Vol. 107, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1982 Pages 492-498

EVIDENCE FOR A HUMORAL FACTOR MEDIATING THE EFFECT OF A PRESSURE LOAD ON LYSINE INCORPORATION IN RABBIT HEART

Yuji Kira, Ken Ebisawa, Tadahiro Koizumi, Etsuro Ogata and Yoshio Ito*

The Fourth Department of Internal Medicine, Faculty of Medicine, The University of Tokyo, Tokyo, Japan
*Sanraku Hospital, Tokyo, Japan

Received June 7, 1982

Mechanisms of an increase in protein synthesis in pressure-loaded heart were studied by measuring rates of ^{3}H-lysine incorporation into cardiac myosin B in a perfused rabbit heart. When a pressure load had been applied to the right ventricle, a significant increase in the incorporation was found not only in the right but also in the left ventricle where pressure load was not applied. Rates of the incorporation in hearts without pressure load increased significantly in both ventricles when they were co-perfused with a heart with a pressure load on the right ventricle. These results suggest that humoral factor(s) is released from the pressure-loaded ventricle which accelerate amino acid incorporation in unloaded ventricles.

INTRODUCTION

Cardiac hypertrophy is a compensatory mechanism in hearts faced with long lasting pressure or volume overload(1, 2) and results from enhancement in the cardiac protein synthesis(3, 4, 5, 6). At present, an increase in cardiac work is thought to be the primary factor accounting for the cardiac hypertrophy, but the precise mechanism of the genesis is still unresolved. Schreiber et al(7) and Zelis et al(8) showed an increase in cardiac protein synthesis in the left ventricle of the hearts where a pressure load was applied only to the right ventricle. We have also reported that the protein synthesis in right ventricle of the hearts with aortic coarctation increased significantly(6).

In an attempt to test the involvement of a humoral factor(s) released from the pressure-loaded ventricle in the genesis of hypertrophy, rates of ^{3}H-lysine incorporation into cardiac structural proteins were examined in isolated rabbit hearts co-perfused with either a pressure-loaded or unloaded heart. Results of the present studies support the role of a humoral factor(s) as a mediator of the effects of pressure overload.

MATERIALS AND METHODS

Perfusion Method:

Young(1 kg in weight and about 5 weeks after weaning) female rabbits were employed. They were fed conventional laboratory rabbit chow and supplied tap water ad lib. Under ether anesthesia, tracheal intubation was perfoumed for respiratory maintenance. The chest was opened and the rabbit was injected with heparin(300 u/kg)

0006-291X/82/140492-07$01.00/0

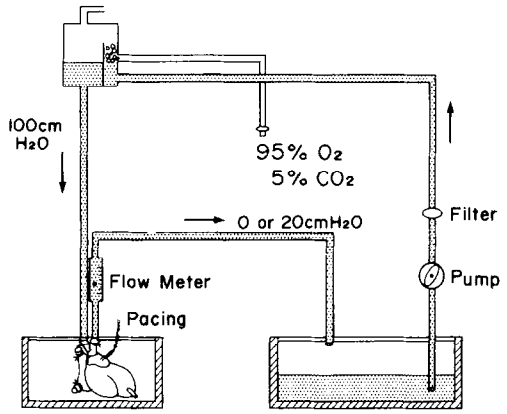

Figure 1. Perfusion apparatus for rabbit heart. Perfusion pressure was maintained at 100 cm H_2O. Pulmonary pressure was at 0 cm H_2O in the control group or 20 cm H_2O in the pressure-loaded group. Hearts were perfused as described in methods.

and curare(0.3 mg/kg) by the ear vein. The heart was removed after ligation of the superior and inferior vena cava. The heart was placed in cold saline to arrest the contraction. The aorta and pulmonary artery were cannulated with a polyethylene tubing(2.5 mm in diameter) followed by the ligation of the pulmonary veins. Blood in the heart chambers was promptly expressed while the aorta was flushed with cold saline. The pericardium was resected. Perfusion was performed by the Langendorff method with minor modifications(Fig. 1). Perfusion pressure was maintained at 100 cm H_2O. The right ventricle was not subjected to any volume load other than that from coronary flow. Pulmonary pressure was maintained at 0 cm H_2O in the control group or 20 cm H_2O in the pressure-loaded group. Imposition of the pressure load was achieved by elevation of the orifice of the pulmonary cannula. Pressure development in the left ventricle was prevented by insertion of an 18 gauge teflon tube through the apex of the ventricle. The heart and cannule assembly were placed in

