A Correlative Study on the Changes of Cardiac Dynamics and Myocardial Energy Liberation in Blood-Let Dogs

Yoshiharu MORITA, M.D.,* Taro ISHIYAMA, M.D.,*
Yosie HATANAKA, M.D.,* Teiichi UENO, M.D.,*
Junichi AZUMA, M.D.,* Takuji TANIMOTO, M.D.,*
Kyoko OGURA, M.D.,* Nozomu TSUKAMOTO, M.D.,**
and Yuichi YAMAMURA, M.D.*

SUMMARY

Blood-letting of 450 to 1,000 ml with or without saline infusion was performed in dogs. In some dogs, right atrial pacing was carried out during blood-letting. Heart rate and isometric time-tension index were measured as the indicators of chronotropism and inotropism, respectively. After 60 min of blood-letting, dogs were sacrificed and mitochondria were isolated from the left ventricular myocardium. Mitochondrial respiration was measured polarographically and respiratory control index was calculated.

As blood-letting advanced, the hearts revealed negative chronotropic with negative inotropism. Mitochondrial respiration was suppressed. When heart rate was forced to increase with atrial pacing, the hearts showed positive chronotropic with negative inotropism. Respiratory control index of mitochondria was deteriorated, showing uncoupling of oxidative phosphorylation.

In consideration with our previous study on the ischemic heart, it is concluded that uncoupling of oxidative phosphorylation appeared in hypoxic myocardium when cardiac dynamics shifted to positive chronotropic with negative inotropism, though suppression of mitochondrial respiration was revealed when cardiac dynamics altered to negative chronotropic with negative inotropism.

Additional Indexing Words:
Chronotropic-inotropic state Mitochondria Respiratory control index Oxidative phosphorylation Hypoxia

It is obvious that myocardial energy metabolism is disturbed in hypoxia. In experimental myocardial infarction, uncoupling of oxidative phos-
phosphorylation was recognized in mitochondria isolated from infarcted myocardium.\textsuperscript{13-4,7} But oxidative phosphorylation was not affected in mitochondria isolated from non-beating heart without coronary perfusion for 1 hour.\textsuperscript{13} Rovetto et al\textsuperscript{5} pointed out a difference in myocardial energy metabolism between myocardial ischemia and anoxia. Moreover, we\textsuperscript{6,7} observed that myocardial energy liberation was influenced by cardiac dynamic state.

In the present study, we tried to prove the disturbance of energy liberation in experimental hypoxic myocardium induced by generalized blood-letting, and also to affirm whether a certain relationship was found between the change of mitochondrial respiration and the alteration of cardiac dynamic state in hypoxic myocardium.

**Material and Methods**

Twelve mongrel dogs weighing 10–12 Kg were anesthetized with 25 mg/Kg of sodium pentobarbital. Respiration was maintained with a cuffed endotracheal tube connected to an artificial respirator. After heparinized, an 8F catheter was placed in the left ventricle via the right carotid artery. Through Statham P23Db transducer, left ventricular pressure (LVP) and the first derivative of LVP (dp/dt) were obtained and recorded on Fukuda Polygraph EMR 100R. Lead II ECG was recorded simultaneously.

Hemorrhagic hypoxia was produced by 450 to 1,000 ml of arterial blood-letting through a polyethylene cannula which was inserted to the femoral artery. In order to avoid severe hypotension, 1,000 to 1,200 ml of saline was infused while blood was let in 7 dogs. In 6 dogs, right atrial pacing was performed prior and during blood-letting with a floating catheter via the right jugular vein in order to induce positive chronotropism.

Heart rate (HR), left ventricular systolic pressure (LVSP), and maximal dp/dt (dp/dt max) were measured, and isometric time-tension index (ITTI)\textsuperscript{8} was calculated. For convenience to evaluate the change of the cardiac dynamics, percent changes of HR and ITTI were calculated sequentially along the time course of experimental procedures, and the values were plotted on a co-ordinate composed of HR (X) axis and ITTI (Y) axis. Thus a chronotropic-inotropic diagram\textsuperscript{6} was constructed. This diagram is useful to evaluate chronotropism and inotropism of the heart.

Arterial blood was sampled at intervals during the experimental procedures. Red blood cells and hemoglobin contents and hematocrit were measured.

