STUDIES ON *ADENYL CYCLASE SYSTEM IN MYOCARDIUM* (PART I) ADENYL CYCLASE SYSTEM IN THE HYPERTROPHIED AND FAILING RABBIT HEARTS

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Many reports state a marked reduction of catecholamine (CA) in cardiac tissue and an increased CA concentration of serum and urine in patients with congestive heart failure[1)-5)]. Thus, it is assumed that the function of the impared heart might be supported by the increased blood CA. It has been reported that the inotropic[6)-12)] and chronotropic response[13)-16)] of CA to the cardiovascular system are mediated by adenyl cyclase-cyclic AMP system.

Adenyl cyclase-cyclic AMP system was initially discovered as the intracellular mediator of the glycogenolytic effects of epinephrine and glucagon in the liver by Sutherland and Rall[17)], but it has since come to be recognized as a second messenger mediating a variety of hormonal effects[18)-20)].

It is proposed that CA activates adenyl cyclase and increases intracellular cyclic AMP by converting ATP to cyclic AMP which mediates inotropic action as a 2nd messenger. Furthermore, the concentration of intracellular cyclic AMP is controlled both by adenyl cyclase, the synthesizing enzyme, and phosphodiesterase, the converting enzyme.

In order to investigate CA and adenyl cyclase-cyclic AMP system in cardiac failure,, the changes of myocardial CA concentration, adenyl cyclase and phosphodiesterase activity in the hypertrophied and failing heart were investigated using experimental aortic stenosis of rabbits.

MATERIALS AND METHODS

1. *Production of cardiac hypertrophy and impending heart failure*

Forty albino rabbits, weighing 2.0 – 3.0 kg, were used. They were divided into five groups, one group served as control. Supravalvular aortic stenosis was produced by a modification of the technique described by Tomomatsu et al[21)]. Adult rabbits were operated under anesthesia induced by intravenous injection of 2.5 ml/kg of urethane. A small piece of laminallia, which was fixed at the tip of the vinyl tube (0.9 mm inside diameter) was inserted into the right carotid artery and positioned just above the aortic valve. After insertion, the laminallia expanded and aortic stenosis was produced. In

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order to add further cardiac burden upon the hypertrophied heart, 100 ml/day of physiological saline solution was infused for 3 days to rabbits 30 days after the operation of aortic stenosis. These rabbits were named animals with impending heart failure. Eight rabbits of 2 days after the operation was abbreviated by AS_2, six of 7 days by AS_7, seven of 30 days by AS_{30} and nine of impending heart failure by IHF. After the animals were sacrificed by a blow on the head, the hearts were quickly excised and the free walls of the left ventricle were removed and used for each assay. The ratio of heart weight to body weight was employed for the evaluation of cardiac hypertrophy.

2. Determination of myocardial water concentration

The method described by Benson[22] was used for the determination of myocardial water concentration. The water concentration was calculated from the difference between 1g of fresh tissue of the left ventricle and dry weight obtained after drying at 110°C for 24 hours.

3. Determination of myocardial CA

Myocardial CA was measured spectrophotofluorometrically by the method of Von Euler et al[23]. The free walls of the left ventricle were weighed and homogenized with 5 volumes of 5% trichloro-acetic acid. The CA in the tissue extract was absorbed onto alminium oxide and oxidized with potassium ferricyanide. The concentration of the resulting trihydroxyindole was measured in a spectrophotofluorometer.

4. Determination of myocardial adenyl cyclase activity

(a) Preparation of tissue fractions

The left ventricle was excised, minced with a fine scissor, then 0.5 gram was homogenized in 6 volumes medium containing 0.001M magnesium sulfate and 0.002M glycylclycine, pH 7.5 in a homogenizer. The crude homogenate was filtered through gauze, and used as an enzyme preparation.

