The Rapid and Reversible Activation of a Calcium-independent Plasmalogen-selective Phospholipase A_2 during Myocardial Ischemia

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Abstract

Recent studies have demonstrated the existence of two members of a novel family of calcium-independent plasmalogen-selective phospholipases A_2 in mammalian myocardium (Wolf, R. A., and R. W. Gross. 1985. *J. Biol. Chem.* 260:7295–7303; and Hazen, S. L., D. A. Ford, and R. W. Gross. 1991. *J. Biol. Chem.* 266:5629–5633). To examine the potential role of these calcium-independent phospholipases A_2 in mediating membrane dysfunction during early myocardial ischemia, the temporal course of alterations in phospholipase A_2 activity during global ischemia in Langendorf perfused rabbit hearts was quantified and compared with traditionally accepted markers of myocytic ischemic injury and anaerobic metabolism. We now report that membrane-associated calcium-independent plasmalogen-selective phospholipase A_2 activity increased over 400% during 2 min of global ischemia ($P < 0.01$), was near maximally activated (> 10-fold) after only 5 min of ischemia, and remained activated throughout the entire ischemic interval examined (2–60 min). Activation of membrane-associated plasmalogen-selective phospholipase A_2 after 5 min of myocardial ischemia was rapidly reversible during reperfusion of ischemic tissue. Both the activation of phospholipase A_2 and its reversibility during reperfusion were temporally correlated to alterations in myocytic anaerobic metabolism. Furthermore, activation of membrane-associated phospholipase A_2 was essentially complete before electron microscopic evidence of cellular damage. Collectively, these results identify dynamic alterations in calcium-independent plasmalogen-selective phospholipase A_2 activity during myocardial ischemia which precede irreversible cellular injury and demonstrate that activation of plasmalogen-selective phospholipase A_2 is amongst the earliest biochemical alterations in ischemic myocardium. (*J. Clin. Invest.* 1991. 88:331–335.) Key words: ischemic injury • phospholipid catabolism • plasmenylcholine • myocardium • Langendorf perfused hearts

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Introduction

Accelerated phospholipid catabolism has been implicated as an important biochemical mechanism underlying electrophysiologic alterations and membrane dysfunction in ischemic myocardium (reviewed in 1–4). Although considerable attention has focused on identification of the phospholipase(s) responsible for accelerated phospholipid hydrolysis in ischemic zones, attempts to document ischemia-induced activation of myocardial phospholipase A_2 in a time frame comparable to the onset of ventricular dysrhythmias (i.e., within 2–5 min after the onset of acute ischemia) have been unsuccessful using conventional techniques (5, 6). Because sarcolemma is the electrophysiologically-active membrane in myocardium and because sarcolemmal phospholipids are predominantly comprised of plasmalogen molecular species (7), the recent demonstration that sarcolemmal phospholipids were the highly selective targets of the phospholipases activated during reversible metabolic deprivation (8) has underscored the potential importance of plasmalogen hydrolysis during ischemic injury.

Prior studies have demonstrated that the majority of phospholipase A_2 activity in mammalian myocardium is catalyzed by a novel family of calcium-independent plasmalogen-selective phospholipases A_2 and that membrane-associated calcium-independent phospholipase A_2 is activated during 15 min of global ischemia in Langendorf perfused rabbit hearts (9–11). To elucidate the potential pathophysiologic significance of this novel activity, we now demonstrate that membrane-associated calcium-independent plasmalogen-selective phospholipase A_2 activity increased over 400% after only 2 min of global ischemia, that activation of this novel phospholipase A_2 was rapidly reversible during reperfusion of ischemic myocardium, that augmentation of calcium-independent phospholipase A_2 activity occurred before the development of ischemia-induced ultrastructural damage, and that alterations in phospholipase A_2 activity during ischemia and reperfusion were temporally correlated to alterations in myocytic anaerobic metabolism.