Sixty min after onset of blood-letting, dogs were sacrificed and pieces of the left ventricular muscle were removed. Mitochondrial fraction was isolated from the muscle, and respiration of mitochondria was measured polarographically according to the methods reported previously.\textsuperscript{1} Glutamate was used as a substrate. Oxygen consumption rates in state 3 (QO\textsubscript{2} state 3) and in state 4 (QO\textsubscript{2} state 4), respiratory control index (RCI) and ADP/O were calculated from the polarograms. The respiration of intact mitochondria was reproduced from data described in the
RESULTS

Cardiac dynamics

Changes of the cardiac dynamics in a typical case of blood-letting with saline infusion were shown in Fig. 1. In the early phase of hemorrhage, HR, LVSP, dp/dt max, and ITTI increased. In advance with bleeding, these parameters gradually decreased. Saline infusion was, however, seemed to be effective to prevent an abrupt cardiac collapse. In a typical case without saline infusion, moderate bradycardia and severe hypotension developed promptly. Changes of the cardiac dynamics in a typical case of blood-letting with saline infusion and atrial pacing were shown in Fig. 2. Atrial
pacing was started 5 min prior to bleeding. HR was set up to 120 to 130% of the initial level. In this phase, LVSP, dp/dt max, and ITTI increased slightly. When bleeding was started, the parameters began to decrease except HR that was fixed in a high rate. In a case of blood-letting with pacing without saline infusion, ITTI declined markedly and ventricular fibrillation occurred 4 min after the onset of bleeding. Data of this case were omitted in this study.

Maximal chronotropic-inotropic vectors, i.e. vectors directed from the zero point of the co-ordinate to the most distant point in the chronotropic-inotropic diagrams, were illustrated in Fig. 3. The maximal chronotropic-inotropic vectors of blood-let dogs fell into the third quadrant, i.e. the negative chronotropic-negative inotropic quadrant. In cases with artificial pacing, the vectors of 4 cases out of 6 deviated into the fourth quadrant, i.e. the positive chronotropic-negative inotropic quadrant. In the remaining 2 animals in which the atrial pacing was able to be maintained for only 8 to 10 min, the chronotropic-inotropic vectors indicated to the first quadrant, i.e. positive chronotropic-positive inotropic quadrant.

ECG and hematology

In all of cases, remarkable ST-segment depression was proved on ECG with progress of bleeding.

Changes of hematology due to blood-letting were showed on Fig. 4. By 450 to 550 ml of blood-letting, decreases of red blood cells, hemoglobin, and hematocrit were 25%, 24%, and 23% respectively. When saline infusion was performed during blood-letting, decreases of red blood cells, hemoglobin,
Fig. 4. Changes of hematology due to blood-letting with or without saline infusion. Range and mean. RBC: red blood cell count, Hb: hemoglobin content, Ht: hematocrit. Open bars: before blood-letting, Hatched bars: after blood-letting. A: blood-letting without saline infusion, B: blood-letting with saline infusion.

Fig. 5. Relationship between mitochondrial respiration (Figs. 6-8) and chronotropic-inotropic co-ordinates.

Mitochondrial respiration

Changes of the mitochondrial respiration were different according to the quadrants where the cardiac dynamics shifted. Respiration of mitochondria isolated from the hearts in which dynamics shifted to the third, the fourth, and the first quadrants were shown in Figs. 6, 7, and 8, respectively.

Mitochondria isolated from the hearts showing negative chronotropism with negative inotropism by blood-letting with or without saline infusion revealed rather decreased $QO_2$ state 3 and $QO_2$ state 4. However, RCI did not lower remarkably, because decreasing rates of $QO_2$ were similar between state 3 and state 4. Mitochondria isolated from the hearts showing positive
Fig. 6. Respiration of mitochondria isolated from the hearts of which chronotropic-inotropic vectors directed to the third quadrant. Range and mean. QO$_2$: oxygen consumption rate, RCI: respiratory control index, Open bars: intact mitochondria, Hatched bars: mitochondria isolated from the blood-let and saline infused hearts, Dotted bars: mitochondria isolated from the blood-let hearts without saline infusion.

Fig. 7. Respiration of mitochondria isolated from the hearts of which chronotropic-inotropic vectors were altered to the fourth quadrant by artificial pacing. See foot-notes of Fig. 6.

Fig. 8. Respiration of mitochondria isolated from the hearts of which chronotropic-inotropic vectors were shifted to the first quadrant by artificial pacing. See foot-notes of Fig. 6.
chronotropism with negative inotropism by blood-letting under the artificial pacing revealed, however, normal or somewhat decreased QO$_2$ state 3 and increased QO$_2$ state 4. Therefore, RCI of these samples was consistently low. When saline was infused, these changes were rather mitigated. Mitochondria isolated from the hearts, in which artificial pacing was unable to be maintained more than 10 min, showed no remarkable respiratory changes.

