Effect of Hypothermic Anoxic Arrest on Myocardial Contractility in the Isolated Blood-Perfused Canine Left Ventricular Muscle

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SUMMARY

The present studies were performed to evaluate the protective effect of topical hypothermia on anoxic heart. The myocardial protection was assessed by myocardial contractility in the isolated blood-perfused electrically driven canine left ventricular muscle. The isometric tension and the rate of rise of tension development (dT/dt) were measured before and after hypothermic anoxic arrest and percent recovery of these values was used as a parameter of myocardial contractility. The percent recovery rates of 100, 95, 90, and 65 were obtained after acute anoxia of 90, 120, 150, and 180 min, respectively at the myocardial temperature of 17°C. These data suggest that the safe limit of acute anoxia at the myocardial temperature of 17°C is defined as 90 min in this experimental model and it can be extended to 120 min at the myocardial temperature below 17°C.

Additional Indexing Words:

Myocardial protection Hypothermic anoxic arrest Myocardial contractility Isolated blood-perfused canine left ventricular muscle

SINCE Shumway,¹⁾ Hurley,²⁾ and Griep³⁾ found that effective myocardial protection could be achieved by means of elective cardiac surface cooling by pericardial irrigation with cold (4°C) physiological saline solution, topical hypothermia has been widely used clinically and a number of studies in which the significant protective effects of hypothermia on the anoxic heart were assessed in morphologic, metabolic, and hemodynamic aspects have been reported. Hitherto, there have been several investigations to examine the recovery of myocardial contractility after acute anoxia^{4),5)} or the possible protection of cardiac muscle from the consequence of acute anoxia in the isolated cardiac muscle preparation.⁶⁾⁻⁸⁾ However, there are few studies in which the myocardial contractility in the isolated blood-perfused cardiac muscle has been employed to assess the protective effect of hypothermia on

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the anoxic cardiac muscle.⁹⁾ Moreover, although several satisfactory preparations have been available for the investigation of acute anoxia in the isolated myocardial preparation,^{5),9)} the well-controlled, reproducible preparation for the studies of hypothermic anoxic arrest has been less easily achieved.

The purpose of this paper is to describe a simple, yet controlled preparation for the assessment of the possible effect of hypothermic anoxic arrest on the myocardial contractility and also to investigate an interrelation between the optimal myocardial temperature and the safe duration of anoxic arrest in this experimental model.

METHODS

The experimental preparation consists of isolated canine left ventricular muscle perfused with blood according to the technique described by Chiba.¹⁰⁾ The mongrel dogs of both sexes weighing 8 to 15 Kg were anesthetized with 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 into cold Tyrode's solution. The wet weight of the excised muscle was approximately 10 to 15 Gm. The left anterior descending artery was cannulated with a small polyethylene catheter and perfused with arterial blood led from a carotid artery of the support dog at a constant rate of 8.4 ml/ml 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 isolated blood-perfused left ventricular muscle was suspended in a cup-shaped glass container, 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 a jugular vein. The muscle was electrically paced at a rate of 70/min with current of 3 mA. The muscle was loaded with a resting tension of 2 Gm. 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 canine left ventricular myocardium is shown in Fig. 1.

In the present studies, coronary perfusion was stopped and the isolated ventricular muscle was bathed in Tyrode's solution at 17°C while anoxic time was varied from 90 min to 180 min. The myocardial temperature rapidly equilibrated with the temperature of the cold Tyrode's solution in which the muscle was bathed. After each anoxic period the muscle was reperfused with arterial blood from the support dog until the myocardial contractility fully recovered at the myocardial

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

temperature of 37°C. The maximal developed tension at this point was referred to as a percent recovery in comparison with the maximal tension before anoxic arrest. Arterial PO_2 , PCO_2 , base excess, and pH of the support dog were measured at intervals using a blood gas analyzer. These values were maintained within normal range by the addition of oxygen and by the administration of 7% sodium bicarbonate solution.

RESULTS

As soon as the isolated ventricular muscle was prepared and perfused with the arterial blood of the support dog, the muscle started to contract with a slow rhythm followed by ventricular fibrillation. Approximately 20 min after the start of perfusion the muscle showed a good response to electrical pacing and the net developed tension defined as total developed tension minus resting tension continued to increase. Approximately 90 min was required before the maximal net developed tension reached a plateau, and the postischemic recovery time required to obtain a plateau of the maximal net developed tension was approximately 60 min.

In the control group the net developed tension showed a plateau at the termination of 4 hours of continuous coronary perfusion. In the experimental

Fig. 2. The interrelation between the percent recovery rate of maximal net developed tension and various anoxic time at the myocardial temperature of 17°C.