Methods

Langendorf perfusion of rabbit myocardium. New Zealand White rabbits were killed by cervical dislocation and their hearts were rapidly removed and perfused at 60 mmHg with modified Krebs-Henseleit buffer utilizing a Langendorf perfused heart model as previously described (12). Hearts were perfused for a 10-min preequilibration interval and were subsequently rendered either globally ischemic (zero-flow) or continuously perfused for the indicated times. For reperfusion studies, hearts were rendered globally ischemic for 5 min (after the 10-min preequilibration interval) and subsequently reperfused for the indicated times. Perfusions were terminated by rapid excision of ventricular tissue and immediate submersion into 0°C homogenization buffer (10 mM imidazole, 10 mM KCl, 0.25 M sucrose [grade 1] [pH 7.8]).

Phospholipase A_2 assays. Ventricular tissue from perfused and ischemic myocardium was rapidly homogenized and separated into subcellular fractions by sequential centrifugations as previously described (11). Phospholipase A_2 activity in myocardial subcellular fractions was assessed by incubating enzyme (typically 8 μg of microsomal protein or 300 μg of cytosolic protein) with 100 μM *sn*-2 radiolabeled choline glycerophospholipid (introduced by ethanolic injection [10 μl]) in assay buffer (final conditions: 100 mM Tris Cl and either 4 mM EGTA or 10 mM $CaCl_2$, pH 7.0) at 37°C for 60 s in a final volume of 210 μl. Released radiolabeled fatty acids, the only radiolabeled products detected, were quantified by established procedures (10). Under these conditions exogenous radiolabeled phospholipid substrate was present in 10-fold molar excess compared with endogenous microsomal and cytosolic phospholipid. Phospholipase A_2 assays were linear with respect to both time and protein under the conditions employed.

Electron microscopic analyses of normal and ischemic myocardium. Electron microscopic analyses of control and ischemic myocardium were performed as previously described (13). Briefly, perfusions were terminated by immersion in and subsequent perfusion of intact hearts with ice-cold modified Karnovsky's fixative. To facilitate comparisons and to sample areas likely to exhibit the greatest damage, tissue from the anterolateral papillary muscle of each heart was processed for electron microscopy and analyzed as previously described (13).

Miscellaneous procedures and sources of materials. Tissue lactate was determined spectrophotometrically from neutralized perchlorate extracts of pulverized freeze-clamped ventricular tissue utilizing yeast lactate dehydrogenase (14). Protein determinations were performed using a Bio-Rad Laboratories (Richmond, CA) protein assay kit. Synthetic homogeneous molecular species of *sn*-2 radiolabeled plasmenylcholine and phosphatidylcholine were prepared as previously described (10). Statistical differences were assessed by analyses of variance using a modified *t* statistic and overall F-test. All radiolabeled starting materials were purchased from DuPont-New England Nuclear (Boston, MA). All polar lipids were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL) and oleic and arachidonic acids were obtained from Nu-Chek Prep, Inc. (Elysian, MN). Yeast lactate dehydrogenase was purchased from Boehringer Mannheim, Inc. (Houston, TX). Most other reagents were obtained from Sigma Chemical Co., St. Louis, MO.

Results

Activation of myocardial microsomal calcium-independent plasmalogen-selective phospholipase A_2 during global ischemia. Myocardial ischemia resulted in the rapid, time-dependent activation of microsomal phospholipase A_2 activity which reached half-maximal levels as early as 2 min and reached near maximal levels by 5 min of ischemia (10-fold increases in specific activity) when assessed with plasmenylcholine substrate containing either arachidonic or oleic acid at the *sn*-2 position (Fig. 1). Phospholipase A_2 activity in microsomes prepared from ischemic myocardium was entirely calcium-independent because maximal enzymic activity was manifest in the presence of EGTA (Fig. 1). In stark contrast to results using plasmenylcholine substrate, incubation of microsomes from control or ischemic myocardium with conventionally employed phosphatidylcholine substrates containing either arachidonic or oleic acid at the *sn*-2 position (in the presence or absence of calcium) failed to demonstrate substantial activation of phospholipase A_2 activity in ischemic myocardium (Fig. 1). No significant differences in phospholipase A_2 activity were observed in control hearts perfused for 0–60 min after the 10-min preequilibration interval using either plasmenylcholine or phosphatidylcholine substrates in the presence or absence of calcium ion (Fig. 1).