(b) Determination of enzyme activities

Adenyl cyclase activity was measured by the method of Krishna et al[24]. The incubation medium consisted of Tris HCl, pH 7.3, 4×10^{-2} moles; $MgCl_2$, 3.3×10^{-3} moles; ^{3}H-ATP 2×10^{-3} moles, 30 $\mu c/\mu$ mole; theophylline, 1.0×10^{-2} moles; epinephrine bitartrate, 1.0×10^{-4} moles. Incubation was performed at 30°C, and was initiated by addition of 100 μl of homogenate in a total incubation volume of 0.6 ml. The reactions were terminated at 2 minutes intervals by the addition of 0.5 mg of carrier cyclic AMP and immediate immersion in a boiling water bath for 3 minutes. Incubations employing heat-denatured enzyme were run as blanks. The carrier cyclic AMP served for calculation of recovery, and tritiated cyclic AMP formed from the labeled ATP was separated by a chromatography on Dowex-50H$^+$ followed by barium-zinc precipitation. The radioactivity in an aliquot of the final supernatant fraction, representing tritiated cyclic AMP, was counted in 15 ml of a BBOT phosphor mixture in a liquid scintillation spectrometer. The reaction was linear with myocardial homogenate over a range 0.05 ml to 0.15 ml. Adenyl cyclase activity was expressed as mμ mole of cyclic AMP formed/h/mg of wet weight.

5. Determination of phosphodiesterase activity

Activity of phosphodiesterase in a crude homogenate was assayed by measurement

of hydrolysis of cyclic AMP[26]. The assay depends on precipitation of material with high absorbance at 260 mμ, including products of hydrolysis, while cyclic AMP remains in the supernatant fraction. The incubation mixture contained Tris HCl, pH 7.4, 4 x 10^{-2} moles; $MgCl_2$, 2 x 10^{-3} moles; and cyclic 3′, 5′ – AMP, 4 x 10^{-4} moles in a final volume of 1.0 ml. Hundred μl aliquot of the initial tissue homogenate was added to initiate the reaction. Incubation was performed at 37°C and terminated by addition of 1.0 ml of 2% $ZnSO_4$ followed by 1.0 ml of 1.8% $Ba(OH)_2$. Following centrifugation at 4000g for 15 minutes, absorbance of the supernatant fraction was measured at 260 mμ, and the rate of degradation of cyclic AMP determined. Phophodiesterase activity was expressed as mμ mole of cyclic AMP degraded/h/mg of wet weight.

ATP-^{3}H (specific activity 5 – 50 mc/m mole) was obtained from New England Nuclear Corporation, Boston, Mass., Dowex-50H$^+$ (50W – X4, 200 – 400 mesh) from Bio-Rad Laboratorus. All other compounds were obtained from commercial sources. Radioactivity was determined with a liquid scintillation spectrometer (Aloka 100). Fluorometric measurement was carried out with a spectrophotofluorometer (Turner Associates).

The results were analyzed using a student's "t" test.

RESULTS

1. Experimental cardiac hypertrophy in rabbits

The cause of death in the operated animals was mainly attributed to heart failure showing edematous lung, enlarged liver and ascites. The ratio of heart weight to body weight in 20 unoperated control animals averaged 2.39 ± 0.13 (±S.E.M.) g/kg. After the operation, this was significantly increased above the control, and averaged 2.53 ± 0.10 g/kg in AS_2, 2.72 ± 0.16 g/kg in AS_7 and 2.84 ± 0.22 g/kg in AS_{30} respectively. In IHF, the ratio, average 2.58 ± 0.09 g/kg, was slightly decreased compared with AS_{30}. Myocardial water concentration was gradually increased after the operation, averaged 778 ± 8.2 mg/g in control animals, 786 ± 5.6 mg/g in AS_2, 797 ± 3.2 mg/g in AS_7 and 802 ± 3.2 mg/g in AS_{30} respectively. In IHF, it was reduced to an average of 783 ± 14.2 mg/g. (Fig. 1)

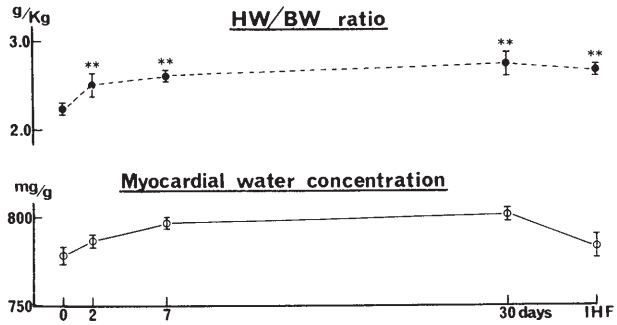

Fig. 1. Time course changes of heart weight/body weight ratio and myocardial water concentration after aortic obstruction.
**: $p<0.01$ HW/BW: heart weight/body weight ratio IHF: impending heart failure