Fig. 3. This rectigram shows the change of the maximal net developed tension and the rate of rise of tension development (dT/dt) before and after acute anoxia of 90 min at the myocardial temperature of 17°C, indicating the percent recovery rate of 100.

groups of anoxic arrest time of 45 and 60 min at the myocardial temperature of 37°C, the percent recovery rates of the net developed tension were $70 \pm 5\%$ (mean $\pm$ SD, $n=5$) and $30 \pm 3\%$ ($n=5$) respectively. In the experimental groups of hypothermic anoxic arrest time of 90, 120, 150, and 180 min at the myocardial temperature of 17°C, as shown in Fig. 2, the percent recovery rates of the net developed tension were 100% ($n=3$), $95 \pm 3\%$ ($n=5$), $90 \pm 5\%$ ($n=5$), and $65 \pm 5\%$ ($n=5$), respectively. Fig. 3 shows a record of the case presenting the percent recovery of the net developed tension and dT/dt of

Table I. Blood Gas Analysis in Support Dog

	Before Ischemia	After Ischemia
pH	7.355 ± 0.087	7.340 ± 0.082 NS
B.E. (mEq)	-8.0 ± 3.3	-7.8 ± 3.4 NS
PaCO ₂ (mmHg)	30 ± 9	31 ± 7 NS
PaO ₂ (mmHg)	96 ± 11	91 ± 23 NS

Values are mean $\pm$ SD, NS=no statistical significance.

100% in the anoxic time of 90 min at the myocardial temperature of 17°C. Through the experiments the change of the rate of rise of tension development (dT/dt) showed a parallel relation to that of the maximal net developed tension.

On the studies of acid-base change in the support dogs, as shown in Table I, there was no significant differences between arterial pH, base excess, PCO₂, and PO₂ during the preischemic perfusion and those during the postischemic perfusion, because these values were maintained within normal ranges by the addition of oxygen and by the administration of sodium bicarbonate. In such a condition of acid-base balance in the support dogs, as shown in Fig. 4, the percent recovery rate of the maximal net developed tension following acute anoxia of 150 min at the myocardial temperature of 17°C was approximately 90. While in metabolic acidosis of the support dog during the postischemic perfusion, as shown in Fig. 5, the percent recovery of the

Fig. 4. This rectigram shows the change of the maximal net developed tension and dT/dt before and after acute anoxia of 150 min at the myocardial temperature of 17°C, indicating the percent recovery rate of 90.

Fig. 5. This rectigram shows an influence of metabolic acidosis on myocardial contractility. In metabolic acidosis of the support dog during postischemic perfusion the percent recovery rate of the maximal net developed tension and dT/dt following the same hypothermic anoxic arrest were reduced to 50.

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DISCUSSION

The effect of hypothermic anoxic arrest on left ventricular function has been experimentally evaluated by means of isovolumetric contractility using a latex balloon in canine whole heart supported by cardiopulmonary bypass,¹¹⁾ aortic flow rate by Langendorff's preparation,¹²⁾ and myocardial contractility in isolated cardiac preparation.⁹⁾ The measurement of isovolumetric contractility of the left ventricle using a latex balloon and aortic pressure or flow rate by Langendorff's preparation were noted to be technically complicated and associated with many uncontrolled variables as mentioned by Ino.⁹⁾ An alternative to evaluate the left ventricular function after acute anoxia is to measure myocardial contractility. There have been a number of investigations along these lines, however much of this work can be criticized on methodological grounds. In some experiments isolated whole heart preparations have been used with the associated problem of adequate oxygenation in the control state,^{4),7),14)} while the use of isolated spontaneously beating atrial preparation is complicated by the interrelation between rate and myocardial

contractile force.^{8),13),15)} A papillary muscle preparation has been widely employed to examine the mechanism of myocardial contractility,^{5),6),16),17)} however, such an excised nonperfused papillary muscle preparation could not be used in the present hypothermic anoxic experiment, because normothermic coronary perfusion is required before and after hypothermic anoxic arrest. Ino⁹⁾ demonstrated a unique method using isolated, blood-perfused and spontaneously beating rabbit papillary muscle preparation to evaluate myocardial contractility after hypothermic anoxic cardioplegia. It is known that the strength of contraction of isolated cardiac muscle is influenced by the frequency of contraction.¹⁸⁾ Therefore, it is important to use an electrically driven muscle preparation.¹⁹⁾ Recently, Shine²⁰⁾ reported that isolated blood-perfused electrically driven rabbit interventricular septa could be adapted for studies of global ischemia by enclosure in a constant-humidity nitrogen atmosphere. The experimental model described here has several characteristics. Namely, the isolated electrically driven left ventricular muscle is perfused with arterial blood led from a carotid artery of the support dog and the myocardial contractility can be precisely measured with stable contractility 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 blood leaving the isolated blood-perfused muscle or Tyrode's solution can be quickly regulated by circulating water pumped from a water bath. The same preparation can be used for studies of metabolic changes and evaluation of cardioplegic solutions.

