Identification and Characterization of Human Myocardial Phospholipase A<sub>2</sub> From Transplant Recipients Suffering From End-Stage Ischemic Heart Disease

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Although numerous studies have implicated accelerated phospholipid catabolism during myocardial ischemia as an important contributor to ischemic membrane dysfunction, no information is currently available on the subcellular distribution, physical properties, or kinetic characteristics of human myocardial phospholipase A<sub>2</sub>. In this report, we demonstrate that the overwhelming majority (98%) of total phospholipase A<sub>2</sub> activity in human myocardium (obtained from transplant recipients) is calcium independent, plasmalogen selective, and is distributed between the microsomal (60–70% of total activity) and cytosolic (30–40% of total activity) fractions. Both human myocardial microsomal and cytosolic phospholipase A<sub>2</sub> enzymes 1) preferentially hydrolyze plasmalogen molecular species containing arachidonic acid at the sn-2 position, 2) are recalcitrant to chemical inactivation by the indole-reactive agent parabromophenacyl bromide, 3) are irreversibly inhibited by covalent modification of an essential thiol residue by 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), and 4) are exquisitely sensitive to mechanism-based inhibition by (E)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one (bromoenol lactone). In sharp contrast, human mitochondrial phospholipase A<sub>2</sub> 1) accounts for only a diminutive amount of total myocardial phospholipase A<sub>2</sub> activity (1–2%), 2) is augmented by calcium ion, 3) exhibits a higher reaction velocity using phosphatidylcholine in comparison with plasmenylcholine substrate, and 4) is not substantially inhibited by either DTNB or bromoenol lactone. Collectively, these results demonstrate that the majority of phospholipase A<sub>2</sub> activity in human myocardium is catalyzed by a novel class of calcium-independent plasmalogen-selective phospholipases A<sub>2</sub> and underscore the potential importance of this class of enzymes in mediating membrane dysfunction during myocardial infarction in humans. (Circulation Research 1992;70:486–495)

KEY WORDS • phospholipase A<sub>2</sub> • myocardium • ischemia

The functional sequelae of acute myocardial ischemia are intimately related to fundamental alterations in the chemical constituents of critical subcellular membranes (e.g., sarcolema, sarcoplasmic reticulum, and mitochondria), which are mediated, at least in part, by the phospholipases that are activated during ischemic injury. Accordingly, extensive efforts have focused on identification of the magnitudes and types of phospholipase activities that contribute to the selective release of arachidonic acid and the accumulation of lysophospholipids in ischemic myocardium. 7–10 Although phospholipases A<sub>2</sub> in myocardium from numerous mammalian species (e.g., dog, 11–15 rabbit, 16 hamster, 17 rat, 18 and pig) 19 have been examined in varying degrees of detail, absolutely no information is available at present on the subcellular distribution, physical properties, or kinetic characteristics of human myocardial phospholipase A<sub>2</sub>. This largely results from the difficulty in procuring sufficient amounts of fresh human myocardial ventricular tissue to allow detailed analyses of human myocardial phospholipases. Furthermore, since the major myocardial phospholipase A<sub>2</sub> activity in many species 13,15,16 selectively hydrolyzes plasmalogen substrate, the utilization of synthetically prepared plasmenylcholine substrate is necessary for detailed analyses of the kinetics and substrate specificities of phospholipase A<sub>2</sub> in human myocardium. To identify the subcellular distribution, kinetic characteristics, and substrate specificities of human myocardial phospholipase A<sub>2</sub>, the phospholipase A<sub>2</sub> activities in fresh human myocardial tissue from transplant recipients were characterized using both plasmenylcholine and phosphatidylcholine substrates under a variety of conditions. We now demonstrate that the majority of phospholipase A<sub>2</sub> activity in human myocardium is calcium independent, selective for the hydrolysis of plasmenylcholine substrate, and present in both the microsomal and cytosolic compartments.

