MECHANISM OF MITOCHONDRIAL DAMAGE AFTER CORONARY REPERFUSION

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We investigated the mechanism of the reperfusion-accelerated mitochondrial dysfunction. To clarify this mechanism, we performed the following experiments using 40 mongrel dogs. Experiment I: Prostaglandin (PG) E and F$_2$α levels in the great cardiac vein (GCV) were examined before, during occlusion and after reperfusion of the left anterior descending coronary artery (LAD). Experiment II: Heart mitochondria were prepared from the normal area and the occluded or the reperfused area after 15 min of the LAD occlusion, or after 5 min of reperfusion following the occlusion with or without premedication of indomethacin. The PG E level in the GCV did not change significantly during occlusion, but increased significantly soon after reperfusion. Mitochondrial dysfunction was caused by occlusion and further accelerated by reperfusion. The PG E level in mitochondria isolated from the reperfused area increased significantly. Indomethacin significantly prevented both the increase in PG E and the acceleration of mitochondrial dysfunction by reperfusion. These results suggest that the increase in PG E level is closely related to the reperfusion-accelerated mitochondrial dysfunction, and that premedication with indomethacin significantly prevented the extension of mitochondrial dysfunction induced by coronary reperfusion.

Since the report of Jennings et al.¹ that reperfusion might not necessarily be always beneficial to the ischemic myocardium, many investigators reported that, even after a relatively short period of ischemia, reperfusion could lead to an extension of ischemia-induced mitochondrial damage.²⁻⁴ Although some possible mechanisms for reperfusion-accelerated mitochondrial dysfunction was suggested, the precise mechanism involved remains unknown.

In our previous study, we observed the significant increase in the plasma prostaglandin (PG) E level in the great cardiac vein (GCV) after reperfusion of the left anterior descending coronary artery (LAD). We also reported that arrhythmias developed after coronary reperfusion are closely related to PG E. The present study was designed to determine whether or not PG plays a key role in reperfusion-accelerated mitochondrial dysfunction using indomethacin, a well-known PG biosynthesis inhibitor.⁵⁻⁹

Key Words:
Reperfusion
Mitochondria
Prostaglandin E
Indomethacin

(Received November 24, 1981; accepted March 20, 1982)
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METHODS

Indomethacin was supplied by Merck Japan, and was dissolved in a vehicle, 5% NaHCO₃. Radioimmunoassay kits for PG E and PG F₂α were purchased from Clinical Assay Inc.

Experiment I: Sixteen adult mongrel dogs of both sexes weighing between 10 and 15 kg were anesthetized with sodium pentobarbital (50 mg/kg) given intraperitoneally. Under artificial respiration, the chest of each dog was opened with a left 4th intercostal incision, and the heart was suspended in a pericardial cradle. Silk ligation of the LAD was placed immediately distal to the first diagonal branch. Dogs were divided into two groups of 8 animals. In all dogs, the GCV was cannulated just beneath the left auricle to gather the blood sample mainly from the ischemic area. The right femoral vein was also cannulated to infuse indomethacin solution. Dogs in the control group received only the vehicle, and dogs in the indomethacin group received indomethacin solution (5 mg/kg) 30 min before the LAD occlusion. In each group, the LAD was occluded for 15 min and then reperfused for 30 min. Blood sampling from the GCV was performed 8 times: before the infusion of indomethacin or the vehicle, before the LAD occlusion, 7.5 and 15 min after occlusion, and 5, 10, 20 and 30 min after reperfusion. Plasma levels of PG E and PG F₂α were measured by radioimmunoassay as reported previously.

Experiment II: Twenty-four mongrel dogs of both sexes, weighing between 10 and 15 kg, were anesthetized, and the chest of each animal was opened as described above. The dogs were divided into three groups. The occlusion group: only the vehicle was infused 30 min before the LAD occlusion, and the occlusion was maintained for 15 min. The reperfusion group: only the vehicle was infused 30 min before the LAD occlusion, and the LAD was reperfused for 5 min following 15 min of occlusion. The indomethacin group: indomethacin (5 mg/kg) was infused 30 min before occlusion, and the LAD was reperfused for 5 min after 15 min of occlusion.

Heart mitochondria were prepared from both the normal and the occluded areas (occlusion group), or from both the normal and the reperfused areas (reperfusion and indomethacin groups), as reported previously.

