CARDIAC MYOFIBRILLAR ATPase ACTIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT*

KYUZO AOKI, M. D., Ph. D., NOBUKO IKEDA, B. SCI.,
AND KEN HOTTA, Ph. D.

Myofibrillar ATPase activity was determined in the hearts of the spontaneously hypertensive, renal hypertensive and normotensive rats. There was no significant difference of myofibrillar ATPase activity per unit protein between the hypertensive and normotensive hearts. However, increases of myofibrillar ATPase activity per heart were found in both the spontaneously and renal hypertensive rats as was to be expected, because the ratio of heart to body weight was significantly increased. Therefore, external cardiac work might be increased in hypertensive hypertrophied heart.

IN HYPERTENSION, the heart must work against elevated pressure, therefore, the external work of hypertensive heart is increased compared to that of normotensive. The energy for this work is supplied by hydrolysis of adenosine triphosphate (ATP) of the contractile proteins. The rate of energy release is controlled by ATPase activity of myofibrils, contractile protein and mitochondria. Since mitochondrial ATPase activity is inhibited by sodium azide, the residual myofibrillar ATPase activity should originate from actomyosin.¹ It has been shown that ventricular myofibrillar ATPase activity is depressed in heart failure, and contractility of the ventricular papillary muscle is correlated with myofibrillar ATPase activity.² Depression of myofibrillar ATPase activity is associated with depressed contractility in the failing heart.^{3,4} The heart in hypertension performs more work than that in normotension, and becomes hypertrophic. Release of energy must be increased in hypertension, therefore, the myofibrillar ATPase activity must also be increased in the hypertensive heart. This investigation has found evidence for such increases of myofibrillar ATPase activity in the

hypertrophied heart in the spontaneously hypertensive rats⁵ and renal hypertensive rats.⁶

EXPERIMENTAL PROCEDURES

Materials

The spontaneously hypertensive rats (Okamoto-Aoki) (SHR)⁵ and Wistar rats were fed commercial chow (Nippon Clea, CE-2) and given tap water to drink. SHR developed hypertension without any treatment. Renal hypertension in rats was produced by ligation of renal artery and removal of the contralateral kidney.⁶ Ages of SHR were from 15 to 25 weeks and those of renal hypertensive rats were 15 to 20 weeks. Operation for induction of renal hypertension was performed two to five weeks before the experiments. The normotensive rats of the same range of ages were used as controls. Systolic blood pressures were determined by tail plethysmography.⁵

Preparation of Myofibrils⁷

Rat hearts weighing approximately 700 to 1100 mg were quickly removed, immersed and transported in 0.1 M tris-HCl, pH 7.5, at 2°C. Connective tissue was removed, and the muscle

(Received for publication; January 5, 1971)

Department of Medicine and Department of Physiology, Nagoya City University, School of Medicine, Nagoya

* This work was supported in part by U. S. Public Health Service Grant HE-11878-01 from National Heart Institute and Grant 7062 from the Educational Ministry of Japan.

TABLE I CARDIAC MYOFIBRILLAR ATPase ACTIVITY

	No. of Rats	Blood Pressure mmHg	Heart Weight mg per 100g Body Weight	ATPase Activity Tris Buffer	ATPase Activity NaN ₃ -Tris
Normotension	13	120 ± 2	284 ± 7	20.3 ± 1.0	11.3 ± 0.2
Renal Hypertension	7	187 ± 4*	380 ± 16*	20.9 ± 1.6	11.4 ± 0.6
Spontaneous Hypertension	8	184 ± 2*	369 ± 17*	18.7 ± 1.0	11.2 ± 0.4

ATPase activity in normotensive, renal hypertensive and spontaneously hypertensive rats. Values are means ± SE. ATPase activity expressed as 10^{-2} μ M Pi/mg protein/min.

Ionic strength μ = 0.086

*Significant difference; $P < 0.01$ compared to control.

Fig.1. ATPase activity changes with ionic strength. Two normotensive and two SHR were used. Values are means. ATPase activity was determined in a medium which contained 5 mM $MgCl_2$, 0.1 M tris-HCl, pH 7.5, KCl at varying concentrations, 1.5–2.0 mg of protein per ml and 5 mM ATP at 25°C. Ionic strength was adjusted by KCl.

was cut into small pieces and homogenized in a waring blender with three volumes of 0.1 M tris-HCl, pH 7.5 for two minutes. The homogenate was centrifuged at 600 x g for 20 minutes, and sediment was suspended in 0.1 M tris-HCl, pH 7.5 and passed through two layers of cheese cloth for removal of coarse material. The myofibrils were washed by repeated suspension in 0.1 M tris-HCl, pH 7.5 and centrifugation at 600 x g. This procedure was repeated six times. Finally, the sediment was suspended in 0.1 M tris-HCl, pH 7.5 for the assay.