A controversy exists on the optimal myocardial temperature for the application of topical cardiac hypothermia in cardiac surgery. Shumway¹⁾ and Griep³⁾ have advocated maintenance of the intramyocardial temperature between 15 to 20°C providing satisfactory myocardial protection in clinical use. In experimental studies Angell²¹⁾ reported that anoxic time of 180 min at the myocardial temperature of 15°C allowed the viability of canine cadaver heart and Ino¹⁾ also noted that the optimal myocardial temperature for satisfactory myocardial protection was 18°C. In our clinical experience the topical cardiac surface cooling with cold (4°C) lactated Ringer's solution and ice slush resulted in the maintenance of intramyocardial temperature of 17°C. Therefore, in the present studies the myocardial temperature of 17°C was chosen as an optimal level of hypothermia.

In the study of a correlation between myocardial temperature and left ventricular function, Greenberg²²⁾ found that less than 30% depression of left ventricular function occurred after acute anoxia of 10 min at 37°C, 15 min at 28°C, 30 min at 18°C, and pronounced depression occurred after 30

min at 37°C. Angell¹¹⁾ also reported that the maximum intervals of allowable anoxia were 35 min at 37°C, 115 min at 24°C, and 230 min at 15°C in canine heart. Ino⁹⁾ and associates showed the significant protective effect of hypothermia on the anoxic heart. They found that 80% recovery of myocardial contractility was estimated after anoxic arrest of 150 min at 18°C from the nomogram expressing anoxic time, myocardial temperature, and percentage recovery of papillary muscle contractility after reperfusion and suggested that the nomogram constructed from rabbit heart experiments could be applied to human beings. The present studies showed that 100%, 95%, 90%, and 65% recovery of myocardial contractility after reperfusion at myocardial temperature of 17°C were obtained after anoxic arrest of 90 min, 120 min, 150 min, and 180 min, respectively, while 70% and 30% recovery at 37°C were obtained after anoxic arrest of 45 min and 60 min, indicating a significant protective effect of hypothermia on the anoxic cardiac muscle.

The safe duration of myocardial anoxia for human beings using topical heart cooling has not been clearly defined. It has been reported that the time limit of safe cardioplegia is as long as 30 min by Willman,²³⁾ 45 min by Sprovieri,²⁴⁾ 60 min by Heimbecker,²⁵⁾ up to 70 min by Robicsek,²⁶⁾ 71 min by Urschel,²⁷⁾ 90 min by Griep,³⁾ and 128 min by Pupello.²⁸⁾ According to Ino,⁹⁾ maximal tolerable anoxic period can be prolonged to nine times of the control by cooling the heart at 18°C. On the bases of the present experimental experience, the safe duration of hypothermic anoxic arrest is as long as 90 min, and it can be even further extended up to 120 min.

It is well documented that myocardial performance is impaired by acidosis. Initially, it was suggested that extracellular pH was the principal determinant of cardiac performance,²⁹⁾ but recently the importance of intracellular pH has been recognized.^{30)–32)} Moreover, a more remarkable decrease in intracellular pH was noted during respiratory acidosis produced by the increase of PaCO_2 than during metabolic acidosis produced by the decrease of bicarbonate concentration. Several investigators also state that intracellular pH is an important determinant of myocardial contractility, because metabolic acidosis results in a smaller negative inotropic effect than respiratory acidosis at the same extracellular pH.^{32)–34)} In the present studies, a remarked metabolic acidosis caused a significant decrease of myocardial contractility. Therefore, arterial pH, PCO_2 , and base excess of the support dog were maintained within normal ranges by the addition of oxygen and the administration of sodium bicarbonate throughout the experiment.

Conclusively, the experimental model described here has several characteristics and can be used for evaluation of the effect of hypothermic anoxic arrest on myocardial contractility. In this experimental model topical hypo-

thermia itself proves to be an effective method for protection against myocardial damage after acute anoxia. Myocardial temperature of 17°C allows anoxic arrest of up to 90 min with safety and the safe limit can be extended to up to 120 min with the recovery of 95% in myocardial contractility.

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