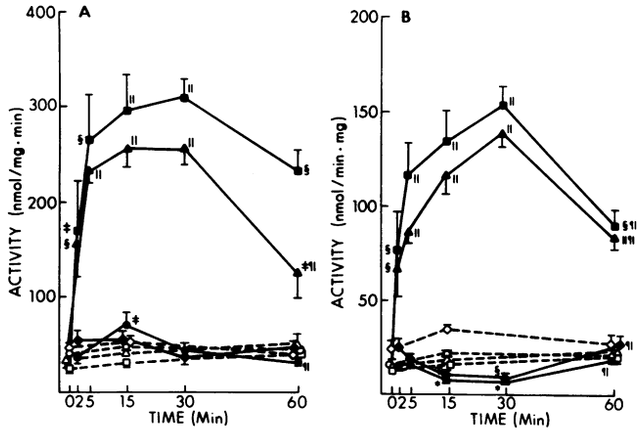

Figure 1. The temporal course of myocardial microsomal phospholipase A_2 activity during global ischemia. Rabbit myocardial microsomes were prepared from myocardium that was either continuously perfused (control) at 60 mmHg (- - -) or rendered globally ischemic (——) for the indicated intervals as described in Methods. Phospholipase A_2 activity in control or ischemic microsomes was subsequently assessed by incubating microsomal protein (8 μg) with 100 μM plasmenylcholine (■, ▲, □, △) or phosphatidylcholine (●, ♦, ○, ◇) substrate in the presence of either 10 mM $CaCl_2$ (▲, ♦, △, ◇) or 4 mM EGTA (■, ●, □, ○) (*A*) (16:0, [^{3}H]20:4 plasmenylcholine or phosphatidylcholine; (*B*) 16:0, [^{3}H]18:1 plasmenylcholine or phosphatidylcholine).[1] Released radiolabeled fatty acid (the only product detected) was subsequently isolated by TLC and quantified by scintillation spectrometry. Each value represents the mean±SEM of four independent determinations. $^*P < 0.025$, $^‡P < 0.01$, $^§P < 0.005$, and $^{\|}P < 0.0005$ for differences between control perfused myocardium at 0 min (i.e., after a 10 min equilibration interval) and globally ischemic myocardium. $^¶P < 0.005$ for differences between 30 min globally ischemic myocardium and 60 min globally ischemic myocardium.

Total calcium-independent plasmalogen-selective phospholipase A_2 activity in the microsomal compartment increased over 10-fold within 5 min after the onset of ischemia (from 37 to 387 nmol/g_{wet} · min), while no change in total cytosolic phospholipase A_2 activity occurred during this interval (Table I). During moderate to prolonged periods of ischemia (15–60 min), total phospholipase A_2 activity in the microsomal fraction remained significantly elevated above control values ($P < 0.01$), while total phospholipase A_2 activity in the cytosolic fraction manifests modest, progressive reductions (Table I).

Reversibility of ischemia-induced microsomal calcium-independent phospholipase A_2 activity and the temporal correlation of alterations in phospholipase A_2 activity with alterations in anaerobic metabolism. To examine the effects of reperfusion on ischemia-induced calcium-independent microsomal plasmalogen-selective phospholipase A_2 activity, rabbit myocar-

1. *Abbreviations used in this paper:* 16:0, [^{3}H]18:1 plasmenylcholine, 1-0-(Z)-hexadec-1′-enyl-2-[9,10-^{3}H]-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine; 16:0, [^{3}H]18:1 phosphatidylcholine, 1-hexadecanoyl-2-[9,10-^{3}H]-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine;16:0, [^{3}H]20:4 plasmenylcholine, 1-0-(Z)-hexadec-1′-enyl-2-[5,6,8,9,11,12,14,15-^{3}H]-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine; 16:0, [^{3}H]20:4 phosphatidylcholine, 1-hexadecanoyl-2-[5,6,8,9,11,12,14,15-^{3}H]-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine.