Assay for ATPase Activity

Myofibrils were preincubated in 0.1 M tris-

HCl, pH 7.5 with 5 mM $MgCl_2$ and 5 mM NaN_3 for about five minutes at 25°C. Details on other additions are given in Results. The reaction was initiated with addition of 5 mM ATP and terminated with 10% trichloroacetic acid. ATPase activity was measured by determining liberation of inorganic phosphate according to the Fiske and Subbarow method⁸ using stannous chloride as the reducing agent. The value for ATPase activity were all initial rates from zero to three minutes after ATP addition when Pi liberation was linear with time. ATPase activity was expressed as μ moles of Pi liberated per milligram protein per minute. Protein concentration was determined spectrophotometrically by modification of Biuret method.

RESULTS

High concentration of sodium azide inhibited the mitochondrial ATPase activity¹. This azide-inhibited fraction of myofibrillar ATPase activity represented about 40 to 45% of the total ATPase activity in all preparations. ATPase activity decreased with increased of ionic strength in both in SHR and control animals as shown in Fig. 1. Accordingly, ATPase activities were measured at the constant ionic strength of 0.086.

The average of systolic blood pressures and heart weight per 100 g body weight of SHR and renal hypertensive rats were significantly higher than those of control animals (Table I). There were no significant differences in ATPase activities per unit protein as shown in the Table I. However, the total amount of ATPase of a heart in hypertensive rat was much higher than that of a normotensive one. When the value of ratio of heart to body weight multiplied by ATPase activi-

ty per unit protein of normotensive rat was taken as 100, the corresponding value of SHR and renal hypertensive rat was 129 and 135, respectively. There were no significant differences between SHR and renal hypertensive rats in blood pressure, ratio of heart to body weight and ATPase activity. Those phenomena might indicate that the contractile protein of the heart muscle might be the same in both hypertensive groups.

Discussion

Both SHR and renal hypertensive rats in the stable stage of hypertension were used in this experiment. Those hypertensive rats have been accompanied by cardiac hypertrophy. The rate of energy release in the heart per unit protein in the stable stage of hypertension is the same as that of normotensive heart. Mechanism of the heart contraction and contractile protein is identical in the hypertensive and normotensive animals.

The energy from hydrolysis of ATP is the source of energy for all the work of myocardial cell. However, in normally functioning heart, considerable energy is expended in the mechanical work of contraction. The release of chemical energy of hypertensive heart increased about 30% over the normotensive heart. This increase of myocardial energy production in hypertension may be used to perform extra work of the heart which is product of multiply high blood pressure^{9,10}. It appears that ventricular hypertrophy due to hypertension, in absence of heart failure, is not associated with depression of the contractile state per unit of myocardium, although the absolute increase of total muscle mass maintains cardiac compensation. Accordingly, the function of hypertrophied heart with hypertension of relatively stable stage is characterized by absolute in-

crease in the useful external work delivered by the myocardium.

Acknowledgement

Thanks to Prof. K. Takikawa, Dept. of Med., and Prof. K. Ohara, Dept. of Physiol., in the Nagoya City University, School of Medicine, and Dr. F. M. Bumpus in the Cleveland Clinic Foundation for many valuable suggestions.

REFERENCES

1. FANBURG, B.: Calcium in the regulation of heart muscle contraction and relaxation. *Federation Proc.* 23: 922, 1964.
2. CHANDLER, B. M., SONNENBLICK, E. H., SPANN, J. F., Jr., & POOL, P. E.: Association of depressed myofibrillar adenosine triphosphatase and reduced contractility in experimental heart failure. *Circ. Res.* 21: 717, 1967.
3. ALPERT, N. R. & GORDON, M. S.: Myofibrillar adenosine triphosphatase activity in congestive heart failure. *Am. J. Physiol.* 202: 940, 1962.
4. GORDON, M. S., & BROWN, A. L., Jr.: Myofibrillar adenosine triphosphatase activity in human heart tissue in congestive failure: Effects of ouabain and calcium. *Circ. Res.* 18: 534, 1966.
5. OKAMOTO, K. & AOKI, K.: Development of a strain of spontaneously hypertensive rats. *Jap. Circ. Jour.* 27: 282, 1963.
6. LOOMIS, D.: Hypertension and necrotizing arteritis in the rat following renal infarction. *Arch. Path.* 41: 231, 1946.
7. FANBURG, B., FINKEL, R. M., & MARTONOSI, A.: The role of calcium in the mechanism of relaxation of cardiac muscle. *J. Biol. Chem.* 239: 2298, 1964.
8. FISKE, C. H. & SUBBAROW, Y.: The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375, 1925.
9. LEDINGHAM, J. M. & PELLING, D.: Cardiac output and peripheral resistance in experimental renal hypertension in rats. *Circ. Res.* 21: Suppl. II to 20 and 21: 187, 1967.
10. OLMSTED, F. & PAGE, I. H.: Hemodynamic changes in trained dogs during experimental renal hypertension. *Circ. Res.* 16: 134, 1965.