EFFECT OF HYPOTHERMIA DURING ISCHEMIA ON MYOCARDIAL CONTRACTILITY IN NONHYPERTROPHIED AND HYPERTROPHIED VENTRICLES

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IT has been suggested that postoperative left ventricular subendocardial necrosis¹⁻⁴ develops more commonly in ventricles that are severely hypertrophic.² Moreover, ventricular fibrillation, in association with myocardial ischemia, has been shown to worsen ventricular function,⁴ particularly in the presence of left ventricular hypertrophy.⁵ Recent studies have also shown that the hypertrophied ventricle is more vulnerable to ischemic damage than is the normal ventricle.⁶ However, it is well-known that the adequacy of myocardial protection during open-heart surgery is the principal determinant of the degree of ischemic myocardial damage. The present studies were performed to investigate the effect of hypothermia on myocardial contractility in nonhypertrophied and hypertrophied canine left ventricle with respect to myocardial tolerance to hypothermic ischemic arrest.

MATERIALS AND METHODS

Mongrel dogs of both sexes weighing 8 to 15 Kg were used. An isolated left ventricular muscle was perfused with the blood of a support dog for determination of myocardial contractility, myocardial c-AMP level, and myocardial calcium level before and after a period of 150 minutes of ischemia at a myocardial temperature of 17°C. Left ventricular hypertrophy was produced by creating supraventricular aortic stenosis

by banding an average of 95 days prior to study. The systolic pressure gradient obtained in left ventricular hypertrophied dogs was approximately 50 mmHg. Two groups were studied: Group I (N): Acute ischemia was maintained for 150 minutes at the myocardial temperature of 17°C in ten left ventricular nonhypertrophied dogs. Group II (AS): Acute ischemia was maintained for 150 minutes at 17°C in nine left ventricular hypertrophied dogs produced by supraventricular aortic stenosis.

The isolated canine left ventricular muscle perfused with blood was prepared according to the technique described by Chiba.⁷ Mongrel dogs of both experimental groups were anesthetized with an intravenous administration of 30 mg/kg of pentobarbital and heparinized with 1 mg/kg of heparin, and the heart was removed. A part of the left ventricular muscle along the anterior descending branch of the left coronary artery was quickly excised and bathed in cold Tyrode's solution. The wet weight of the excised muscle was approximately 10 to 15 g. The left anterior descending artery was cannulated with a small polyethylene catheter and perfused with arterial blood led from the carotid artery of the support dog at a constant rate of 8.4 ml/min. by means of a Harvard pump. A pneumatic resistance was placed in parallel with the perfusion system to obtain a constant pressure of 100 mmHg. The actual coronary flow measured by the blood leaving the isolated ventricular muscle ranged from 7.1 to 8.3 ml/min. with an average of 7.6 ± 0.3 . The isolated blood-perfused left ventricular muscle was suspended in a cup-shaped glass con-

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Fig.1. Schematic presentation of the isolated canine left ventricular muscle perfused with the blood introduced from the carotid artery of support dog.

tainer, which was maintained at a constant temperature of 37°C , with the two parts of the muscle fixed to a stainless steel bar. The upper part of the isolated ventricular muscle was attached by a silk thread to a force-displacement transducer (Nihon Kohden SB-1TA). The volume of the container was 65 ml and was initially filled with warmed Tyrode's solution, which was gradually displaced by the blood leaving the

isolated ventricular muscle. Two streams of blood, overflowing from the container and passing through the pneumatic resistance, were collected in a blood reservoir and then returned to the support dog through the jugular vein. The muscle was electrically paced at a rate of 70/min. in 3 mA. The muscle was loaded with a resting tension of 2 g. The isometric tension and the rate of rise of tension development (dT/dt) were continuously recorded on an ink-writing rectigraph (Nihon Kohden WI-680G) through a carrier amplifier (Nihon Kohden AP-600G) and an electronic differentiator (Nihon Kohden ED-600G). The diagram of the blood-perfused system of the left ventricular canine myocardium is shown in Fig. 1.