Table I. Total Phospholipase A_2 Activity in Subcellular Fractions from Ischemic and Control Perfused Rabbit Hearts

Experimental condition	Total phospholipase A_2 activity ($nmol/g_{wet} \cdot min$)			
	16:0, [^{3}H]20:4 Plasmenylcholine substrate		16:0, [^{3}H]18:1 Plasmenylcholine substrate	
	Cytosol	Microsomes	Cytosol	Microsomes
Zero-time control	114.4±7.8	37.0±7.9	44.9±3.3	18.2±4.0
Ischemia (2 min)	111.3±12.1	247.4±77.7‡	46.8±4.7	111.7±30.3§
Ischemia (5 min)	106.3±1.4	386.5±67.7§	42.2±0.6	168.9±25.1‖
Ischemia (15 min)	70.2±7.7§	408.9±50.2‖	32.8±3.6*	184.2±22.7‖
Ischemia (30 min)	65.5±4.5§	436.0±24.5‖	34.9±2.1*	215.6±12.2‖
Ischemia (60 min)	33.1±1.4‖	325.6±29.3§	16.9±0.8‖	122.2±11.0§
Control perfusion (15 min)	119.5±5.3	43.9±3.8	49.1±2.2	22.4±1.2
Control perfusion (60 min)	100.7±5.8	57.3±10.3	40.2±1.9	28.9±5.8

Rabbit hearts were initially perfused for a 10-min preequilibration interval (zero-time control) and were subsequently either perfused at 60 mmHg (control) or rendered ischemic for the indicated time intervals. Following subcellular fractionation, calcium-independent phospholipase A_2 activity in the cytosolic or microsomal fractions was subsequently quantified by incubation of either cytosolic (300 μg) or microsomal (8 μg) protein with the indicated molecular species of *sn*-2 radiolabeled plasmenylcholine substrate (100 μM) in the presence of 10 mM Tris Cl, 4 mM EGTA, pH 7.0, for 60 s at 37°C. Radiolabeled fatty acids (the only radiolabeled product detected) were subsequently isolated and quantified by TLC and scintillation spectrometry as described in Methods. Each value represents the mean±SEM for four independent determinations. * $P < 0.025$, ‡ $P < 0.01$, § $P < 0.005$, and ‖ $P < 0.0005$, respectively, for comparisons between zero-time controls and globally ischemic hearts at each experimental interval as described in Methods.

dium was rendered globally ischemic for 5 min and subsequently reperfused for either 10 or 55 min before quantitation of enzymic activity. After only 10 min of reperfusion, ischemia-induced microsomal phospholipase A_2 activity decreased by 60% ($P < 0.005$, compared with 5 min ischemic values) and completely returned to control values after 55 min of reperfusion (Fig. 2). Reperfusion had no significant effects on either the hydrolysis of phosphatidylcholine by microsomes, or cytosolic phospholipase A_2 activity utilizing plasmenylcholine or phosphatidylcholine substrates in the presence or absence of calcium (data not shown).

To compare the temporal course of microsomal phospholipase A_2 activation during ischemia with a traditionally used marker of anaerobic metabolism, tissue lactic acid content in control, ischemic, and reperfused myocardium was determined. The activation of phospholipase A_2 temporally paralleled tissue lactic acid accumulation in ischemic myocardium (Fig. 2). Furthermore, both membrane-associated phospholipase A_2 activity and tissue lactic acid content declined with similar time courses during reperfusion of ischemic tissue (Fig. 2).

Ischemia-induced activation of microsomal phospholipase A_2 activity precedes irreversible myocytic injury. To determine whether the activation of ischemia-induced microsomal phospholipase A_2 activity occurred before the development of irreversible ischemic injury, electron microscopic analyses of control, and ischemic myocardial tissue were performed. Examination of hearts rendered ischemic for 5 min revealed no evidence of myocytic injury and samples of these hearts were morphologically indistinguishable (at the electron microscopic level) from those of control perfused hearts (Fig. 3). Thus, activation of microsomal phospholipase A_2 precedes irreversible ischemic injury and did not result from processes that are activated during cellular necrosis. As anticipated, myocardium rendered globally ischemic for 60 min demonstrated profound alterations in myocytic ultrastructure indicative of irreversible ischemic injury, including disruption of mitochondrial cristae, formation of dense amorphous granules in the mitochondrial ma-