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Materials and Methods

Preparation of Human Myocardial Subcellular Fractions

Fresh human myocardium from transplant recipients was rapidly placed in ice-cold isotonic saline solution (typically within 2 minutes). Left ventricular tissue was rapidly trimmed of epicardial fat and visible fibrotic lesions, weighed, and placed (25% [wt/vol]) in homogenization buffer (0.25 M sucrose [grade 1], 10 mM imidazole, and 10 mM KCl, pH 7.8, at 0°C). All subsequent isolation procedures were performed at 4°C. Ventricular tissue was finely minced into 0.2×0.4-cm strips with sharp scissors and homogenized with three strokes of a loose-fitting Potter-Elvehjem apparatus operated at 2,000 rpm. Human myocardial subcellular fractions were subsequently prepared by methods previously established.\textsuperscript{15,20}\ Briefly, homogenate was centrifuged at 1,000g for 10 minutes to remove nuclei and cellular debris, and the lipidic upper portion of the supernatant was gently removed by aspiration. The remaining supernatant was centrifuged at 10,000g for 20 minutes, yielding a crude mitochondrial pellet that was washed, repelleted (as above), and resuspended in homogenization buffer at a concentration of 1 mg protein/ml. The supernatant from the initial 10,000g centrifugation was centrifuged at 100,000g for 60 minutes, yielding a crude microsomal fraction (pellet) and a cytosolic fraction (supernatant). Microsomal protein was washed, repelleted (as above), and resuspended in homogenization buffer at a concentration of 200 µg/ml.

Preparation of Synthetic Phospholipids

Homogeneous 16:0,\textsuperscript{[3]H}18:1 (1.4×10^6 dpm/nmol) and 16:0,\textsuperscript{[3]H]20:4 (6.7×10^5 dpm/nmol) phosphatidylcholine and plasmenylcholine molecular species were synthesized from either homogeneous 1-hexadecanoylsn-glycero-3-phosphocholine or 1-O-(Z)-hexadec-1′-enylnsn-glycero-3-phosphocholine and the appropriate radiolabeled fatty acid as previously described.\textsuperscript{15}\ Briefly, dicyclohexylcarbodiimide-mediated synthesis of radiolabeled fatty acyl anhydride was followed by its condensation to the sn-2 hydroxyl of the appropriate lysophospholipid. To facilitate direct kinetic comparisons between diacyl and vinyl ether subclasses of choline glycerophospholipids, radiolabeled molecular species of identical specific activities were synthesized using common preparations of radiolabeled fatty acyl anhydride. Each radiolabeled choline glycerophospholipid molecular species was initially purified by preparative thin-layer chromatography (TLC) and subsequently purified by Partisil SCX-high-performance liquid chromatography (HPLC) as previously described.\textsuperscript{15}\ Individual molecular species of unlabeled phosphatidylcholine and plasmenylcholine were prepared similarly.

Polar head group–labeled plasmenylcholine ([\textsuperscript{3}H]Me-choline–labeled; specific activity, 200,000 dpm/nmol) was prepared using ([\textsuperscript{3}H]Me-choline)lysoplasmenylcholine and oleoyl anhydride as described above. Briefly, ([\textsuperscript{3}H]Me-choline)lysoplasmenylcholine was prepared by exhaustive methylation of reverse-phase–purified lysoplasmenylethanolamine\textsuperscript{21} using [\textsuperscript{3}H]CH\textsubscript{3}I with benzyltrimethylammonium chloride as catalyst.\textsuperscript{22}\ Polar head group–labeled plasmenylcholine and lysoplasmenylo-
labeled starting materials were purchased from Du-
pont—New England Nuclear, Boston. Bovine heart
decithin, dipalmitoyl phosphatidylycholine, and palmitoyl
ilysosphatidylycholine were purchased from Avanti
Polar Lipids. Oleic and arachidonic acids were obtained
from Nu Chek Prep, Inc. Bromonol lactone (generously
provided by Dr. R.H. Weiss, Monsanto Chemical Co.,
St. Louis, Mo.) was prepared according to the pro-
cedure of Daniels et al.24 and further purified by prepar-
ative reverse-phase HPLC.23 Most other reagents were
obtained from either Sigma Chemical Co., St. Louis,
Mo., or Aldrich Chemical Co., Milwaukee, Wis. Sta-
tistical differences were assessed by analyses of variance
using a modified t statistic and overall F test.25

