特 集 (special edition)

Myocardial free fatty acids during ischemia and reperfusion

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Contents

Key Word: Free Fatty Acids (FFA), Ischemia, Reperfusion, Arachidonic acid, ATP:AMP ratio, Phospholipase, Plasmalogen, Cardioprotection

Abbreviations: FFA, free fatty acids; PL, phospholipids; TG, triacylglycerol; ATP, adenosine triphosphate; AMP, adenosine monophosphate; CrP, creatine phosphate; lysoPL, lysophospholipids; HPLC, high performance liquid chromatography; ADAM, 9-anthryldiazomethane; GSH, reduced glutathione; GSSG, oxidized glutathione; DAG, diacylglycerol;

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1. Introduction

Accumulation of tissue free fatty acids (FFA) occurs in the ischemic heart and its relation to severity of ischemic cell damage has been described[11,32,57]. Studies with isolated, perfused hearts have revealed that the myocardial levels of FFA increase markedly not only during ischemia but also during post-ischemic reperfusion[4,22,43,57,58]. The FFA that have accumulated during ischemia and reperfusion (such as arachidonic acid) are considered to be degradation products of membrane phospholipids[8,11,27,50,61,62], which produce a deleterious effect on the myocardial cellular function[10,11,32,45,57,65]. Therefore, if a drug attenuates of accumulation of the tissue FFA, it would be evaluated as a substance that has myocardial protective effect on ischemic cellular damage[43,58]. Previous studies have demonstrated that propranolol[38,40], pindolol[44], lidocaine[41] and diltiazem[42] attenuate accumulation of the tissue FFA during ischemia and reperfusion and hence protect the heart from ischemia reperfusion-induced damage[38,41,43,44].

The biochemical mechanism of accumulation of myocardial FFA during ischemia and reperfusion, however, has not been fully elucidated. In the present article, a possible mechanism by which the myocardial FFA accumulate during ischemia and reperfusion and importance of myocardial lipid metabolism in the ischemic-reperfused heart are discussed.

2. Ischemic injury and reperfusion injury

In the ischemic heart, in which oxygen supply is reduced or stopped due to reduction of coronary flow, changes of metabolism and contractile dysfunction occur; myocardial acidosis, a decrease in the levels of tissue high energy phosphate such as adenosine triphosphate (ATP) and creatine phosphate (CrP), an increase in unaerobic glycolytic metabolic intermediates such as lactate (Fig. 1), and a decrease in contractile force. The metabolic alterations are regarded as a reflection of myocardial "ischemic damage".

"Reperfusion-induced damage" is not synonymous with ischemia-induced damage. Rapid restoration of oxygen supply following ischemia results in massive calcium overload in the cell, free radical generation and change in membrane permeability leading to both leakage of intracellular enzymes such as creatine kinase and disturbance of transmembrane ion distribution. Carbohydrate and energy metabolism that has changed during ischemia returns to the pre-ischemic status during reperfusion, though the recovery is not always complete. As shown in fig. 1, the levels of ATP, CrP and lactate that had changed during ischemia recovered to the non-ischemic levels after reperfusion incompletely. As a result, the ischemia-induced metabolic changes of ATP, CrP and lactate do not reflect the reperfusion-induced myocardial damage.

In contrast to the levels of ATP, CrP and lactate, the level of tissue free fatty acids (FFA) such as arachidonic acid increased during both ischemia and reperfusion (Fig. 1). Because arachidonic acid is a major component of membrane phospholipids (PL), accumulation of arachidonic acid in the myocardium reflects degradation of cell membrane, and therefore arachidonic acid would be a sensitive indicator that reflects the degree of tissue damage during ischemia and reperfusion. In addition, accumulation of myocardial FFA occur not only when the ischemia reperfusion-induced damage is reversible but also when it is irreversible[22].