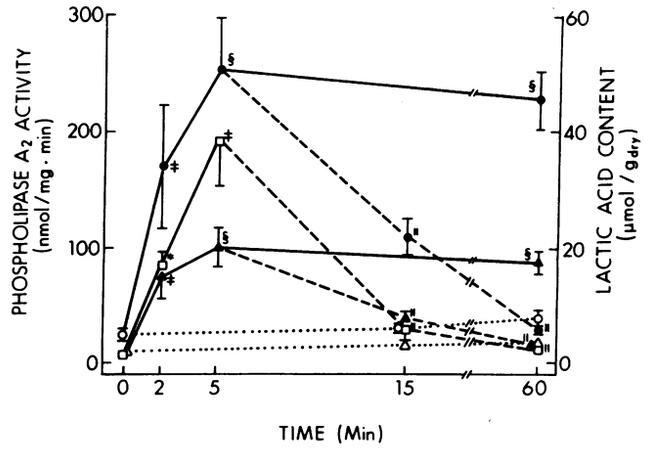

Figure 2. Reversibility of the activation of membrane-associated phospholipase A_2 activity by reperfusion and correlation of changes in phospholipase A_2 activity with alterations in anaerobic metabolism. Control, ischemic, and reperfused rabbit hearts were initially perfused for a 10-min equilibration interval before a 0–60-min experimental period. After the initial 10-min preequilibration interval, experimental hearts were: (*a*) rendered ischemic for 2, 5, or 60 min (——); (*b*) rendered ischemic for 5 min and subsequently reperfused for an additional 10 min (– – –) (i.e., 15 min data points); (*c*) rendered ischemic for 5 min and reperfused for an additional 55 min (---) (i.e., 60 min data points); or (*d*) continuously perfused at 60 mmHg for the indicated intervals (0, 15, and 60 min) (· · · · ·). Phospholipase A_2 activity was assessed by incubating microsomes (8 μg) from either control, ischemic, or reperfused rabbit hearts with 16:0, [^{3}H]18:1 plasmenylcholine (▲, △) or 16:0, [^{3}H]20:4 plasmenylcholine (●, ○) in the presence of 4 mM EGTA as described in Methods. Tissue lactic acid content (□) was quantitated from perchlorate-extracts of pulverized ventricular tissue spectrophotometrically utilizing yeast lactate dehydrogenase. Each value represents the mean±SEM for three independent lactate determinations and four independent phospholipase A_2 determinations. *$P < 0.025$, ‡$P < 0.005$, and §$P < 0.0005$ for comparisons between 0 min and myocardium rendered ischemic. ‖$P < 0.005$ for comparisons between myocardium rendered ischemic for 5 min and that reperfused for 10 or 55 min.