Results

Substrate Specificity and Calcium Requirements of
Human Myocardial Phospholipase A2

Incubation of 100 μM radiolabeled plasmenylcholine
or phosphatidylcholine (specifically labeled at the sn-2
fatty acid) with human myocardial microsomes, cytosol,
or mitochondria resulted in the production of radio-
abeled free fatty acid without substantial production
(<2% of released fatty acid) of radiolabeled lysospho-
lipid, diglyceride, monoglyceride, or phosphatidic
acid. The possibility that the release of sn-2 fatty acid
from phospholipid substrate occurred by sequential
enzymatic activities (e.g., phospholipase A1, C, or D
and subsequent lipase activities) was considered unlikely
since inclusion of 100 μM lysophospholipid, diglyceride,
or phosphatidic acid attenuated the rate of [3H]fatty
acid release from sn-2-radiolabeled substrate by <30%.
Since the maximum concentration of putative radio-
abeled intermediates in sequential pathways would be
more than two orders of magnitude less than the
concentration of exogenously added unlabeled inter-
diate, these results mitigate against sequential reactions
as substantial contributors to the observed release of sn-2
[3H]fatty acid. To unequivocally identify the observed
activities as phospholipase A2 activities, polar headgroup-
labeled 16:0,18:1 plasmenylcholine was synthesized. Incu-
bation of [3H]cheoline—labeled 16:0,18:1 plasmenylcholine
with each subcellular fraction resulted in the production of
only [3H]Me-choline-lyosphasmeylncholine (confirmed by
TLC in two dimensions and by its acid lability) and not
radiolabeled 2-acyl-lyosphosphatidylcholine, glyceropho-
osphorycholine, phosphocholine, or choline. Collectively,
these results demonstrate that the majority of phospholi-
pase activity present in human myocardium was catalyzed
by phospholipase A2 under the conditions used.

Human myocardial microsomal phospholipase A2
could not be solubilized by addition of high salts,
chelators, submicellar concentrations of detergent, or
sonication. Microsomal phospholipase A2 activity was
calium independent, exhibiting maximal enzymatic
activity in the presence of EGTA when multiple
different phosphatidylycholine and plasmenylcholine
molecular species were used as substrates (Figure 1).
Comparisons of different phosphatidylycholine and
plasmenylcholine substrates demonstrated that human
myocardial microsomal phospholipase A2 preferen-
tially hydrolyzed choline glycerophospholipids con-
taining a vinyl ether linkage and choline glycerophos-
pholipids containing arachidonic acid esterified to the
sn-2 position (Figure 1).

Characterization of human myocardial cytosolic
phospholipase A2 cofactor requirements and substrate
selectivity was performed similarly. Human myocardial
cytosolic phospholipase A2 exhibited maximum hydro-
lytic rates in the absence of calcium ion (Figure 2). In
fact, enzymatic activity was modestly inhibited (~20%)
in the presence of supraphysiological concentrations of
calcium ion (i.e., 10 mM), which have been routinely
used for analyses of extracellular phospholipases A2.
The choline glycerophospholipid subclass selectivity of

![Figure 1. Bar graph showing choline glycerophospholipid subclass selectivity and calcium requirements of human myocardial microsomal phospholipase A2 activity.](http://circres.ahajournals.org/DownloadedFrom)
human myocardial cytosolic phospholipase A2 activity was also remarkable for its preferential hydrolysis of 16:0,18:1 and 16:0,20:4 plasmenylcholine molecular species in direct comparisons with their diacyl phospholipid counterparts. Cytosolic phospholipase A2 hydrolysis of plasmenylcholine substrate containing sn-2-esterified arachidonic acid was twofold greater than that of plasmenylcholine containing sn-2 oleic acid. In contrast, only modest differences in hydrolytic rates catalyzed by the soluble enzyme were observed using phosphatidylcholine molecular species differing in their sn-2 acyl constituents (Figure 2).