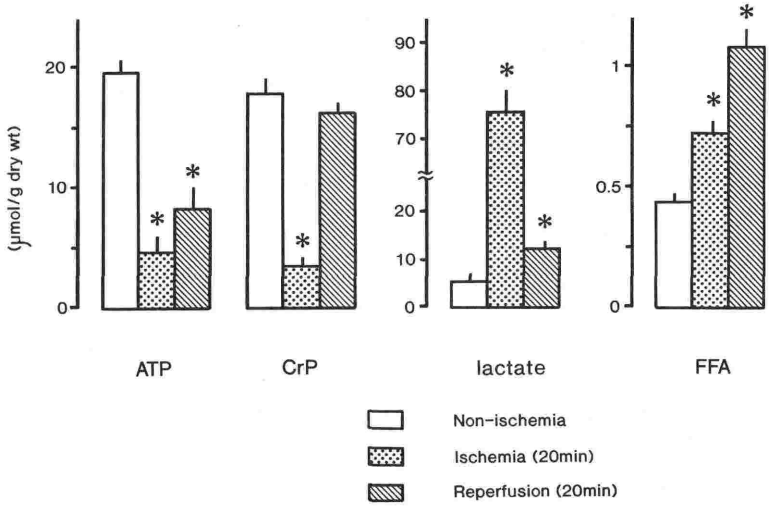

Fig. 1 Changes in the levels of tissue ATP, creatine phosphate (CrP), lactate and free fatty acid (FFA) in the non-ischemic, ischemic and reperfused hearts. Values are mean ± SEM. *; $p < 0.05$ vs. non-ischemic group

3. Determination of myocardial FFA

It is quite sure that there is degradation of membrane PL during ischemia and/or reperfusion[8,11,57]. However, it is difficult to detect loss of tissue PL and elevation of lysophospholipids (lysoPL), one of the degradation product of PL, even in the post-ischemic reperfused heart[8,57], because the level of tissue PL is extremely high and that of lysoPL is very low. On the other hand, changes in the tissue level of FFA in the ischemic-reperfused myocardium can be detected, and therefore the changes of FFA level could be used as a sensitive indicator of integrity of membrane PL and hence cellular damage.

The levels of tissue FFA were determined by a simple method using a reverse-phase high-performance liquid chromatography (HPLC) connected with a fluorescence detector[38,43]. Immediately after the end of perfusion experiments, hearts were freeze-clamped with aluminum Wollenberger clamps previously cooled with liquid nitrogen. The tissue FFA were extracted from the frozen and pulverized cardiac sample with chloroform/methanol and converted to their fluorescence-derivatives with 9-anthryldiazomethane (ADAM). After incubation at room temperature for 1 hour, the fatty acyl fluorescence-derivatives were separated by a reverse-phase HPLC system using an ODS column and methanol/water (1000:90) as a mobile phase. The level of individual FFA was determined by comparing the peak height with that of an internal standard (heptadecanoic acid). Tissue PL and triacylglycerol (TG) were also detected after saponification with KOH/ethanol. Each of the constituent fatty acids in PL and TG was converted to its fluorescence-derivative with the ADAM reagent.

4. FFA in aerobic heart and ischemic heart

Because both exogenous and endogenous FFA serve as an essential substrate for energy production in the myocardial cell, a decrease in myocardial oxygen supply increases the tissue levels of FFA; fatty acid β-oxidation in the mitochondria ceases during ischemia, leading to an increase in the tissue levels of FFA intermediates (such as fatty acyl-CoA and fatty acyl-carnitine ester) that induce further myocardial damage[11,32]. Studies with isolated, perfused rat hearts have demonstrated that the tissue levels of saturated FFA (such as lauric, myristic and palmitic acids) decrease after 60 min of aerobically perfused working heart, but the decrease is not detected after 60 min of aerobically perfused Langendorff heart (Fig. 2), suggesting that some of the myocardial saturated FFA serve as an endogenous substrate for the production of ATP in the perfused working heart[42]. Cardiac work of the working heart is greater than that of the Langendorff heart, and therefore the working heart requires more oxygen and substrates to keep the heart work normal. This is an explanation for the difference in the saturated FFA levels between working and Langendorff hearts that are aerobically perfused[42].

In the ischemic heart, however, the tissue levels of unsaturated FFA (such as arachidonic and linoleic acids) increased significantly (Fig. 2), suggesting that the ischemia-induced accumulation of the tissue FFA is not due to inhibition of mitochondrial β-oxidation but probably due to degradation of membrane PL, because arachidonic acid is one of the major constituent fatty acids of membrane PL[19,27,41,43]. Consequently, inhibition of mitochondrial β-oxidation may not contribute directly to accumulation of FFA during ischemia.