2. CA concentration in the left ventricle

In 34 normal rabbits, the left ventricular CA concentration averaged 1.00 ± 0.04 μg/g (±S.E.M.). In AS_2, no significant change was observed, average 0.94 ± 0.10 μg/g. In AS_7 and AS_{30}, it decreased to values which averaged 0.84 ± 0.08 μg/g and 0.90 ± 0.09

μg/g respectively. IHF showed the most significant decrease in concentration, averaged 0.71 ± 0.07 μg/g ($p < 0.01$) compared with control. (Fig. 2)

3. Adenyl cyclase activity in the left ventricle

In 10 normal rabbits, the adenyl cyclase activity in the left ventricle averaged 1.12 ± 0.11mμ mole/h/mg (±S.E.M.). The activities in AS_2 and AS_7 decreased significantly, averaging 0.69 ± 0.13mμ mole/h/mg ($p < 0.05$) and 0.67 ± 0.14mμ mole/h/mg ($p < 0.05$) respectively. The values in AS_{30} showed further significant decrease, averaging 0.26 ± 0.12mμ mole/h/mg ($p < 0.01$). IHF showed rather an increases ober AS_{30}, averaging 0.39 ± 0.13mμ mole/h/mg, which showed also a significant decrease compared with normal. ($p < 0.01$). (Fig. 3)

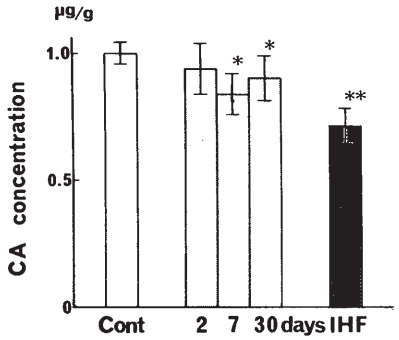

Fig. 2. CA concentration in the left ventricle of rabbits in five groups.
*: $p<0.05$ **: $p<0.01$

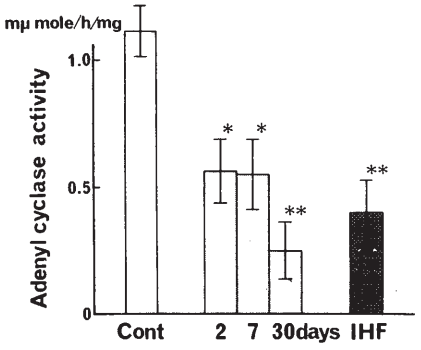

Fig. 3. Adenyl cyclase activity in the left ventricle of rabbits in five groups.
*: $p<0.05$ **: $p<0.01$

4. Phosphodiesterase activity in the left ventricle

The phosphodiesterase activity in the left ventricle of 10 normal rabbits averaged, 42.36 ± 11.0mμ mole/h/mg. The activity in AS_2, AS_7, and AS_{30} showed no remarkable change, averaging 36.75 ± 7.56mμ mole/h/mg, 37.08 ± 5.97mμ mole/h/mg and 36.2 ± 6.6mμ mole/h/mg respectively. The activity in IHF averaged 29.7 ± 9.8mμ mole/h/mg, and also showed no significant decrease. (Fig. 4)

DISCUSSION

The experimental method of Tomomatsu used in this study to produce cardiac hypertrophy is simple and dependable. Cardiac hypertrophy was already observed 2 days after the operation of aortic obstruction and progressed gradually. It is difficult in the experimental animal to produce heart failure similar to that in humans. In this study, impending heart failure was produced by imposing 4 days volume overload infusion of physiological saline solution on the animals of 30 days after the operation of aortic obstruction. The animals with IHF showed a clinical picture which resembles patients with congestive heart failure. In spite of some difference of seriousness in these IHF, all cases were treated as a whole, because of difficulty of detailed classification.