Examination of human myocardial mitochondrial phospholipase A2 activity revealed that the specific activity of mitochondrial phospholipase A2 was substantially less than that present in the cytosolic fraction and was nearly three orders of magnitude less than that present in the microsomal fraction (Figure 3). In stark contrast to human myocardial microsomal and cytosolic phospholipase A2 activities, mitochondrial phospholipase A2 activity was augmented in the presence of calcium ion (Figure 3). Furthermore, comparisons between 16:0,18:1 and 16:0,20:4 molecular species of phosphatidylcholine and plasmenylcholine revealed that human myocardial mitochondrial phospholipase A2 exhibited higher reaction velocities using phosphatidylcholine substrates. Finally, human myocardial mitochondrial
phospholipase A₂ activity displayed no selectivity for hydrolysis of sn-2-esterified oleic or arachidonic acids using either choline glycerophospholipid subclass (Figure 3).

**Distribution of Total Phospholipase A₂ Activity in Human Myocardium**

Comparisons of total phospholipase A₂ activity in human myocardium (in nanomoles per gram wet weight per minute) in each subcellular compartment demonstrated that 1) the overwhelming majority of human myocardial phospholipase A₂ activity is calcium independent, 2) approximately two thirds of human myocardial phospholipase A₂ activity is located in the microsomal compartment, 3) cytosol contains nearly one third of human myocardial phospholipase A₂ activity, 4) only minimal amounts of phospholipase A₂ activity are present in the mitochondrial compartment, 5) the total amount of phospholipase A₂ activity in human myocardium is substantially greater when assessed using plasmenylcholine substrate in comparison with phosphatidylcholine substrate, and 6) total measurable phospholipase A₂ activity in human myocardium is nearly twofold greater using choline glycerophospholipids containing sn-2-esterified arachidonic acid in comparison with sn-2-esterified oleic acid (Table 1). Taken together, these results demonstrate that the polypeptide(s) catalyzing the vast majority of phospholipase A₂ activity in human myocardium is calcium independent, plasmalogen selective and preferentially hydrolyzes choline glycerophospholipids containing sn-2-esterified arachidonic acid.

**Kinetic Analyses of Human Myocardial Phospholipase A₂ Activities**

Detailed kinetic analyses of the choline glycerophospholipid subclass selectivities of the major human myocardial phospholipase A₂ activities (i.e., microsomal and cytosolic) were performed to gain further insight into the mechanisms underlying the substrate specificity of myocardial phospholipase A₂. To this end, mixed vesicles composed of binary mixtures of synthetic 16:0,18:1 plasmenylcholine and 16:0,18:1 phosphatidylcholine (to minimize differences in the physical properties of vesicles composed entirely of a single subclass) were prepared that contained different mole percentages of plasmenylcholine and phosphatidylcholine. Incubation of vesicles consisting of equimolar mixtures of plasmenylcholine and phosphatidylcholine with human myocardial microsomal phospholipase A₂ (at saturating bulk lipid concentrations*3) resulted in the preferential hydrolysis of plasmenylcholine, demonstrating that the selectivity of human myocardial microsomal phospholipase A₂ for vinyl ether–containing choline glycerophospholipids is independent of the physical properties and interfacial characteristics of aggregated substrate (Figure 4, left panel). Whether 16:0,13H]18:1 plasmenylcholine was varied within a phosphatidylcholine lipid matrix or 16:0,13H]18:1 phosphatidylcholine was varied within a plasmenylcholine lipid matrix, the initial rate of [3H]fatty acid release from each subclass increased linearly as a function of its mole percent within the vesicle (i.e., human microsomal phospholipase A₂ activity exhibited surface dilution kinetics) (Figure 4, left panel). Human microsomal phospholipase A₂ efficiently catalyzed the hydrolysis of plasmenylcholine, even when