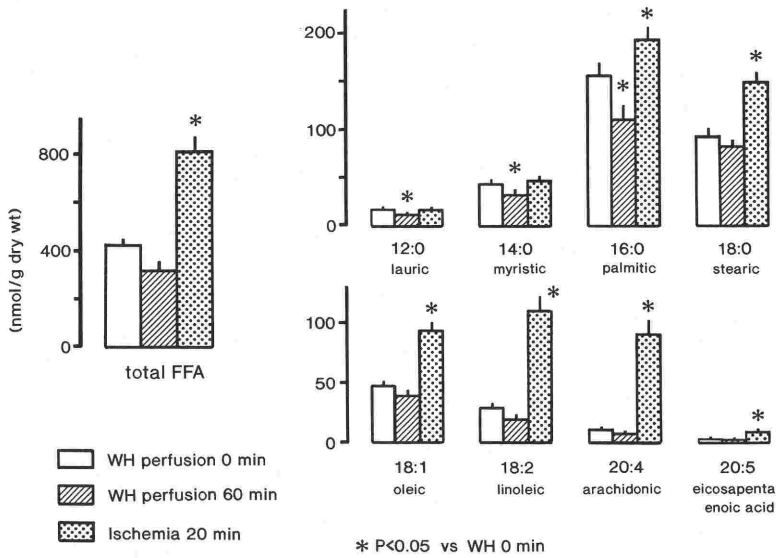

Fig. 2 Changes in the levels of tissue total FFA and individual FFA before working heart perfusion, after aerobic 60 min of working heart perfusion and after 20 min of ischemia. Values are mean ± SEM. WH are working heart. *; $p<0.05$ vs. before WH perfusion (WH perfusion 0 min)

5. PL and TG in ischemic heart

There is ample evidence showing that the tissue FFA such as arachidonic acid accumulate during ischemia and reperfusion[4,8,22,43,50,57], but the source of the FFA that have been released during ischemia is not clear. If the tissue TG and/or PL are sources of the FFA[58], the content of the constituent FFA of TG and/or PL must decrease in response to ischemia and reperfusion. It is difficult, however, to determine the source of the FFA accumulated during ischemia and reperfusion, because the tissue levels of TG and PL are very high compared with those of FFA. It must be noted that arachidonate in TG increases significantly during ischemia (our personal observation). These findings suggest that myocardial TG plays an important role in the regulation of lipid metabolism especially in the ischemic heart probably as a reservoir or an acceptor of the FFA[27,35]. Since the total level of TG also increased during ischemia, TG may not be a source of the FFA accumulated during ischemia[58,60].

This view is supported by similar results obtained by Burton and his co-workers[4]; TG content increases during ischemia-reperfusion in rat hearts. The fact that glycerol-3-phosphate, an essential substrate for TG biosynthesis, increases during ischemia in concert with the accumulation of FFA[57], and another fact that exogenous palmitate is incorporated into myocardial TG to a greater extent in the ischemic reperfused heart than in the nonischemic heart[37], also support the foregoing view though indirectly.

The levels of tissue total PL and their major constituent fatty acids such as arachidonate do not change greatly during ischemia, but the levels of myocardial phosphatidylcholine and phosphatidylethanolamine decrease during a prolonged period of ischemia and reperfusion in in vitro[48], in situ[30,46,54] and in vivo[8,66] preparations. These results suggest that the ischemia-induced accumulation of tissue FFA is probably due to degradation of the tissue PL rather than the tissue TG. It has been reported that the FFA themselves have an inhibitory effect on the cardiac function[36] to pro-

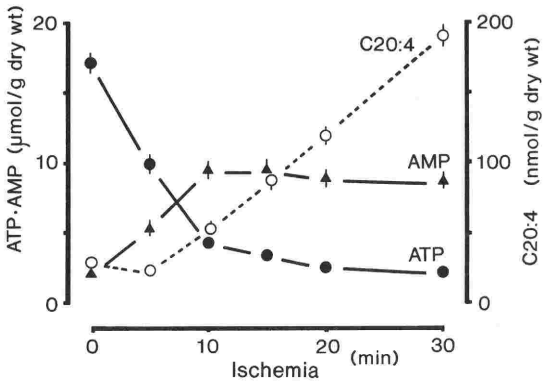

Fig. 3 Time-course change in the tissue levels of ATP, AMP and C20:4 (arachidonic acid) during ischemia. Values are mean ± SEM. This figure shows that accumulation of tissue arachidonic acid begins in the ischemic myocardium when tissue ATP level becomes lower than AMP level.

duce a deleterious effect on the myocardial cells[11] including inhibition of the Na^+-K^+ ATPase[10,31].