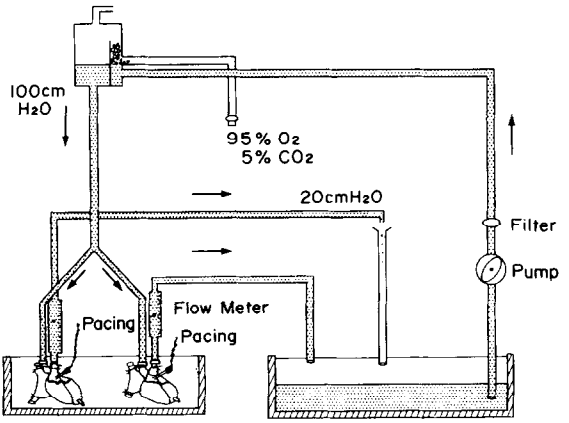

Figure 2. Co-perfusion apparatus for paired young rabbit hearts. The conditions were same as in Fig. 1. The right ventricular load was applied to only one of the two hearts as described in methods.

a temperature-controlled chamber. The heart was perfused at 37 and the heart rate was adjusted at 250/ min by atrial pacing with an electrical stimulator(DPS-100, DIAMEDICAL SYSTEM Co.). Coronary flow rate was assessed by measuring the outflow from the pulmonary artery with a flow meter. After 20 to 30 minutes of perfusion, ^{3}H-lysine(100 μCi, 1.35 μmol) was added to perfusate(250 ml) and perfusion was continued for another 150 minutes. The rate of incorporation of lysine into cardiac structural protein was compared between the right and left ventricles by isolation of cardiac myosin B. To examine wheather a humoral factor is involved in the regulation of amino acid incorporation in hearts under pressure load, two young rabbit hearts were co-perfused with 500 ml of a perfusate(Fig. 2). The pressure load was applied to only one of the two hearts.

Perfusate:

In an attempt to secure an adequate supply to the organ, a blood substitute, Fluosol DA(Green Cross Company, Osaka, Japan), was employed. The contents were as follows: 14 g perfluorodecalin/100 ml, 6.0 g perfluorotripropylamine/100 ml, 3.0 g hydroxyethylstarch/100 ml, 0.6 g NaCl/100 ml, 0.034 g KCl/100 ml, 0.02 g $MgCl_2$/100 ml, 0.028 g $CaCl_2$/100 ml, 0.21 g $NaHCO_3$/100 ml, 2.7 g pluronic F-68/100 ml, 0.4 g yolk phospholipid/100 ml, 0.8 g glycerol/100 ml and 12.5 mM glucose. A mixture of amino acids was added to make the final concentration basically the same as in the paper(9) with slight modifications.

Measurement of Lysine Incorporation into Structural Protein:

Perfused hearts were cut into two portions, the right ventricular wall and the left ventricular wall with septum. Myosin B was extracted by the method of Sugita et al(10) with slight modifications. Briefly, heart wall was minced and homogenized with polytron(PT 10/35m KINEMATIKA) for 30 seconds in a solution containing 0.1 M NaCl and 5 mM NaOH. The homogenate was centrifuged at 15,000 x g for 10 minutes. The supernatant was decanted and the residue was rehomogenized with a teflon homogenizer in a solution containing 0.1 M NaCl and 2 mM $NaHCO_3$. The homogenate was centrifuged at 15,000 x g for 10 minutes. The same procedure was repeated 5 times. The final residue was dissolved in a solution of 1.0 M NaCl and 2 mM $NaHCO_3$ and centrifuged at 15,000 x g for 30 minutes. The supernatants were dialysed exhaustively against 2 mM $NaHCO_3$ and used for estimation of lysine incorporation into myosin B.

Radioactivity Measurements:

Radioactivity was measured using a liquid scintillation counter(TRI-CARB 460 CD, PACKARD). The scintillation fluid was composed of 6 g 2,5-diphenyloxazole, 0.4 g of p-bis(2-(5-phenyloxazolyl)-benzene, and 167 ml of Isolab Scintisol-GP solubilizer per liter of toluene. The rate of ^{3}H-lysine incorporation was calculated and expressed as cpm/mg protein. L-(4, 5-^{3}H)lysine monohydrochloride(74.3 Ci/mmol) was purchased from New England Nuclear.