In the present experiments coronary perfusion was stopped and the isolated ventricular muscle was bathed in Tyrode's solution during a period of 150 minutes at 17°C . The myocardial temperature rapidly equilibrated with the temperature of the cold Tyrode's solution in which the muscle was bathed. After the ischemic period of 150 minutes the muscle was reperfused with arterial blood from the support dog until the myocardial contractility fully recovered at the myocardial temperature of 37°C . The maximal developed tension at this point was referred to as a percent recovery rate in comparison with the maximal tension before ischemic arrest. Blood gas analysis of the support dog was performed at intervals using a Radiometer analyzer. These

Fig.2. Percent recovery rate of net developed tension after ischemia in nonhypertrophied (Normal dog) and hypertrophied (AS dog) canine left ventricular muscles.

Fig.3. Changes of rectigram of net developed tension and dT/dt before and after ischemia (ischemic period of 150 minutes at myocardial temperature of 17°C) in hypertrophied left ventricular muscle. The percent recovery rate is 70 in this case.

values were maintained within the normal range by the addition of oxygen and by the administration of sodium bicarbonate solution. As soon as the isolated ventricular muscle was prepared and perfused with the arterial blood of the support dog, the muscle started to contract in a slow rhythm followed by ventricular fibrillation. Approximately 20 minutes after the start of perfusion the muscle showed a good response to electrical pacing and the net developed tension, which was defined as total developed tension minus resting tension, continued to increase. Approximately 90 minutes were required before

the maximal net developed tension reached a plateau; the postischemic recovery time required to obtain a plateau of the maximal net developed tension was approximately 60 minutes. Myocardial calcium content was measured by atomic absorption spectrometry and myocardial c-AMP level was assessed by radioimmunoassay⁸ before the ischemia and after the termination of reperfusion. A statistical analysis was performed by means of Student's test with $p < 0.05$ as the level of significance. The values experimentally obtained were expressed as mean $\pm$ SD.

Fig.4. Changes of myocardial cyclic AMP contents before and after ischemia in the both groups.

RESULTS

The percent recovery rate of net developed tension following an ischemic period of 150 minutes at the myocardial temperature of 17°C was $90 \pm 9\%$ (mean $\pm$ SD) in N group, while it was $70 \pm 19\%$ in AS group (Fig. 2). Comparing the two groups, a significant decrease ($p < 0.01$) of percent recovery rate of net developed tension was seen in AS group. The percent recovery rate of dT/dt also showed the same change as that of net developed tension in both groups. Fig. 3 shows a record of the case presenting a percent recovery rate of net developed tension and dT/dt of 70% in an ischemic period of 150 minutes at 17°C in an AS dog.

In N group myocardial c-AMP level was 0.63 ± 0.46 p mole/mg tissue before the ischemia and 0.47 ± 0.21 after the reperfusion, showing no statistical significance. While, in AS group myocardial c-AMP level was 0.73 ± 0.33 p mole/mg tissue before the ischemia and 0.43 ± 0.42 after the reperfusion, indicating a significant decrease ($p < 0.05$) of myocardial c-AMP as consequence of an ischemic period of 150 minutes at 17°C (Fig. 4).

In N group myocardial calcium level was 0.048 ± 0.013 mg/g tissue before the ischemia and 0.059 ± 0.008 after the reperfusion. The trend towards an increase of myocardial calcium was investigated after the reperfusion, however no statistical significance was found. In AS group the myocardial calcium level was 0.048 ± 0.011 mg/g tissue before the ischemia and 0.056 ± 0.016 after the reperfusion, indicating no statistical significance.

DISCUSSION

The experimental model described here to evaluate myocardial protection has several characteristics. The isolated electrically driven left ventricular muscle perfused with arterial blood led from the carotid artery of the support dog resembles extracorporeal circulation and the myocardial contractility can be precisely measured with the stability of contractility for over 4 hours. The coronary perfusion through the cannulated anterior descending artery of the isolated left ventricular muscle can be precisely regulated. In addition, the temperature of the ventricular muscle suspended in the glass container filled by the blood leaving the isolated blood-perfused muscle or Tyrode's solution can be quickly regulated by circulating water

pumped from a water bath.