6. Relationship between high energy phosphates and FFA in ischemic heart

Time-course changes in the tissue levels of ATP, adenosine monophosphate (AMP), and arachidonic acid are shown in fig. 3. ATP decreased and AMP increased markedly during the first 10 min after ischemia, and both ATP and AMP levels decreased slightly and gradually during 15 to 30 min after ischemia. The level of arachidonic acid tended to decrease 5 min after ischemia followed by an almost lineal increase. As shown in fig. 3, the tissue FFA began to increase after the level of ATP became lower than that of AMP. Recent studies have revealed that there is an exponential inverse correlation between the tissue level of arachidonic acid and that of ATP in the ischemic heart[22] and in the cultured myocytes treated with metabolic inhibitors[9,21,28].

According to by Gunn et al.,[21] depletion of ATP is an essential factor in the accumulation of tissue arachidonic acid during ischemia, suggesting that inhibition of the ATP-requiring reacylation process contributes to the accumulation of tissue FFA during ischemia[8]. Furthermore, if the energy-sparing drug such as diltiazem is applied to the heart before ischemia, the tissue level of ATP would be preserved at the level higher than that of AMP even in the ischemic heart[43]. Therefore, it is likely that the ischemia-induced accumulation of FFA is closely related to both decrease in tissue ATP and the increase in AMP.

7. PL as a source of FFA accumulated during ischemia

PL metabolism changes during ischemia and reperfusion. It is logical to assume that an increase in deacylation and/or a decrease in reacylation accounts for the increase in FFA during ischemia and reperfusion. According to Burton et al.,[4] accumulation of tissue arachidonic acid during ischemia is associated with depletion of membrane PL, which might be resulted from inhibition of reacylation of FFA into PL. It has also been demonstrated that activity of the deacylation enzymes, such as the phospholipase A_2, is enhanced and that of reacylation enzymes, such as the acyl-CoA synthetase and the lysophospholipid acyltransferase, is depressed in the ischemic and reperfused myocardium[12], suggesting that the deacylation-reacylation cycle in PL biosynthesis is impaired during ischemia and reperfusion[8,12,48]. Both increased deacylation and decreased reacylation contribute to degradation of the membrane PL, leading to accumulation of FFA in the myocardium[8,12]. Hazen and his co-workers have reported[24] that there is an increased activity of the phospholipase A_2 in microsomal fraction of myocardium, which is the most plausible enzyme that contributes to accumulation of the tissue FFA (such as arachidonic acid) during ischemia[5,12].

7-1 ATP:AMP ratio and acyl-CoA synthetase

The acyl-CoA synthetase, one of the reacylation enzymes that exists predominantly in outer membrane of mitochondria, requires ATP as a co-factor in the process of synthesis of acyl-CoA and is inhibited by the end-products such as AMP and fatty acid-CoA esters[64]. The Km value of the acyl-CoA synthetase for ATP and the Ki value of the acyl-CoA synthetase for AMP are 0.8 mM and 0.2 mM (in vitro assay), respectively[64]. The calculated concentrations of the minimal levels of ATP and AMP in the myocardium in the present study are 3.3 mM and 0.7 mM, respectively, when calculated by an assumption[57] that intracellular volume is 60 % of the total tissue volume and a dry-to-wet weight ratio is 0.2. Even under ischemic conditions, the lowest calculated concentration of the tissue ATP in the heart is higher than the Km value of the acyl-CoA synthetase for ATP, and the Ki value for AMP is lower than the lowest calculated concentration of the tissue AMP in the non-ischemic heart. Therefore, even when there is inhibition of the acyl-CoA synthetase during ischemia, the inhibition cannot be explained merely by the tissue levels of ATP and AMP only. It is possible, however, to assume that the tissue levels of both ATP and AMP (i.e. ATP:AMP ratio) regulate intracellular activity of the acyl-CoA synthetase in the ischemic heart. This is because the tissue levels of FFA did not increase in the early phase of the ischemic heart or in the diltiazem-treated heart, in which the ATP:AMP ratio was preserved at the value greater than 1 (Fig. 2). These results suggest that there is a mechanism of FFA accumulation, which is regulated by the ATP:AMP ratio (Fig. 5).

Adenosine is another endogenous inhibitor of the acyl-CoA synthetase[64], and the level of adenosine increases during ischemia[57] as does the level of AMP. Adenosine that has increased in the ischemic myocardial cell penetrates through sarcolemma into the extracellular space and be washed out even during ischemia[2]. Therefore, contribution of adenosine to the metabolic inhibition of the acyl-CoA synthetase during ischemia seems to be unlikely.