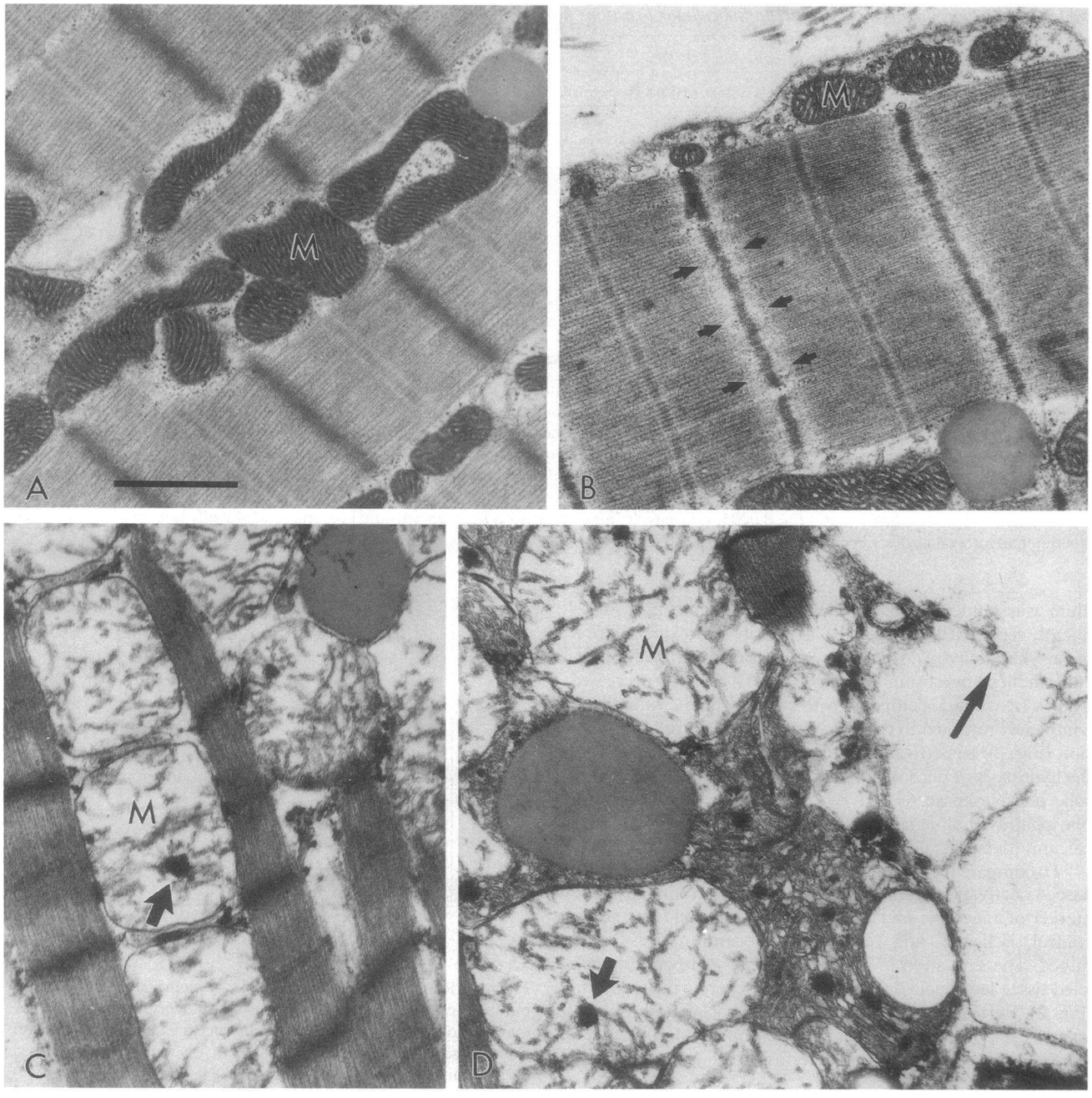

Figure 3. Representative electron micrographs of papillary muscle samples obtained from a control heart perfused with oxygenated buffer for 5 min (*A*) or hearts made ischemic for 5 min (*B*) or 60 min (*C* and *D*). In controls (*A*), mitochondria (*M*) exhibited compact cristae and electron dense matrix. After 5 min of ischemia (*B*), mitochondria were indistinguishable from controls and the sarcolemma was structurally intact. The only morphologic change was the appearance of I-bands (*arrowheads*), consistent with cessation of contractile activity. In contrast, 60 min of ischemic injury (*C* and *D*) produced ultrastructural features of irreversible injury including marked swelling of mitochondria, amorphous electron dense particles in mitochondria (*short arrows*), and focal disruption of the sarcolemma (*long arrow* in *D*). Original magnification in all panels, 26,000. Bar in *A* = 1 μm.

trix, myofibrillar edema, and sarcolemmal discontinuities (Fig. 3).

Discussion

These results demonstrate that: (*a*) a microsomal calcium-independent, plasmalogen-selective phospholipase A_2 is activated within 2 min of myocardial ischemia; (*b*) near-maximal activation of calcium-independent phospholipase A_2 occurs within 5 min of ischemia; (*c*) activation of phospholipase A_2 activity precedes the development of irreversible injury; (*d*) activation of microsomal phospholipase A_2 is reversible by reperfusion of ischemic myocardium; and (*e*) a temporal correlation exists between alterations in phospholipase A_2 activity and

alterations in anaerobic metabolism during ischemia and reperfusion.