In these experiments with rabbits, the left ventricular CA concentration was not significantly depressed in 2 days after the operation of experimental cardiac failure, but was fairly depressed in 7 and 30 days, and was distinctly depressed in IHF. These

results resembled that of many reporters who studied the myocardial CA in experimental heart failure. Adenyl cyclase activity was fairly depressed in 2 and 7 days after the operation, and further depression occured in 30 days. The degree of the depression decreased in IHF than in 30 days without significant differences. Phosphodiesterase activity showed a slight decrease in 2, 7, and 30 days after operation, but no significant difference compared to normal. Many reports[1)-5)] show decrease of myocardial CA and increase of blood CA in cardiac failure. Increases of blood CA suggest augmented activity of the sympathetic nervous system which is considered to serve the maintenance of cardiac performance in heart failure. Fig. 5 shows the relation between CA and

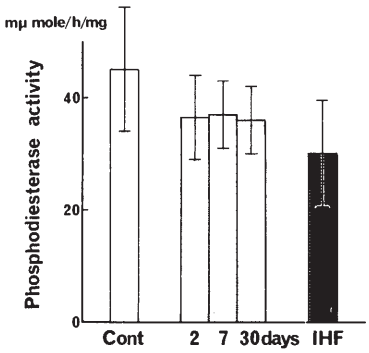

Fig. 4. Phosphodiesterase activity in the left ventricle of rabbits in five groups.

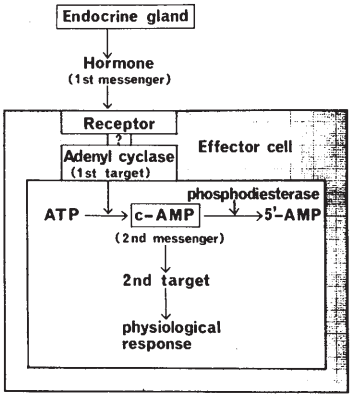

Fig. 5. The second messenger system (from Sutherland et.al.[25)]).

adenyl cyclase-cyclic AMP system[25)]. Myocardial cyclic AMP which play an important role as a second messenger in the inotropic effect of myocardial contraction is controlled by adenyl cyclase and phosphodiesterase. There are few reports on myocardial adenyl cyclase activity in cardiac hypertrophy and heart failure. On one hand, Sobel et al.[26)]. have reported a depressed adenyl cyclase activity in failing guinea pig heart, while on the other hand, Gertler, Saluste, and Spencer[27)] failed to observe such a change.

In this study, in spite of no changes observed on converting enzyme phosphodiesterase in cardiac hypertrophy and IHF, significant decreases of synthesizing enzyme adenyl cyclase activity suggest an important defect of cyclic AMP producing system in cardiac hypertrophy and IHF. However a direct measurement of my ocardial cyclic AMP would be in cardiac hypertrophy and cardiac failure. The mechanism of depletion of myocardial adenyl cyclase activity in cardiac hypertrophy and heart failure is not clear at present. The enzyme dilution theory due to increased myocardial protein in heart failure by Sobel et al.[26)] cannot be neglected, but all can not be explained by it, because there was no depression of myocardial phosphodiesterase activity. Furthermore, though the depletion of myocardial CA was noticed in cardiac hypertrophy and impending heart failure, it seemed that the depressed myocardial adenyl cyclase activity in this stage was not due to decrease of myocardial CA store. It is supported by the result of Sobel's experiment using a cat that no diminntion of adenyl cyclase activity was observed when myocardial CA was reduced by denervation. From the above reasons adenyl cyclase itself seemed to receive primary qualitative change in cardiac hypertrophy and heart failure. In any case, prominent depression of

myocardial adenyl cyclase activity in cardiac hypertrophy and heart failure indicates disturbance of myocardial cyclic AMP producing system which play an important role on inotropic action in heart failure.

Two biochemical defects in myocardial cyclic AMP producing system, the decrease of myocardial CA and the depression of adenyl cyclase activity were demonstrated in cardiac hypertrophy and heart failure and it is suggested that the diminished myocardial inotropic effects would be explained by them.

SUMMARY

In order to investigate the pathophysiological significance of myocardial CA and adenyl cyclase-cyclic AMP system in cardiac failure, myocardial CA, adenyl cyclase and phosphodiesterase activity in cardiac hypertrophy and impending heart failure were measured by experimental aortic stenosis in rabbits.

1. A reduction of left ventricular CA concentration was observed in the stage of cardiac hypertrophy and significant reduction in the stage of impending heart failure.
2. Adenyl cyclase activity decreased gradually after the production of experimental hypertrophy and most significantly in 30 days after and the stage of impending heart failure.
3. Phosphodiesterase activity showed no significant changes in both the stage of cardiac hypertrophy and impending heart failure.

These results suggest that at least two biochemical defects in the supporting mechanism of sympathetic systems in cardiac failure – the depletion of myocardial CA and the decrease of adenyl cyclase activity – may contribute to the reduction of myocardial inotropic effect.

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