Determination of Protein Concentration:

Protein concentration was determined by Lowry's method using bovine serum albumin as the standard.

RESULTS

Incorporation of ^{3}H-lysine into Ventricular Myosin B:

In the young rabbit hearts, the rate of ^{3}H-lysine incorporation into ventricular myosin B increased linearly during 150 minutes of perfusion(data not shown). The specific activity of lysine in the perfusate was constant during 150 minutes of perfusion. When the coronary perfusion pressure was maintained at 100 cm H_2O, coronary flow rates varied from 15 to 29 ml/min depending on the coronary vascular resistance. Incorporation of ^{3}H-lysine was relatively constant over this range of coronary flow(Fig. 3).

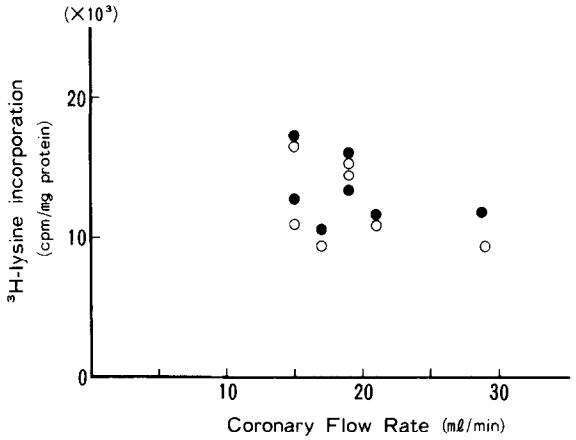

Figure 3. Correlation between incorporation of ^{3}H-lysine into myosin B and coronary flow rate in hearts with no right ventricular pressure load. There was no correlation between the rates of ^{3}H-lysine incorporation and the coronary flow rate over this range(r=0.43 in right ventricle(o) and r=0.31 in left ventricle(●))

Effects of Right Ventricular Load on ^{3}H-lysine Incorporation into Ventricular Myosin B:

Incorporation of ^{3}H-lysine into right and left ventricular myosin B were 12623±3083 and 13299±2522(mean±S.D., n=5) cpm/mg protein respectively in hearts without a right ventricular pressure load. The ratio of lysine incorporation between right and left ventricle was 0.94±0.11. When the pressure load was applied to the right ventricle, the incorporation rates in both right and left ventricle increased significantly to 28892±7216($p < 0.001$) and 23539±5914($p < 0.005$) cpm/mg protein respectively. The relative incorporation ratio between right and left ventricles increased to 1.23±0.085, significantly different($p < 0.005$) from unloaded hearts (Fig. 4). Under these conditions, enhancement of ^{3}H-lysine incorporation in the right ventricle might be a consequence of the increase in the right ventricular wall tension. However, the increase in lysine incorporation found in left ventricle, that was free from pressure load, may have involved another mechanism, perhaps a humoral factor(s), for the mediation of pressure load information from right ventricle to the left ventricle. To provide evidence for a humoral factor(s), the effect of a heart with pressure load on its right ventricle on lysine incorporation in another heart without any pressure load was examined in a co-perfusion system(Fig. 2).

^{3}H-lysine Incorporation in Co-perfused Hearts:

^{3}H-lysine incorporation in a heart without a pressure load on its right ventricle significantly increased($p < 0.001$) in both the right and left ventricles when co-perfused with another heart with a load on its right ventricle(Fig. 5, (a)).

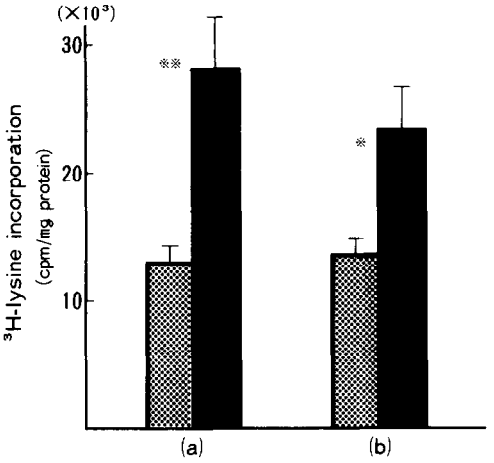

Figure 4. Rate of ^{3}H-lysine incorporation into myosin B in perfused young rabbit hearts with or without a pressure load on the right ventricle. The incorporation rates were increased significantly in (a) the right(**$p < 0.001$) and (b) the left ventricle(*$p < 0.005$) in hearts with a pressure load on the right ventricle. Dotted column; heart without pressure load on the right ventricle. Black column; heart with pressure load on the right ventricle.