Many of the deleterious changes in cardiac function after coronary occlusion occur within 2 min after the onset of myocardial ischemia (15). Thus, true enzymic mediators of these alterations must necessarily undergo substantial changes in their kinetic properties within this time frame. The identification of fourfold increases in microsomal phospholipase A_2 activity after only 2 min of ischemia demonstrates dynamic alterations in an enzymic mediator of phospholipid catabolism concurrent with the evolution of electrophysiologic dysfunction in ischemic zones. Although these results do not demonstrate a cause and effect relationship between these phenomena, they do demonstrate that the catalytic potential for profound alterations in the phospholipid constituents of critical subcellular membranes is present within minutes of myocardial ischemia.

Recently, the translocation of calcium-dependent phospholipases A_2 during cellular activation has received considerable attention (16, 17). Because activation of microsomal phospholipase A_2 activity during 2–5 min of ischemia occurs before, and independent of, measurable changes in cytosolic phospholipase A_2 activity, these results demonstrate that simple stoichiometric translocation of cytosolic phospholipase A_2 activity to the microsomal compartment cannot account for the observed augmentation in microsomal phospholipase A_2 activity. However, concomitant translocation and activation of a latent cytosolic phospholipase A_2 or translocation of a cytosolic regulatory element to the membrane compartment are compatible with the observed increase in membrane-associated phospholipase A_2 activity. Alternatively, these findings are also consistent with the reversible activation of a latent membrane-associated phospholipase A_2 (or phospholipase A_2 regulatory element), which could be mediated by reversible posttranslational modification (e.g., phosphorylation and dephosphorylation).

Both membrane-associated and cytosolic phospholipases A_2 possess distinctive kinetic characteristics (e.g., calcium independence and plasmalogen selectivity), which suggest that they are both members of a novel class of intracellular phospholipases A_2. However, their differential subcellular distribution (cytosolic vs. microsomal), pH optima (pH 6.5 vs. 8.5), and magnitude of plasmalogen specificity (4-fold vs. 16-fold) suggest that microsomal and cytosolic phospholipase A_2 activities are catalyzed by distinct members of this class of intracellular calcium-independent phospholipases A_2 (10, 11). The molecular differences underlying the differential subcellular localization and kinetic properties of these phospholipases A_2 are unknown, but could result from the transcription of different gene products, alternative splicing, posttranslational modification, or differential association with regulatory elements.

It has long been recognized that reversibility is an essential element of biologically significant regulatory processes. Although numerous studies have described myocardial phospholipase A_2 activities in varying degrees of detail (5, 6, 9–11, 18–20), the results of this study are the first to demonstrate an activity that undergoes dynamic alterations during ischemia and reperfusion. Because activation of membrane-associated phospholipase A_2 occurs in concert with activation of anaerobic metabolism, these results indicate that activation of phospholipase A_2 is one of the earliest biochemical manifestations of acute myocardial ischemia. Accelerated phospholipid catabolism has been implicated as a mediator of physiologic and pathophysiologic responses to cellular perturbation in a multiplicity of cell types. Accordingly, the role of these novel calcium-independent plasmalogen-selective phospholipases A_2 as enzymic mediators of cellular responses to acute ischemia in other tissues, or as enzymic initiators of cellular responses to physiologic perturbations in mammalian cells merits consideration.

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References

1. Katz, A. M., and F. C. Messineo. 1981. Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. *Circ. Res.* 48:1–16.

2. Corr, P. B., R. W. Gross, and B. E. Sobel. 1984. Amphipathic metabolites and membrane dysfunction in ischemic myocardium. *Circ. Res.* 55:135–154.

3. Hazen, S. L., and R. W. Gross. 1991. Principles of membrane biochemistry and their application to the pathophysiology of cardiovascular disease. *In* The Heart and Cardiovascular System. 2nd Edition. H. A. Fozzard, E. Haber, R. Jennings, A. M. Katz, and H. E. Morgan, editors. Raven Press, Ltd., New York. In press.