According to Das et al.,[12] the enzymatic activity of the acyl-CoA synthetase itself decreases during ischemia. These considerations lead to a view that the accumulation of tissue FFA during ischemia is probably due to inhibition of the acyl-CoA synthetase, which is produced by metabolic inhibition by the endogenous substrates (i.e., an increase in AMP and a decrease in ATP) and by reduction of the enzymatic activity of the acyl-CoA synthetase itself caused by ischemia[12].

7-2 lysophospholipid acyltransferase

In the deacylation-reacylation cycle in the PL metabolic cycle, two kinds of enzymes contribute to the reacylation process; one is the acyl-CoA synthetase and the other is the lysophospholipid acyltransferase, the latter being sensitive to alterations in the redox ratio of reduced glutathione (GSH): oxidized glutathione (GSSG)[3]. The lysophospholipid acyltransferase is inhibited when the GSH:GSSG ratio decreases. Ischemia lowers the total glutathione content[14,55] and reperfusion reduces the GSH:GSSG ratio resulting in a decrease in the mitochondrial PL contents[30]. Therefore, it is possible that the activity of the lysophospholipid acyltransferase decreases during ischemia and reperfusion[12], and that the depression of this enzyme activity is partially responsible for accumulation of the myocardial FFA during both ischemia and reperfusion.

8. Accumulation of tissue FFA during reperfusion

Biochemical changes in the myocardium during reperfusion are very different from those during ischemia. One of these differences is the relation between the ATP:AMP ratio and

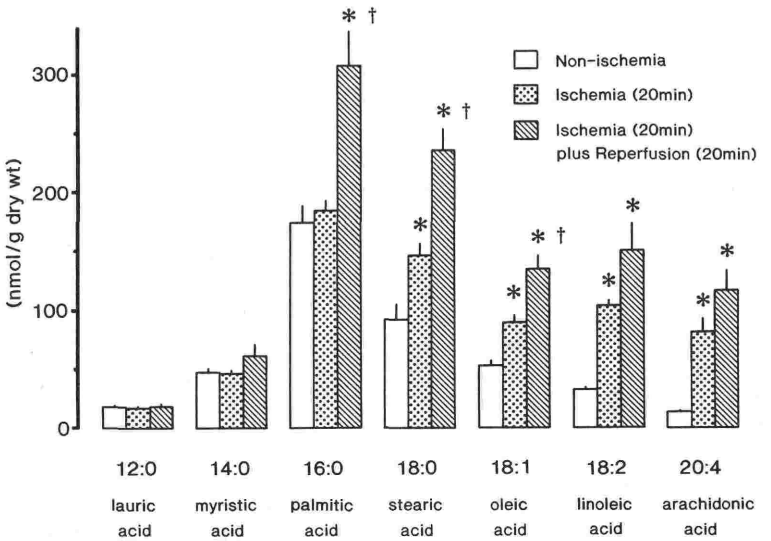

Fig. 4 The myocardial levels of FFA in the non-ischemic, ischemic and reperfused hearts. Values are mean ± SEM. Hearts were subjected to 20 min of no-afterload ischemia (Ischemia) followed by 20 min of reperfusion (Ischemia plus reperfusion). *; $p<0.05$ vs. non-ischemic hearts. †; $p<0.05$ vs. ischemic group.

accumulation of FFA; during ischemia the tissue FFA begin to increase when the ATP:AMP ratio becomes the value lower than 1, whereas during reperfusion they still continue to increase even when the ATP:AMP ratio returns to the value greater than 1. Similar findings are obtained even when hearts are treated with diltiazem; the ischemia-induced accumulation of FFA is prevented by the pre-ischemic treatment with diltiazem at the concentration that attenuates the loss of ATP and the rise of AMP in the ischemic hearts, whereas the reperfusion-induced accumulation of FFA is not always prevented by diltiazem[43]. It should also be noted that the FFA that had accumulated during ischemia were not exactly the same as those accumulated during reperfusion; the levels of arachidonic, linoleic and oleic acids increased during ischemia whereas those of palmitic, stearic and oleic acids increased during reperfusion (Fig. 4). These results indicate that the mechanism by which the FFA accumulate in the myocardium during ischemia and during reperfusion must be different. Therefore, it is possible to assume that there is another mechanism by which FFA accumulate during reperfusion; enhanced activity of the deacylation enzymes such as phospholipase A_2 and/or C may be involved (Fig. 5). Experimental findings have shown that activities of the phospholipase A_2 and the phospholipase C increase during reperfusion of the ischemic myocardium[12,47,49], resulting in an increased degradation of membrane PL during reperfusion[8,12,48]. Consequently, the underlying mechanism of accumulation of the tissue FFA during ischemia and that during reperfusion is not the same.