Our previous studies⁹ indicated that the percent recovery rates of net developed tension of 100, 95, 90, and 65 were obtained after an ischemic period of 90, 120, 150, and 180 minutes respectively at the myocardial temperature of 17°C, suggesting that the safe limit of ischemia at 17°C is defined as up to 90 minutes in this experimental model and can be extended to up to 120 minutes. Controversy exists over the optimal myocardial temperature for the application of topical cardiac hypothermia in cardiac surgery. Shumway¹⁰ and Griepp¹¹ have advocated maintenance of the intramyocardial temperature at 15 to 20°C providing satisfactory myocardial protection in clinical use. In experimental studies Angell¹² reported that an ischemic period of 180 minutes at the myocardial temperature of 15°C allows the viability of canine cadaver heart, and Ino¹³ also noted that the optimal myocardial temperature for satisfactory myocardial protection is 18°C. In our clinical experiences it has been recognized that topical cardiac cooling with cold (4°C) lactated Ringer's solution and ice slush keeps intramyocardial temperature at 17°C with a safe ischemic period of 90 minutes. Therefore, in the present studies the myocardial temperature of 17°C and the ischemic period of 150 minutes beyond a safe limit to exactly evaluate the myocardial damage were chosen as the experimental conditions to compare the change of myocardial contractility after ischemia in N group and AS group.

Many investigations have already been carried out on the influence of ischemia upon hypertrophied heart. Fundamentally, Meerson¹⁴ indicated that the wear complex which consists of the disturbances of metabolism and structure of myocardial cells, along with changes in regulatory mechanism of the hypertrophied myocardium represents an important factor in the decrease of force and velocity of contraction in hypertrophied heart muscle and suggested that the presence of an additional load or some additional cardiac lesion may precipitate heart failure. Clinically, it has been reported that ischemic contracture of the heart or postoperative low cardiac output syndrome after the ischemia frequently occur in hypertrophied heart and failing heart.^{2,3,15} Hottenrott⁵ has also indicated that ventricular fibrillation may be safe in normal hearts during cardiopulmonary bypass, but may cause ischemic damage to hypertrophied hearts.

From the above-mentioned results, it is generally accepted that hypertrophied ventricle is more vulnerable to ischemic damage than is the normal ventricle. Therefore, it is assumed that myocardial protection for ischemia is more necessary for hypertrophied ventricle than for the normal ventricle. Mundth¹⁶ has reported that hypertrophied canine hearts increased the severity of depression of left ventricular function after ischemia as compared to nonhypertrophied canine hearts, but potassium cardioplegia and myocardial hypothermia (10 to 14°C myocardial temperature) prevent this with an ischemic period of up to 75 minutes. In the present studies, the results obtained support the above-mentioned results by other investigators, that is, hypertrophied canine left ventricle showed a decrease of tolerance to ischemia as compared to nonhypertrophied ventricle in the investigation of myocardial contractility, suggesting the necessity of more intense myocardial protection in addition to cardiac hypothermia.

On the morphological and biochemical changes of hypertrophied heart, it has been reported that morphologically, in the damage stage, two days after the creation of supra-ventricular aortic stenosis in rabbits numerous mitochondria in the myocardium are impaired, and in the stage of relatively stable hyperfunction, 45 days after the creation of aortic stenosis the normalization of mitochondrial structure occurs. In the stage of gradual exhaustion of the myocardium, eight months after the onset of hyperfunction the number of mitochondria undergoing destruction is increased.¹⁷ While, biochemically, in the damage stage concentrations of both creatine phosphate (CP) and ATP are decreased with a more remarkable decrease of CP than of ATP, and in the stage of stable hyperfunction both CP and ATP are normal. However, in the stage of gradual exhaustion both CP and ATP again decreased, suggesting a gradual impairment of metabolism of high energy phosphate according to the development of myocardial hypertrophy.^{18,19} Moreover, on the changes of high energy phosphate after myocardial ischemia, it has been reported that low levels of high energy phosphates prevent the recovery of contractility following normothermic ischemic arrest and reperfusion.^{20,21} However, a great deal of uncertainty has centered about exactly how ATP levels relate to contractile function. Thus, it is suggested that there is no direct relation between ATP levels and contractile activity.²⁰ Left ven-

tricular hypertrophy used in the present studies was produced by creating supra-ventricular aortic stenosis an average of 95 days prior to the experiment. Therefore, it is likely that the morphological and biochemical changes of canine left ventricular muscle used corresponded to those in the stage of relatively stable hyperfunction described by Meerson.¹⁷