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<th>Cell fraction</th>
<th>Total phospholipase A₂ activity (nmol/g wet wt · min)</th>
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<td>EGTA</td>
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<td>Phosphatidylcholine</td>
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Values are mean±SEM. Data were compiled from eight determinations from four separate hearts (microsomes and cytosol) and from four determinations from two separate hearts (mitochondria). Human myocardial microsomes, cytosol, and mitochondria were prepared by differential centrifugation as described in “Materials and Methods.” Synthetic 16:0,[3H]18:1 and 16:0,[3H]20:4 plasmenylcholine or phosphatidylcholine substrates (100 µM) were incubated with the indicated subcellular fraction for 60 seconds at 37°C in 100 mM Tris chloride buffer (pH 7.0; final volume, 210 µl) containing either 4 mM EGTA or 10 mM CaCl₂ as indicated. Reaction products were extracted into butanol and separated by thin-layer chromatography. [3H]fatty acid was quantified by scintillation spectrometry as described in “Materials and Methods.”

*p<0.0005, †p<0.005, and §p<0.05 for comparisons between plasmenylcholine and phosphatidylcholine substrates containing identical constituents at the sn-2 position.

the physical characteristics of the vesicles were largely those of phosphatidylcholine (i.e., 10 mol% [3H]plasmenylcholine in a phosphatidylcholine matrix). Likewise, phosphatidylcholine was not substantially hydrolyzed even when present in vesicles possessing the physical properties of the preferred subclass of substrate (i.e., 10 mol% [3H]phosphatidylcholine in a plasmenylcholine matrix) (Figure 4, left panel). The ratio of the slope of plasmenylcholine/phosphatidylcholine hydrolysis was 6.0 (Figure 4), reflecting the sixfold selectivity of human microsomal phospholipase A₂ for plasmenylcholine substrate at multiple different surface concentrations. Replots of the surface dilution data expressing initial reaction velocity as a percentage of maximum hydrolysis for each subclass demonstrated linear relations that intersected at 50 mol% of each substrate (Figure 4, right panel). Thus, human microsomal phospholipase A₂ selectively hydrolyzed plasmenyl-
choline substrate in a variety of lipid matrices and followed strict surface dilution kinetics.

The choline glycerophospholipid subclass selectivity of human myocardial cytosolic phospholipase A₂ was further characterized by Lineweaver-Burk analyses. For both 16:0,18:1 and 16:0,20:4 molecular species of phosphatidylcholine and plasmenylcholine, the enhanced catalytic turnover rate of vinyl ether-containing choline glycerophospholipids arose almost exclusively from increased maximum reaction velocity and not from differing affinity of the enzyme for each subclass of substrate (i.e., choline glycerophospholipid subclasses containing similar sn-1 and sn-2 aliphatic constituents possessed similar apparent $K_m$ values). Human myocardial cytosolic phospholipase A₂ possessed a low apparent $K_m$ for hydrolysis of all choline glycerophospholipids examined (Figure 5).

Comparisons of the pH profiles of human myocardial microsomal, cytosolic, and mitochondrial phospholipases A₂ demonstrated substantial differences in each subcellular fraction (Figure 6). Human myocardial microsomal phospholipase A₂ exhibited a pH optimum of 8.5 using either 16:0,18:1 plasmenylcholine or 16:0,18:1 phosphatidylcholine as substrate. In contrast, cytosolic phospholipase A₂ activity was optimal at pH 7.0 using either 16:0,18:1 plasmenylcholine or 16:0,18:1 phosphatidylcholine as substrate. Comparisons of the pH profiles of cytosolic phospholipase A₂ using either plasmenylcholine or phosphatidylcholine as substrate demonstrated substantial differences from pH 7.5–9.0, suggesting (but not proving) the presence of two enzymes possessing distinct pH optima. Finally, human myocardial mitochondrial phospholipase A₂ activity possessed a basic pH optimum (pH 8.5–9.0) using either choline glycerophospholipid as substrate. At all pH values examined, microsomal and cytosolic phospholipase A₂ activities selectively hydrolyzed plasmenylcholine substrate, whereas mitochondrial phospholipase A₂ selectively hydrolyzed phosphatidylcholine substrate.