The myocardial tissue pH decreases rapidly in response to ischemia and returns to the initial value immediately after the onset of reperfusion[1]. Because an optimum pH range for the myocardial phospholipase A_2 is neutral to alkaline[6,59], the ischemia-induced tissue acidosis is considered one of the myocardial defensive responses that protect the cellular membrane against attack of the phospholipase A_2 during ischemia. In addition, enzymatic activity of the phospholipase A_2 and C does not alter or decreases during ischemia or

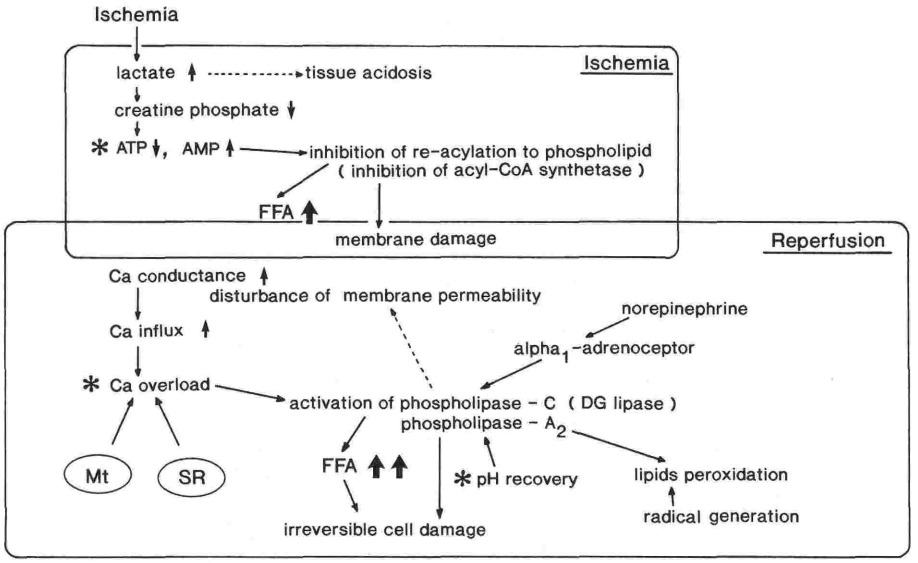

Fig. 5 Possible mechanism of the accumulation of FFA during ischemia and reperfusion and the relationship between tissue FFA and myocardial cell damage. *; possible metabolic modulators responsible for accumulation of tissue FFA. Mt; mitochondria, SR; sarcoplasmic reticulum

hypoxia[5,12,52,53], except for the plasmalogen-specific phospholipase A_2 as demonstrated by Hazen and Gross[24,25]. These considerations lead to a view that the mechanism by which the tissue FFA accumulate during ischemia cannot be explained by the increased activity of the phospholipases alone.

During reperfusion, however, the tissue pH increases toward the optimum pH range of the phospholipase A_2, leading to activation of the enzyme to produce further accumulation of the tissue FFA. Moreover, because the phospholipase A_2 activity increases with an increase in intracellular calcium concentration[17,34,56], and reperfusion increases the intracellular level of calcium (i.e. calcium overload), the phospholipase A_2 activity would increase during reperfusion[12,53] (Fig. 5).

Recently, Davies and his co-workers[13] have demonstrated a significant accumulation of lysoplasmenylethanolamine during reperfusion, suggesting that plasmenylethanolamine may serve as an important repository of arachidonic acid that is to be released upon reperfusion.

9. Contribution of phospholipases to breakdown of PL and accumulation of FFA

9-1 Phospholipase A_2

Circumstantial evidence indicates that myocardial phospholipase A_2 catalyses breakdown of membrane PLs and contributes to post-ischemic reperfusion injury[12,49]. Das and his co-workers[12] has demonstrated that activity of the phospholipase A_2 increases in the post-ischemic reperfused heart, but not in the ischemic heart. Similar results are obtained by Schwertz and Halverson[53], who have shown that there is an increase in the phospholipase A_2 and C activities in the post-ischemic reperfused heart. It is also interesting that the phospholipase A_2 itself is stimulated during reperfusion but not during ischemia[12]. Kawaguchi and Yasuda[33] have reported that there is an increase in the activity of the phospholipase A_2 in the hypoxic myocardium, which is found only when the tissue is incubated under the physiologic pH conditions for more than 24 hours[33]. Under these conditions,

the tissue may be in a state of autolysis because of a long period of hypoxic incubation and most of the lypolytic enzymes must be activated by disruption of lysosomes. Therefore, it is uncertain whether the increase in the phospholipase A_2 activity is specific for hypoxia.