4. Chien, K. R., A. Han, A. Sen, L. M. Buja, and J. T. Willerson. 1984. Accumulation of unesterified arachidonic acid in ischemic canine myocardium. *Circ. Res.* 54:313–322.

5. Das, D. K., R. M. Engelman, J. A. Rousou, R. H. Breyer, H. Otani, and S. Lemeshow. 1986. Role of membrane phospholipids in myocardial injury induced by ischemia and reperfusion. *Am. J. Physiol.* 251:H71–H79.

6. Bentham, J. M., A. J. Higgins, and B. Woodward. 1987. The effects of ischemia, lysophosphatidylcholine and palmitoylcarnitine on rat heart phospholipase A_2 activity. *Basic Res. Cardiol.* 82:127–135.

7. Gross, R. W. 1984. High plasmalogen and arachidonic acid content of canine myocardial sarcolemma: a fast atom bombardment mass spectroscopic and gas chromatography-mass spectroscopic characterization. *Biochemistry.* 23:158–165.

8. Miyazaki, Y., R. W. Gross, B. E. Sobel, and J. E. Saffitz. 1990. Selective turnover of sarcolemmal phospholipids with lethal cardiac myocyte injury. *Am. J. Physiol.* 259:C325–C331.

9. Wolf, R. A., and R. W. Gross. 1985. Identification of neutral active phospholipase C which hydrolyzes choline glycerophospholipids and plasmalogen selective phospholipase A_2 in canine myocardium. *J. Biol. Chem.* 260:7295–7303.

10. Hazen, S. L., R. J. Stuppy, and R. W. Gross. 1990. Purification and characterization of canine myocardial cytosolic phospholipase A_2-A calcium-independent phospholipase with absolute *sn*-2 regiospecificity for diradyl glycerophospholipids. *J. Biol. Chem.* 265:10622–10630.

11. Hazen, S. L., D. A. Ford, and R. W. Gross. 1991. Activation of a membrane-associated phospholipase A_2 during rabbit myocardial ischemia which is highly selective for plasmalogen substrate. *J. Biol. Chem.* 266:5629–5633.

12. Ford, D. A., and R. W. Gross. 1989. Differential accumulation of diacyl and plasmalogenic diglycerides during myocardial ischemia. *Circ. Res.* 64:173–177.

13. Hoyt, R. H., M. L. Cohen, and J. E. Saffitz. 1989. Distribution and three-dimensional structure of intercellular junctions in canine myocardium. *Circ. Res.* 64:563–574.

14. Schon, R. 1965. A simple and sensitive enzymic method for determination of L(+)-lactic acid. *Anal. Biochem.* 12:413–420.

15. Downar, E., M. J. Janse, and D. Durrer. 1977. The effect of acute coronary artery occlusion on subepicardial transmembrane potentials in the intact porcine heart. *Circulation.* 56:217–224.

16. Gronich, J. H., J. V. Bonventre, and R. A. Nemenoff. 1988. Identification and characterization of a hormonally regulated form of phospholipase A_2 in rat renal mesangial cells. *J. Biol. Chem.* 263:16645–16651.

17. Channon, J. Y., and C. C. Leslie. 1990. A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A_2 with membrane in the macrophage cell line RAW 264.7. *J. Biol. Chem.* 265:5409–5413.

18. Franson, R. C., D. L. Weir, and J. Thakkar. 1983. Solubilization and characterization of a neutral-active, calcium-dependent, phospholipase A_2 from rabbit heart and isolated chick embryo myocytes. *J. Mol. Cell. Cardiol.* 15:189–196.

19. Tam, S. W., R. Y. K. Man, and P. C. Choy. 1984. The hydrolysis of phosphatidylcholine by phospholipase A_2 in hamster heart. *Can. J. Biochem. Cell Biol.* 62:1269–1274.

20. Nalbone, G., and K. Y. Hostetler. 1985. Subcellular localization of the phospholipases A of rat heart: evidence for a cytosolic phospholipase A_1. *J. Lipid Res.* 26:104–114.