Meanwhile, an heart without pressure load on its right ventricle showed no increase in ^{3}H-lysine incorporation rate when co-perfused with another heart with no pressure load on its right ventricle(Fig. 5, (b)). These results suggest the existence of a humoral factor(s) which may accelerate lysine incorporation in cardiac muscle.

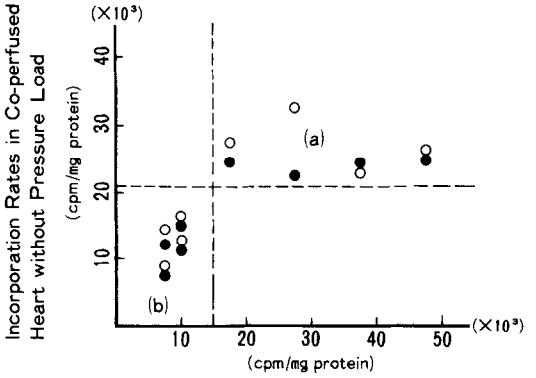

Figure 5. Rate of ^{3}H-lysine incorporation into myosin B in co-perfused hearts. ^{3}H-lysine incorporation was measured in hearts without a pressure load on the right ventricle (vertical axis) when co-perfused with another heart either with a 20 cm H_2O load on the right ventricle(a) or with no pressure load on its right ventricle(b). Horizontal axis shows the ^{3}H-lysine incorporation rate of a right ventricle of a heart either with the pressure load(a) or with no pressure load(b). o; right ventricle or •; left ventricle in hearts without a pressure load on the right ventricle.

DISCUSSION

In the present study, control of ^{3}H-lysine incorporation into proteins by a pressure load was examined in perfused rabbit hearts. For the perfusate, we employed a perfluorochemical blood substitute. This enabled us to supply an adequate amount of oxygen to the perfused heart. The substitute has a capacity to release 5 volume % oxygen when the pO_2 drops from 550 to 50 mmHg at pH 7.4 and 37 (11), while water and blood have the capacity to release 1.6 and 5.9 volume %, respectively. In our experimental conditions, the arterial perfusate had a pO_2 of 450 mmHg, and the coronary effluent had a pO_2 of 200 mmHg. Therefore, it appears that tissue oxygenation was adequate. We used ^{3}H-lysine as marker substrate for the overall process of amino acid uptake, protein synthesis and release of nonradioactive lysine from protein. Incorporation of ^{3}H-lysine into myosin B increased linearly for at least 150 minutes(data not shown, and Ref.(4)). Under these conditions the specific activity of intracellular lysine reached only 70 % of the specific activity of extracellular lysine after 3 hours of perfusion(12, 13). However, changes in coronary flow between 15 and 29 ml/min did not affect lysine incorporation indicating not only that oxygen supply was not rate-determing but also that the specific activity of ^{3}H-lysine at the loci of protein synthesis was relatively constant. Similar observation were reported by Schreiber et al(7). Therefore, it seems reasonable to use this system for comparison of incorporation rates.

The effects of right ventricular pressure load on protein synthesis in the left ventricle are still controvertial. Zelis et al(8) and Schreiber et al(7) showed a coincidental increase in the left ventricular protein synthesis in hearts with a right ventricular pressure load, while Everret et al(14) could not find any increase. Our results agree well with the former authors who suggested that mechanical effects on the left ventricular myocardium by loading the right ventricle increased the rate of left ventricular protein synthesis. However, as recognized by Taylor et al(15), the effect of right ventricular pressure on the left ventricular myocardium could be negligible when the left ventricle was empty as in our heart perfusion system. Therefore, it seemed appropriate to invoke another factor to account for the development of increased lysine incorporation in the left ventricle. As shown in the co-perfusion experiments(Fig. 5), ^{3}H-lysine incorporation in both ventricles of heart without pressure load on the right ventricle increased when it was co-perfused with another heart with a load on the right ventricle. This result suggests the presence of a factor(s) in the perfusate which was released from the heart subjected to pressure load and was able to accelerate lysine incorporation. Recent reports (16, 17) indicate that mechanical load, such as pressure development might not be the sole factor for cardiac hypertrophy. Actually, humoral factors such as norepinephrine(18), prostaglandins(19), angiotensin(20), and aldosterone(21) were