ADP/O ratios were ranged within 2.42 to 2.86 in all cases, showing no significant difference with 2.77 in intact mitochondria.

**DISCUSSION**

The greater part of myocardial energy liberation depends on oxidative phosphorylation. Therefore, it is easily predicted that the mechanism of energy liberation is severely affected in the state of anoxia. We$^{1,7}$ and other investigators$^{2-4}$ have reported on the alteration of oxidative phosphorylation in experimental myocardial infarction. We$^1$ also pointed out that the uncoupling of oxidative phosphorylation revealed in the myocardium when the extirpated hearts without coronary perfusion were forced to beat with artificial pacing. On the contrary, oxidative phosphorylation remained within normal range in mitochondria isolated from non-beating extirpated hearts without coronary perfusion. Moreover, when HR was suppressed by propranolol in dogs of which heart was infarcted, uncoupling of oxidative phosphorylation in the heart mitochondria was mitigated due to suppression of QO$_2$ state 4.

From these results, it is considered that the disturbance of energy liberation in anoxic myocardium due to coronary ischemia is not always uniform but it is influenced by the change of dynamic states, i.e. the states of chronotropism and inotropism, of the heart. Even in the intact canine heart without myocardial infarction, uncoupling of oxidative phosphorylation was also recognized by isoproterenol-induced positive chronotropic and positive inotropic state, while the suppression of mitochondrial respiration was proved by propranolol-induced negative chronotropic and negative inotropic state.$^6$

Rovetto et al$^5$ pointed out a difference of energy liberation between anoxia and whole heart ischemia from the standpoint of carbohydrate utilization. We intended to know another difference of energy liberation between hypoxemia and coronary ischemia from the standpoint of mitochondrial respiration. Moreover, we attempted to prove in the hypoxic hearts that the difference of myocardial energy liberation was attributed to the difference of induced cardiac dynamic states.

In this study, the relationship between the changes of mitochondrial
respiration and the dynamic states of the hearts was affirmed in the anoxic myocardium due to hypoxemia as follows. HR and ITTI were gradually decreased in advance with blood-letting. Thus, the chronotropic-inotropic vectors directed to the third, negative chronotropic-negative inotropic quadrant. Then, respiration of the left ventricular mitochondria revealed a slight suppression in both QO₂ state 3 and QO₂ state 4, whereas RCI was rather well maintained, even though considerable hypoxia was strongly suggested in the myocardium from the electrocardiographic findings of marked ST-segment depression and the changes of hematology. These changes of the cardiac dynamics and the mitochondrial respiration were different from those of the infarcted myocardium, where the positive chronotropism with negative inotropism, followed by increased QO₂ state 4 and lowered RCI were proved.¹)

In the next step, when HR was forced to maintain a high level with artificial pacing during blood-letting, the chronotropic-inotropic vector was deservedly shifted to the fourth quadrant except 2 cases in which pacing was able to be continued for less than 10 min and the cardiac dynamics shifted to the first quadrant. As predicted, respiration of mitochondria isolated from the artificially paced blood-let heart was accelerated in state 4, followed by deterioration of RCI, showing a typical pattern of uncoupling of oxidative phosphorylation. This change was just same with that of experimental myocardial infarction. Therefore, the difference of the mitochondrial respiration between the blood-let myocardium and the infarcted myocardium may not be attributed to the pathophysiological condition of the heart, that is hypoxemic in the former and ischemic in the latter.

In the previous study,¹) we had experienced some cases in which HR increased spontaneously during blood-letting. The discrepancy of the change of HR between the previous and the present studies was thought to be interpreted by the quantity or rate of bleeding and saline infusion. If either quantity or rate of infusion had been inadequate, dogs had fallen into hypovolemic shock states. Though ITTI was not been measured in the previous cases, the chronotropic-inotropic vectors were thought to direct to the fourth quadrant. It was noteworthy that the oxidative phosphorylation of the myocardium in the previous cases had shown uncoupling. Hypovolemia was protected with a proper saline infusion in the present study. Hereupon, HR did not increase but decrease and uncoupling of oxidative phosphorylation did not occur.

It is concluded from these results that the disturbance of energy liberation in the hypoxic heart in situ depends on the concurrent changes of cardiac dynamic state. In the hypoxic heart, uncoupling of oxidative phosphorylation emerged when the cardiac dynamics shifted to the positive chronotropism
with negative inotropism, while suppression of the mitochondrial respiration revealed when the cardiac dynamics directed to the negative chronotropism with negative inotropism.

REFERENCES