PG E and PG F₂α in mitochondria were extracted according to the method of Blondin. Two or 3 ml of mitochondria (20–30 mg protein) were extracted with 5.7–8.6 ml of a solution (CHCl₃ : MeOH : H₂O = 1:2:0.8) and the phase separation was carried out by addition of CHCl₃ and aq. 5% K₂CO₃ (75 ml/g protein, each). The lower phase was re-extracted with 5.7–8.6 ml of a solution (MeOH : H₂O : aq. 5% K₂CO₃ = 2:0.8:1). The combined upper phases were dried with an evaporator at 50°C. The dried extract was redissolved in 2 ml of 0.9% NaCl and was acidified to pH 3 with HCl and then extracted twice with 10 ml of diethylether. Combined ether extracts were evaporated to dryness in a water bath (40°C–50°C), and then the dried extract was redissolved in 0.5 ml of 20 mM Tris-HCl buffer, pH 7.6, containing gelatin (1 mg/ml) for the radioimmunoassay of PG E and PG F₂α as described previously.

The indexes of mitochondrial function measured were the respiratory control index (RCI), the rate of oxygen consumption in state III (St. III O₂) and ADP/O. RCI was taken as the ratio between the rate of oxygen consumption after and before the addition of ADP. St. III O₂ was calculated from the mitochondrial oxygen consumption in n atoms of oxygen consumed per mg of mitochondrial protein per min during state III respiration. ADP/O was calculated from the ratio of μ moles of ADP phosphorylated to atoms of oxygen consumed. These indexes were measured immediately after the preparation of mitochondria. Oxygen consumption in mitochondria was measured polarographically as described previously using an oxygen electrode and a closed cell.

Statistical analyses were done by Student’s t-test.

RESULTS

Experiment I

Figure 1 shows the time courses of the plasma PG E level in the GCV for the control and indomethacin groups. In the control group, the initial plasma PG E level of 0.25 ± 0.06 ng/ml (mean ± SE) did not change significantly during occlusion. However, the level of PG E was elevated significantly reaching a value of 0.56 ± 0.07 ng/ml after 5 min of reperfusion. Thereafter its level gradually decreased to a value of 0.48 ± 0.09 ng/ml after 10 min of reperfusion, and after 20 min of reperfusion no significant elevation of the PG E level was observable. In the indomethacin group, the initial plasma PG E level of 0.20 ± 0.03 ng/ml did not change significantly during occlusion or
Fig. 1. Time courses of plasma PG E level in GCV. In the control group, plasma PG E level in GCV increased significantly 5 and 10 min after reperfusion of LAD. In the indomethacin group, significant increase in plasma PG E level in GCV was prevented by premedication with indomethacin.

Fig. 2. Time courses of plasma PG F_{2α} level in GCV. There was no significant change in plasma PG F_{2α} level throughout the experiment in the two groups, with or without premedication of indomethacin.

after reperfusion. That is, in the indomethacin group, the increase in the PG E level after reperfusion was prevented significantly. Figure 2 shows the time courses of the plasma PG F_{2α} level in the GCV for the two groups. In the control and indomethacin groups, the respective initial PG F_{2α} levels of 0.26 ± 0.07 ng/ml and 0.20 ± 0.03 ng/ml did not change significantly throughout the experiment.

**Experiment II**

PG E levels in mitochondria are presented in Fig. 3. In the occlusion group, there was no significant difference between the PG E level in mitochondria isolated from the occluded area (39.7 ± 14.9 pg/mg protein, mean ± SD) and that from the normal area (27.3 ± 4.1 pg/mg protein). In the reperfusion group, the PG E level in mitochondria from the reperfused area showed a strikingly increased value of 97.3 ± 20.5 pg/mg protein compared with that from the normal area (39.8 ± 14.1 pg/mg protein). In the indomethacin group, the increase in the PG E level from the reperfused area was effectively diminished by premedication with indomethacin with a value of 35.0 ± 8.4 pg/mg protein. In this group, there was no significant difference between the PG E level in mitochondria from the normal area and that from the reperfused area. On the other hand, as represented in Fig. 4, the PG F_{2α} level
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in mitochondria did not show any significant difference among the three groups.