It is recognized that cyclic AMP is considered to be a mediator of both the metabolic and the inotropic effects through its stimulation of phosphoprotein formation or through some as yet undefined role in controlling calcium ion movement.²² An increase of cyclic AMP level in the heart also can be brought about either by acceleration of its formation from ATP through increased adenyl cyclase activity or by slowing of its hydrolysis to 5'-AMP through a decrease of phosphodiesterase activity.²³ Therefore, a definite relation between changes in cyclic AMP and changes in myocardial contractility has been suggested, but the mechanism remains to be defined.²⁴⁻²⁷ Generally, on the mechanism of myocardial contractility it is clear that adenylate cyclase, which catalyzes the production of cyclic AMP from ATP in the presence of Ca^{++} , is activated by catecholamine which attaches to beta receptors on the myocardial cell membrane, and intracellular cyclic AMP regulates protein kinase system and calcium transport. In the experimental model used plasma catecholamine of the support dog is considered to be a definite level. Therefore, in order to biochemically examine the difference between hypertrophied muscle and nonhypertrophied muscle myocardial cyclic AMP and myocardial calcium were investigated. In the present studies, although there was no difference between the cyclic AMP levels in hypertrophied left ventricular muscle and in nonhypertrophied muscle before the ischemia, the cyclic AMP level in hypertrophied muscle showed a significant decrease during reperfusion after the ischemia as compared to the changes of nonhypertrophied muscle. This decreased myocardial cyclic AMP level supports a depressed myocardial contractility in hypertrophied muscle after the ischemia as compared to that in nonhypertrophied muscle.

A reperfusion injury that has been noticed is that the swollen mitochondria obtained from ischemic myocardium contain deposits of calcium phosphate and amorphous matrix densities, and particularly, many of these changes become more intense when blood flow is restored.²⁸⁻³⁰ The mechanism of the uptake of

calcium and matrix density has not been established. It appears to be related to a defect in cellular permeability or mitochondrial function.^{29,31} Moreover, it is possible that the reperfusion injury may be a contributing factor in the transient myocardial dysfunction.³² In the present studies, a trend towards an increase of myocardial calcium content was found at the termination of reperfusion after the ischemia in the hypertrophied and nonhypertrophied left ventricular muscle, however, there was no definite relation between contractile damage in hypertrophied left ventricular muscle and myocardial calcium contents after an ischemic period of 150 minutes at the myocardial temperature of 17°C. From the above-mentioned results in this experimental studies, it is suggested that in hypertrophied muscle an impairment of myocardial contractility biochemically results from a decrease of myocardial cyclic AMP and an abnormal increase of myocardial calcium at the reperfusion. Further investigations concerning pH of perfusate and histochemical assessment of intracellular calcium deposit are required to define an interrelation between them.

SUMMARY

The effect of hypothermic ischemic arrest on myocardial contractility was investigated with use of isolated blood-perfused canine heart preparation in the hypertrophied left ventricle created by supra-aortic stenosis and the nonhypertrophied ventricle. The following results were obtained: The percent recovery rate of net developed tension during reperfusion after an ischemic period of 150 minutes at the myocardial temperature of 17°C was $90 \pm 9\%$ in the nonhypertrophied muscle, while it was $70 \pm 19\%$ in the hypertrophied muscle, that is, hypertrophied left ventricle was considered to be more vulnerable to ischemia as compared to nonhypertrophied left ventricle, suggesting the necessity of more intense myocardial protection in addition to cardiac hypothermia against ischemia in the hypertrophied heart.

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