**Differential Susceptibility of Human Myocardial Phospholipase A₂ to Covalent Modification**

To further compare phospholipase A₂ activities within each human myocardial subcellular fraction, the sensitivity of each activity to selected chemical modification of essential histidine or thiol residues was examined. Para-bromophenacyl bromide inhibits numerous calcium-dependent phospholipases A₂ enzymes (e.g., *Naja naja* and pancreatic phospholipase A₂) by covalent modification of an essential histidine residue within the active site, whereas DTNB inhibits numerous calcium-independent lipases (e.g., canine myocardial cytosolic phospholipase A₂, rat heart cytosolic phospholipase A₂, and human plasma lecithin-cholesterol acyltransferase) through covalent modification of reactive thiol residues. Both human cytosolic and microsomal phospholipases A₂ were completely and irreversibly inhibited by DTNB, whereas treatment with para-bromophenacyl bromide had no effect (Figure 7). In stark contrast, the

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**Figure 4.** Graphs showing kinetic analysis of human myocardial microsomal calcium-independent phospholipase A₂ activity using binary mixtures of plasmenylcholine and phosphatidylcholine. Left panel: Calcium-independent phospholipase A₂ activity in human myocardial microsomes was assessed in vesicles composed of the indicated mole percent of either 16:0,[³H]18:1 plasmenylcholine within a 16:0,18:1 phosphatidylcholine bilayer (●) or 16:0,18:1 phosphatidylcholine within a 16:0,18:1 plasmenylcholine bilayer (○). Mixed bilayers were prepared by injection of appropriate amounts of previously codissolved mixtures of phospholipids in ethanol. Human myocardial microsomal protein (13 µg) was incubated with 100 µM substrate (total lipid) in assay buffer at pH 7.0 for 60 seconds at 37°C. Released sn-2-[³H]labeled fatty acid was isolated and quantified by thin-layer chromatography and scintillation spectrometry as described in “Materials and Methods.” Data points represent the mean of duplicate determinations. Right panel: Data from the left panel replotted as the fractional percent of hydrolytic rates present at 100 mol% of each choline glycerophospholipid subclass relative to their mole fraction in mixed bilayers.

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**Figure 5.** Graphs showing pH dependence of human myocardial microsomal calcium-independent phospholipase A₂ activity using binary mixtures of plasmenylcholine and phosphatidylcholine. The pH profiles of human myocardial microsomal phospholipase A₂ activity were determined using either 16:0,18:1 plasmenylcholine or 16:0,18:1 phosphatidylcholine as substrate. Each graph shows the percentage hydrolysis (100%) of the appropriate choline glycerophospholipid substrate at pH values ranging from 6.0 to 9.0.
majority of human mitochondrial phospholipase \( A_2 \) activity was inhibited by parabromophenacyl bromide, whereas only modest inhibition of enzymatic activity was present after exposure to DTNB (Figure 7). The differential sensitivity of either human myocardial microsomal or cytosolic phospholipase \( A_2 \), compared with human myocardial mitochondrial phospholipase \( A_2 \), to group-specific chemical modification further underscores the differences in the chemical moieties that mediate catalysis by the phospholipases in these fractions.