Figure 5 shows RCI of mitochondria in the occlusion, reperfusion and indomethacin groups. In the occlusion group, RCI of mitochondria prepared from the occluded area decreased significantly to 3.62 ± 0.32 (mean ± SD) compared with that from the normal area (4.25 ± 0.27). In the reperfusion group, the decrease in RCI of mitochondria from the reperfused area accelerated to 3.29 ± 0.28. This value was revealed to be significantly lower than RCI of mitochondria from the occluded area in the reperfusion group. In the indomethacin group, RCI from the normal and reperfused areas were 4.16 ± 0.14 and 3.73 ± 0.26, respectively. Though RCI from the reperfused area was significantly lower than that from the normal area, premedication with indomethacin manifested significant protection against the decrease in RCI accelerated by reperfusion. There was no significant difference in RCI of mitochondria prepared from the normal area among the three groups. Change in St. III O₂ was revealed the similar tendency to RCI as represented in Fig. 6. In the occlusion group, St. III O₂ of mitochondria prepared from the occluded area decreased significantly to 315.2 ± 28.5 n atoms/mg protein/min (mean ± SD) compared with that from the normal area (377.6 ± 16.0 n atoms/mg protein/min). In the reperfusion group, the decrease in St. III O₂ from the reperfused area accelerated to 254.6 ± 21.7 n atoms/mg protein/min, and this value was significantly lower than that from the occluded area in the occlusion group. The striking finding, however, was the effectiveness of indomethacin premedication against the decrease in St. III O₂ from the reperfused area. In the indomethacin group, the decrease in St. III O₂ from the reperfused area diminished significantly by indomethacin to 318.1 ± 23.9 n atoms/mg protein/min. Figure 7 shows ADP/O of mitochondria in each group. There was no significant change in ADP/O of mitochondria among the three groups.

Typical respiratory traces of mitochondria prepared from the occluded or the reperfused area of each group are presented in Fig. 8.

**DISCUSSION**

Recent advances show that even after relatively brief periods of acute coronary occlusion, the ischemic myocardium is not capable of fully utilizing the blood reflow of reperfusion.
In particular, the early phase of reperfusion was reported to be associated with a state of hazardous metabolic, electrophysiologic and mechanical dysfunction. However, there is no general agreement concerning the mechanism involved.

Kraemer and Fols15 observed that PG-like material was released into the GCV after the LAD reperfusion. In this study, we confirmed that the PG E level in the GCV increased soon after reperfusion, and that indomethacin prevented this increase. We also demonstrated that the PG E level in the reperfused mitochondria increased significantly after reperfusion. Biosynthesis of PG E could be activated after reperfusion, because oxygen, which is essential for PG biosynthesis16,17 can be sufficiently supplied from the reflowing blood. Since PG is considered to be biosynthesized in microsomal fractions of vessel walls and myocardium18,19 the increased PG E level in the reperfused mitochondria could be a reflection of the increased biosynthesis of PG E in cardiac vessels and/or myocardium. While it has been suggested3,4 that the reperfusion damage of mitochondria is attributed to Ca\(^{2+}\)-influx induced by reperfusion. Since PG E has a Ca\(^{2+}\)-ionophoretic action5,11,20 the accumulated PG E in mitochondria could induce Ca\(^{2+}\)-influx into the reperfused mitochondria, resulting the extension of mitochondrial dysfunction after coronary reperfusion. Sobel and colleagues21,22 and Chien et al23,24 have reported that phospholipids in membrane systems of ischemic cell were widely degraded by phospholipase which was activated by influxed Ca\(^{2+}\). From these results, they suggested that the degraded membrane systems are related closely to the development of irreversible damage in ischemic cell. Our findings that PG E, an end-product of phospholipase digestion of membrane phospholipids, was accumulated in the reperfused mitochondria could be interpreted as a result of the degradation of membrane systems in myocardium. However, indomethacin, an inhibitor of cyclo-oxygenase7-9 was demonstrated to prevent dysfunction of the reperfused mitochondria (Figs. 5 and 6) as well as to prevent the increase in the PG E.

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level in them. Therefore, it is suggested that the increase in the PG E level in the reperfused mitochondria is not merely as a result of membrane degradation by phospholipase, but also one of the causes of mitochondrial dysfunction.

In accordance with the findings of Jennings et al. and Kane et al., we confirmed in this study that ischemic damage of the mitochondrial function was accelerated by reperfusion. Our results presented in this paper with the effect of indomethacin on the function and the PG level in mitochondria suggest that the increase in the PG E level is closely related to the extension of ischemia-induced mitochondrial dysfunction after coronary reperfusion.

Acknowledgment

We would like to express our appreciation to Mr. Adelbert G. Smith of the Language Center, University of Nagoya, for reading previous drafts of this paper and giving us numerous suggestions concerning language and style.

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