proposed as stimulators of protein synthesis in heart. Thus it is possible that the concomitant increase in ^{3}H-lysine incorporation in the left ventricle of hearts with right ventricular pressure load was induced by a humoral factor rather than by a pressure load via the systemic circulation. Recently the existence of a digitalis-like substance was reported in the plasma of an animal with a pressure-loaded heart. Similarly, preliminary study from our laboratory found the perfusate of a heart with a right ventricular pressure load to have the ability to increase tension development of the cardiac muscle from another heart. Further delineation of the humoral factor is currently under investigation. In any event, the present study provides evidence supporting the concept that a humoral factor may be involved in the genesis of myocardial hypertrophy that occurs in response to pressure load.

ACKNOWLEDGEMENT

We are thankful to Mrs. T. Kuroda for her technical assistance. We wish to also express our gratitude to Prof. H. E. Morgan, Chairman of Department of Physiology, Pennsylvania State University, for the comments on this manuscript.

REFERENCES

1. Linzbach, A. J. (1969) Am. J. Cardiol. 5, 370-382.
2. Badeer, H. S. (1964) Am. J. Cardiol. 14, 133-138.
3. Schreiber, S. S., Oratz, M., Evans, C., Gueyikian, I., and Rothschild, M. A. (1970) Am. J. Physiol. 291, 481-486.
4. Morkin, E., Kimata, S., and Skillman, J. J. (1972) Circ. Res. 30, 690-702.
5. Zak, R., and Fischman, D. A. (1971) Cardiac Hypertrophy, pp. 247-258. Academic Press, New York.
6. Ito, Y., Ebisawa, K., Kira, Y., and Koizumi, T. (1980) Adv. Myocardiol. vol. 1, pp. 509-518, University Park Press, Baltimore.
7. Schreiber, S. S., Rothschild, M. A., Evans, C., Reff, F., and Oratz, M. (1975) J. Clin. Invest. 55, 1-11.
8. Zelis, R., Wikman-Coffelt, J., Kamiyama, T., Peng, C. L., Salel, A. F., Amsterdam, E. A., and Mason D. T. (1973) Rec. Adv. Stud. Cardiac Struct. Metabol. vol. 3, pp. 625-634, University Park Press, Baltimore.
9. Rannels, D. E., Kao, R., and Morgan, H. E. (1975) J. Biol. Chem. 250, 1694-1701.
10. Sugita, H., Katagiri, T., Shimizu, T., and Toyokura, Y. (1973) Basic Res. in Myol. vol. 294, pp. 291-297, Excepta Medica, Amsterdam.
11. Naito, R., and Yokoyama, K. (1978) The Green Cross Co. Technical Inform. Ser. 5, pp. 73-75, The Green Cross Co., Osaka.
12. Morgan, H. E., Earl, D. C. N., Broadus, A., Wolpert, E. B., Giger, K. E., and Jefferson, L. S. (1971) J. Biol. Chem. 246, 2152-2162.
13. Schreiber, S. S., Oratz, M., Evans, C., Reff, F., Klein, I., and Rothschild, M. A. (1973) Am. J. Physiol. 224, 338-346.
14. Everett, A. W., Sparrow, M. P., and Taylor, R. R. (1979) J. Mol. Cell. Cardiol. 11, 1253-1263.
15. Taylor, R. R., Covell, J. W., Sonnenblick, E. H., and John Ross, JR. (1967) Am. J. Physiol. 213, 711-718.
16. Frolich, E. D., and Tarazi, R. C. (1979) Am. J. Cardiol. 44, 959-963.
17. Sen, S., Tarazi, R. C., Khairallah, P. A., and Bumpus, F. M. (1974) Circ. Res. 35, 775-781.
18. Laks, M. M., and Morady, F. (1976) Am. Heart J. 91, 674-675.
19. Chazov, E. I., Pomoinetsky, V. D., Geling, N. G., Orlova, T. R., Nekrasova, A. A., and Smirnov, V. N. (1979) Circ. Res. 45, 205-211.
20. Robertson, A. L., and Khairallah, P. A. (1971) Science 172, 1138-1139.
21. Liew, C. C., Liu, D. K., and Gornall, A. G. (1972) Endocrinol. 90, 488-495.
22. Schreiber, v., Kolbel, F., Stepan, J., Gregorova, I., and Priibyl, T. (1981) J. Mol. Cell. Cardiol. 13, 107-110.