**Mechanism-Based Inhibition of Human Myocardial Phospholipase \( A_2 \) Activities**

Bromoenol lactones have recently been identified as potent irreversible mechanism-based inhibitors of calcium-independent phospholipase \( A_2 \).\(^{23}\) Accordingly, the susceptibility of human myocardial phospholipase \( A_2 \) activity in each subcellular fraction to suicide inhibition was examined. Both human microsomal and cytosolic phospholipase \( A_2 \) activities were specifically and irreversibly inhibited by the suicide inhibitor, bromoenol lactone, demonstrating an IC\(_{50}\) of 60–90 nM under the conditions used (Figure 8). In stark contrast, the majority of human mitochondrial phospholipase \( A_2 \) activity was resistant to inhibition by bromoenol lactone, since the majority of activity was present at 10 \( \mu \)M inhibitor (Figure 8). Taken together, the results substantiate the conclusion that the calcium-independent plasmenylcholine-selective phospholipase \( A_2 \) activities in human myocardial microsomes and cytosol are separate and distinct from the calcium-dependent phosphatidylcholine-selective phospholipase \( A_2 \) activity in human myocardial mitochondria.

**Discussion**

Because of their potential importance as enzymatic mediators of a diverse array of physiological and pathophysiological perturbations,\(^{33–39}\) phospholipase \( A_2 \) activities in fresh human myocardial tissue were examined. The present study is the first to identify and characterize the subcellular distribution, physical properties, and kinetics of human myocardial phospholipases \( A_2 \). Distinct phospholipase \( A_2 \) activities were identified in human myocardium; these activities were functionally distinguishable based on their differential subcellular localization, calcium requirements, substrate specificities, pH profiles, and susceptibilities to covalent modifications. The overwhelming majority of phospholipase \( A_2 \) activity in human myocardium was calcium independent. The majority of calcium-independent phospholipase \( A_2 \) activity (~65% of total activity) in human myocardium was present in the microsomal fraction and was tightly bound to the microsomal membrane, since neither high salt concentrations, chelators, submicellar concentrations of detergents, nor sonication resulted in the solubilization of enzymatic activity. Human myocardial microsomal phospholipase \( A_2 \) activity was also remarkable for its preferential hydrolysis of choline glycerophospholipids containing a vinyl ether linkage and choline glycerophospholipids containing sn-2-esterified arachidonic acid. Human myocardial cytosol also contained substantial amounts of phospholipase \( A_2 \) activity (~35% of total activity) that was likewise calcium independent, plasmenylcholine selective, and preferentially hydrolyzed plasmenylcholine containing arachidonic acid at the sn-2 position. In contrast, human myocardial mitochondrial phospholipase \( A_2 \) repre-
several inositides (e.g., 1,4,5-trisphosphate, inositol 1,4,5-trisphosphate, and inositol 1,3,4,5-tetrakisphosphate) in the myocardium. We have determined that both phospholipase A2 activities are integral membrane proteins. These experiments do not rule out the possibility that both cytosolic and microsomal calcium-independent phospholipase A2 activities are catalyzed by the same gene product that has undergone differential intracellular processing (e.g., posttranslational processing and proteolysis).

The abundance of phospholipase A2 activity in the microsomal compartment of myocardium from transplant recipients is substantially different from that observed in normal (i.e., nonischemic) myocardium from several mammalian species in which the majority of total calcium-independent phospholipase A2 activity was present in the cytosolic fraction. We have reported the presence of a second phospholipase A2 activity in the microsomal compartment of myocardium from normal and transplant recipient pigs. These results suggest that the two phospholipase A2 activities may be biologically distinct and may have different substrates.

FIGURE 6. Graphs showing pH profiles of human myocardial phospholipases A2. Human myocardial microsomal (9 mg), cytosolic (24 mg), and mitochondrial (50 mg) proteins were incubated with either 16:0-[3H]18:1 plasmenylcholine (○) or 16:0-[3H]18:1 phosphatidylcholine (●) (100 umol each) in buffer (200 mM K2HPO4 at the indicated pH) for 60 seconds at 37°C under conditions yielding optimal enzymatic activity (i.e., 4 mM EGTA for microsomes and cytosol and 10 mM CaCl2 for mitochondria). Released radiolabeled fatty acid was subsequently extracted into butanol, isolated by thin-layer chromatography, and quantified by scintillation spectrometry as described in "Materials and Methods." Data points represent the mean of duplicate determinations.