Recent findings indicate that selective accumulation of lysoplasmenylethanolamine, but not lysoplasmenylcholine, occurs in the perfused rabbit heart during reperfusion when exogenous fatty acid concentrations are high, probably due to an activation of the membrane-associated, plasmalogen-selective phospholipase A_2[13]. Alternatively the lysoplasmenylethanolamine accumulation may be due to an increase in fatty acid intermediates that may inhibit lysoplasmenylethanolamine catabolism[13]. In this respect, the experimental findings of Hazen and his co-workers[24] are worth to mention; they found a plasmalogen (1-alkenyl-2-acyl PL)—specific, calcium-independent phospholipase A_2 activity in the membrane-associated fraction of the myocardium that is rapidly increased by ischemia[24].

Furthermore, the calcium-independent, plasmalogen-selective phospholipase A_2 has been found in the human myocardium, and importance of this phospholipase in mediating damage of membrane function during myocardial infarction has been suggested[25].

Although the plasmalogen-specific phospholipase A_2 is responsible for accumulation of arachidonic acid derived from plasmalogen during ischemia, it is possible to assume that the released arachidonic acid may be incorporated to TG and/or PL again if the tissue level of ATP is still higher than that of AMP. Furthermore, because accumulation of FFA can not be detected during ischemia when the ATP:AMP ratio is greater than 1[22] (Fig. 3), the underlying mechanism of accumulation of FFA during ischemia may not be explained merely by the activation of the plasmalogen-specific phospholipase A_2. Of course, conversion of arachidonic acid, at least in part, to its active metabolites such as prostaglandin analogous[15] may be one of reasons why an elevation of arachidonic acid was not detected during an early period of ischemia. We think that the activation of this type of phospholipase A_2 plays an important role for "release of arachidonic acid" from membrane-associated plasmalogen in response to ischemia and that inhibition of the acyl-CoA synthetase contributes to accumulation of arachidonic acid in the ischemic myocardium. Recent findings show that decreased reacylation rather than increased deacylation accounts for ischemia-induced PL degradation[12,53]. Reduction of the myocardial total phospholipase A_2 activity in the ischemic heart may attenuate accumulation of the membrane perturbing metabolites and prevent cleavage of myocardial PL[53]. According to Schwertz and Halverson[53], the phospholipase activity increases during reperfusion only when heart was subjected to ischemia lasting long enough to produce calcium overload or resulting in irreversible injury. Furthermore, they suggested that if the ischemic damage does not result in irreversible myocardial injury, the phospholipase activity appears to recover to the pre-ischemic level[53].

9-2 Phospholipase C activity

Phosphatidylinositol-specific phospholipase C activity has been shown to decrease during ischemia[52], and therefore contribution of phospholipase C to accumulation of arachidonic acid in the ischemic myocardium seems to be unlikely. However, in the ischemic-reperfused heart, Prasad and his co-workers[49] have demonstrated that the phospholipase C specific antibody has the cardioprotective effect against ischemic damage, suggesting possible contribution of phospholipase C to ischemic-reperfused cell injury. Diacylglycerol (DAG) released from phosphatidylinositol by the action of the phospholipase C also increases during reperfusion of the ischemic myocardium[47]. The fact that α_1-adrenoceptor-mediated

phosphatidylinositol turnover cycle is stimulated in the reperfused myocardium, also supports the foregoing proposal that the activity of the phospholipase C increases during reperfusion but not during ischemia[49].

Since the FFA that had accumulated markedly during reperfusion were palmitic, stearic and oleic acids being bound preferentially to the SN-1 position of glycerophospholipids (Fig. 4), the increased activity of the phospholipase A_2 may not directly relate to further accumulation of the tissue FFA during reperfusion. Recent findings indicate that reperfusion enhances the phosphatidylinositol-specific phospholipase C activity[47], and therefore, an increase in the activity of the phospholipase C followed by the diacylglycerolipase could be responsible for PL cleavage that produces the FFA during reperfusion. According to Grynberg et al.,[20] the cultured rat ventricular myocytes exhibit an alkaline phospholipase A activity, which has SN-2 specificity and calcium dependency, and also has an acid phospholipase A activity that is SN-1 specific, indicating the presence of the phospholipase A_1 activity in the myocardium. Nevertheless contribution of the phospholipase A_1 to the release of FFA during reperfusion is unlikely, because about 40 % of the PL in the sarcolemmal membrane, which is considered to serve as a source of the FFA released during ischemia and reperfusion[7,23], are plasmalogens[19], that do not release FFA from their SN-1 position. It has been suggested that plasmalogen-selective neutral-active phospholipase C contributes to the ischemia-induced membrane disruption, since plasmalogenic DAG accumulates in ischemic isolated rabbit hearts[67]. Higgins et al.[26] has suggested that the cardiac cell membrane becomes more susceptible to attack of the phospholipase C, when the intracellular ATP falls. These considerations lead to a view that a possible contribution of the activation of the phospholipase C, at least in part, to membrane PL disruption during both ischemia and reperfusion.

