascular insufficiency; –·–·– death of coli infection complicated with toxic pneumonia and cardiovascular insufficiency with normal myosin. Experimental conditions: 3×10^{-1} mg/ml of hybrid actomyosin. Medium: 2×10^{-2} mol . l^{-1} Tris-HCl buffer; pH 7.2; 5×10^{-2} mol . l^{-1} KCl; 2×10^{-3} mol . l^{-1} $MgCl_2$; 2×10^{-4} mol . l^{-1} ATP; $t = 20$ °C.

The second series of experiments: The extent of SP of hybrid actomyosins containing Straub actin from the hearts of infants with toxic pneumonia complicated with acute heart failure was significantly decreased in comparison with the extent of SP of control, conditionally normal actomyosin (by 28%, $P < 0.001$). The extent of SP of actomyosin, containing actin from the hearts of infants with coli infection, complicated with pneumonia and cardiovascular insufficiency, was by 19% lower ($P < 0.01$). The SP rates of these hybrid actomyosins were lower by 63% and 65% respectively, as compared to the control group ($P < 0.01$) (Fig. 2).

There were no considerable differences in Mg-ATPase activity of the first, second and third types of hybrid actomyosins in the second series of experiments, in spite of changes in the rate and extent of SP (0.933 ± 0.3; 0.839 ± 0.11; 0.955 ± 0.22 μmol P_i/mg protein/min, respectively).

Discussion

Data presented in this study as well as previous findings obtained at the Republican Centre of Medical Biophysics (Karsanov et al. 1974; 1976a; 1980; 1981; Karsanov and Eristavi 1981; Karsanov and Dzhinchvelashvili 1981 a, b) all point to an important role of thin myofilament proteins in the decrease of heart myofibrillar protein system contractility in acute and congestive heart failure.

These data, once again, have confirmed the earlier evidence on a decrease in Straub actin polymerization ability in heart failure (Karsanov et al. 1974; 1976a) and coincide with the experimental findings on a decrease in the extent of SP of actomyosin containing Straub actin from the failing heart (Karsanov and Eristavi 1981, 1983; Karsanov et al. 1981). We can thus conclude that in heart failure in

adults and infants changes in Straub actin properties occur during the lifetime and not postmortem and, therefore, this actin plays an important role in the decrease in contractility of the myofibrillar protein system in acute and congestive heart failure in man and experimental animals.

In this case, as it follows from results of the second series of experiments, pathologic Straub actin fully preserves its ability to activate normal myosin Mg-ATPase and, consequently, hybrid actomyosin Mg-ATPase activity undergoes no changes. Similar phenomenon is observed in experimental inflammatory disease of the heart muscle (Karsanov et al. 1981) and athyreosis (Karsanov and Eristavi 1983). Thus, the decreased myofibrillar Mg-ATPase activity in congestive heart failure in man (Alpert and Gordon 1962; Gordon and Brown 1966) and experimental animals in various heart diseases (Chandler et al. 1967; Berson and Swynghedauw 1973; Tomlinson et al. 1976; Medugorac 1980) is due to changes in properties of myosin only.

In contrast to results obtained in experimental heart diseases (Karsanov and Eristavi 1981, 1983; Karsanov et al. 1981), in human heart failure both the extent and the rate of SP decrease. This fact shows that, in heart failure in man and infants, physico-chemical properties of Straub actin are more profoundly altered (still to be determined) than it is the case in experimental pathologies and, on the other hand, it provides evidence that the decrease in the myocardial contraction rate can be contributed by a damage of both myosin (Lompre et al. 1979) and thin myofilaments, at least under human pathological conditions.

It has been established that in heart failure, changes in optical activity of Straub actin are due to changes in the main protein of the thin myofilament (sites responsible for polymerization and interaction with myosin are changed), and these changes do not affect minor regulatory proteins (Karsanov and Dzhinchvelashvili 1981b).

It may therefore be suggested that changes in hybrid actomyosin SP in adults and infants with acute and congestive heart failure are also due to changes in the properties of the main protein of thin myofilaments. However, the decrease in SP rate may also be related to changes in the properties of the tropomyosin-troponin complex, since this complex is able to increase sharply the SP rate and activate the Mg-ATPase of actomyosin.

However, in athyreosis, when a decrease of SP extent was observed, the pathologic tropomyosin-troponin complex stimulated the SP rate to the same degree as the normal one, in spite of a lower activation of Mg-ATPase by pathologic complex (Karsanov and Eristavi 1983). Nevertheless, in case of heart failure in adults and infants, the role of the tropomyosin-troponin complex in the decrease of SP rate should be turned attention to in special studies.

Thus, results obtained in both series of experiments show that in heart failure both in adults and infants properties of thin myofilament proteins, i. e. probably

those of actin change. Therefore, both changes in myosin properties (which seem to occur at a more advanced pathology) and changes in properties of the main protein of thin myofilament, actin, underlie the reduced myofibrillar protein system contractility, actin still being out of consideration and drawing no serious attention of heart failure investigators.

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