FIGURE 7. Bar graphs showing chemical modification of human myocardial phospholipases A2. Accessible histidine and thiol residues in human myocardial microsomal, cytosolic, and mitochondrial phospholipase A2 were covalently modified by incubation with either parabromophenacyl bromide (pBr0Br) or 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) as described in "Materials and Methods." Phospholipase A2 activity remaining in each subcellular fraction was subsequently quantified using 100 tmol 16:0-[3H]18:1 plasmenylcholine substrate in assay buffer (100 mM Tris chloride [pH 7.4]) under optimal reaction conditions (i.e., 4 mM EGTA for microsomes and cytosol and 10 mM CaCl2 for mitochondria) as described in "Materials and Methods." Values are expressed as the percent maximum phospholipase A2 activity remaining in comparison with incubations performed in the absence of an inhibitor (i.e., buffer controls) and represent the mean of triplicate determinations. *p<0.0005, †p<0.005, and ‡p<0.025 for comparisons between chemically modified subcellular fractions and their controls.
FIGURE 8. Graph showing mechanism-based inhibition of human myocardial phospholipases A2. Human myocardial microsomal, cytosolic, and mitochondrial proteins were preincubated with the indicated concentrations of (E)-6-(bromomethylenetetracyclon-3-(1-naphthalenyl)-2H-pyran-2-one (bromoenol lactone) for 5 minutes at 20°C. Phospholipase A2 activity in each subcellular fraction was subsequently quantified after dilution using 100 μM 16:0[3H]18:1 plasmalogen-choline substrate as described in “Materials and Methods.” Activity is expressed as the percent maximal phospholipase A2 activity relative to incubations performed in the absence of inhibitor (i.e., buffer controls). Data points represent the mean of duplicate determinations.

cently identified the selective activation of a microsomal calcium-independent phospholipase A2 activity in ischemic rabbit myocardium16 (which accounts for as much as 90% of total phospholipase A2 activity in ischemic myocardium) with kinetic characteristics (substrate selectivity, pH profile, and surface dilution kinetics) that are similar to the human myocardial microsomal phospholipase A2 activity characterized herein. Human myocardial tissue used in this study was obtained from transplant recipients who suffered from end-stage ischemic heart disease. Accordingly, the subcellular distribution and relative magnitudes of each of the phospholipase A2 activities quantified herein do not necessarily reflect those present in normal human myocardium. Thus, the abundance of microsomal calcium-independent phospholipase A2 activity in myocardium from transplant recipients may reflect alterations resulting from activation of enzymes in compromised tissue. For obvious reasons, direct comparisons of the phospholipases A2 present in these tissue samples with those present in normal human ventricular myocardium cannot be performed.

Although it was traditionally assumed that intracellular phospholipase A2 possessed physical and kinetic properties similar to those of the rigorously characterized extracellular phospholipases A2 (low molecular weight, calcium dependence, and broad substrate specificities), recent studies have demonstrated that many mammalian intracellular phospholipases A2 possess separate and distinct characteristics.15,15,40–42 The present results document the presence of microsomal and cytosolic calcium-independent phospholipase A2 activities that collectively account for the overwhelming majority of measurable phospholipase A2 activity in human myocardium and that possess the catalytic potential for hydrolysis of the major phospholipid constituents (i.e., plasmalogens containing arachidonic acid esterified to the sn-2 position) in critical myocardial subcellular membranes (e.g., sarcolemma and sarcoplasmic reticulum) during myocardial ischemia.20,43 Although calcium is not an obligatory cofactor for catalysis by this class of phospholipases A2, the possibility that calcium can trigger one or more events that result in the activation (e.g., phosphorylation or proteolysis) of one or more members of this class of enzymes is tenable. Accordingly, identification of the regulatory mechanisms that modulate the activity of this class of phospholipases A2 should provide important insight into the physiological and pathophysiological significance of human myocardial calcium-independent phospholipase A2.

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