10. lipid peroxidation and reperfusion injury

Oxygen free radicals increase during reperfusion in the isolated perfused heart[68] and attack unsaturated fatty acids such as arachidonic acid at double bounds in PL[51], leading to oxidative degradation. In order to cleave the peroxidized acyl-residues of the membrane phospholipids, the phospholipase A_2 should be activated[63] (Fig. 5). Therefore, it is uncertain whether activation of the phospholipase A_2 during reperfusion is favorable or unfavorable for preventing ischemic-reperfused membrane disruption. It has been hypothesized that the change in molecular shape produced by the vinyl-ether bound at the SN-1 position might increase accessibility of the unsaturated SN-2 fatty acids of plasmalogen molecular species to free radical attack[39]. Functional importance of plasmalogens in the myocardial cell has been shown; plasmalogens are abundant in sarcolemmal PL[19], asymmetrically distribute in the sarcolemma[19], contain arachidonic acid at the SN-2 position[16], have an influence on membrane fluidity and stability, and modulate membrane enzymes and ion channel function[29].

It has been shown that the phospholipase C also contributes to the cleavage of peroxidized membrane phospholipids[18]. Consequently, both phospholipase A_2 and phospholipase C play an important role in cleavage of the peroxidized unsaturated FFA in membrane PL including plasmenylethanolamine.

11. Cardioprotective drugs and myocardial FFA

As described previously, the level of tissue arachidonic acid increases in response to ischemia and increases further during post-ischemic reperfusion. Since arachidonic acid is one of the major components of membrane PL, its ac-

cumulation in the myocardium suggests degradation of myocyte membranes, and therefore, it would be a parameter that reflects the degree of myocardial tissue damage. Since tissue FFA increase during both ischemic and reperfused periods, if a drug prevents the FFA accumulation during either ischemia or reperfusion, or both, the drug would exert its effect 1) during ischemia, 2) during reperfusion, 3) during both ischemia and reperfusion.

For example, a cardioprotective drug having an energy-sparing effect in ischemic heart such as diltiazem attenuates ischemia-induced FFA accumulation, because diltiazem preserves the ATP:AMP ratio in the ischemic heart[43] (class 1). However, diltiazem does not prevent the reperfusion-induced FFA accumulation completely[43]. In fact, the calcium channel blockers were effective when they were applied to the heart before ischemia, but not after ischemia[43]. Amiloride, a specific Na^+/H^+ exchange inhibitor, prevents only the post-ischemic reperfusion-induced FFA accumulation, if it is applied to the heart during both ischemic and reperfused periods (our personal observation) (class 2). Pindolol, a β-adrenoceptor antagonist with intrinsic sympathomimetic activity, prevents both ischemia and reperfusion-induced FFA accumulation when the drug is applied to the heart during preischemic and post-ischemic periods[44] (class 3).

Our previous results have suggested that if the increased level of the tissue FFA is below a critical level during ischemia, the heart can be protected from the post-ischemic reperfused damage, even when the level of ATP decreases, suggesting that attenuation of the ischemia-induced accumulation of FFA is a sensitive index of the cardioprotective effect of the drug[43].

13. Conclusion

In conclusion, the ischemia-and reperfusion-induced derangement of PL metabolism may be one of the critical biochemical alterations leading to accumulation of the tissue FFA. The biochemical alterations include both inhibition of the reacylation process and enhancement of the deacylation process in PL biosynthesis. In addition, the main mechanism by which the FFA accumulate during ischemia may be inhibition of the reacylation process, and the main mechanism by which the FFA accumulate during reperfusion may be enhancement of the deacylation process. Furthermore, the drug-induced inhibition of the accumulation of FFA during ischemia and reperfusion is associated with its cardioprotective effect, suggesting that an increase in the tissue FFA serve as a sensitive indicator that reflects both ischemia-and reperfusion-induced cellular damage.

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