Cardiac Ischemia Activates Calcium-independent Phospholipase A₂β, Precipitating Ventricular Tachyarrhythmias in Transgenic Mice

RESCUE OF THE LETHAL ELECTROPHYSIOLOGIC PHENOTYPE BY MECHANISM-BASED INHIBITION

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Murine myocardium contains diminutive amounts of calcium-independent phospholipase A₂ (iPLA₂) activity (<5% that of human heart), and malignant ventricular tachyarrhythmias are infrequent during acute murine myocardial ischemia. Accordingly we considered the possibility that the mouse was a species-specific knockdown of the human pathologic phenotype of ischemia-induced lethal ventricular tachyarrhythmias. Transgenic mice were generated expressing amounts of iPLA₂β activity comparable to that present in human myocardium. Coronary artery occlusion in Langendorff perfused hearts from transgenic mice resulted in a 22-fold increase in fatty acids released into the venous eluent (29.4 nmol/ml in transgenic versus 1.38 nmol/ml of eluent in wild-type mice), a 4-fold increase in lysophosphatidylcholine mass in ischemic zones (4.9 nmol/mg in transgenic versus 1.1 nmol/mg of protein in wild-type mice), and malignant ventricular tachyarrhythmias within minutes of ischemia. Neither normally perfused transgenic nor ischemic wild-type hearts demonstrated these alterations. Pretreatment of Langendorff perfused transgenic hearts with the iPLA₂ mechanism-based inhibitor (E)-6-(bromomethylene)-3-(1-naphthalenyl)-2H-tetrahydropyran-2-one (BEL) just minutes prior to induction of ischemia completely ablated fatty acid release and lysolipid accumulation and rescued transgenic hearts from malignant ventricular tachyarrhythmias. Collectively these results demonstrate that ischemia activates iPLA₂β in intact myocardium and that iPLA₂β-mediated hydrolysis of membrane phospholipids can induce lethal malignant ventricular tachyarrhythmias during acute cardiac ischemia.

Electrophysiologic dysfunction is the major cause of death during myocardial infarction in humans (1). Years ago we suggested that activation of myocardial phospholipases during acute cardiac ischemia resulted in the generation of amphiphilic metabolites that alter ion channel function, thereby precipitating lethal ventricular dysrhythmias (2–10). Since myocytic electrophysiologic function is influenced by the physiochemical properties of the lipids surrounding ion channels (11–13), accelerated hydrolysis of sarcolemmal phospholipid constituents during acute ischemia could potentially provide a foundation for understanding the biochemical basis of ischemia-induced arrhythmias. However, a cause and effect relationship between ischemia-induced phospholipolysis and malignant ventricular arrhythmias has not previously been established. Moreover, myocardium contains at least three distinct intracellular phospholipase A₂ (PLA₂) activities encoded by discrete genes (iPLA₂β, iPLA₂γ, and cPLA₂γ) (14–16), and the molecular identity of the phospholipase A₂ activated during cardiac ischemia is unknown. Finally, although each of these enzymes has been cloned, expressed, purified from recombinant systems, and subjected to detailed in vitro kinetic analyses, definitive proof of the ability of any of these discrete covalent entities (in the cPLA₂ or iPLA₂ families) to hydrolyze phospholipids in intact organs has not been directly demonstrated.

Calcium-independent phospholipase A₂ activity was initially discovered in the cytosolic and membrane fractions of canine myocardium and identified as a novel phospholipase A₂ by its ability to hydrolyze fatty acids from the sn-2 position of vinyl ether or diacyl phospholipids in the presence of EGTA (5). These studies unambiguously demonstrated that this activity was a bona fide phospholipase A₂ since release of the sn-2 acyl chain from other lipids could not result from sequential phospholipase A₁ followed by lysophospholipase activities. Purification of canine myocardial cytosolic iPLA₂ resulted in the isolation of a high specific activity 40-kDa polypeptide (6) whose identity was substantiated by its robust radiolabeling with the mechanism-based inhibitor (E)-6-(bromomethylene)tetrahydro-3-1-[4-³H]naphthalenyl)-2H-pyran-2-one (17). Likewise purification of iPLA₂ from human myocardium also identified a high specific activity 40-kDa isoform (as in canine myocardium) and a larger 85-kDa polypeptide of lower specific activity (18). Subsequent work has demonstrated that the 40-kDa polypeptide was a proteolytic fragment of a larger gene that has now been cloned and had its 85-kDa protein product expressed and
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**EXPERIMENTAL PROCEDURES**

**Materials**—ECL reagents were purchased from Amersham Biosciences. A DNA thermocycler and PCR reagents were purchased from Perkin-Elmer Life Sciences. EGTA, Beliphat, and sodium dodecyl sulfate (SDS)-PAGE were obtained from Calbiochem. Most other reagents were obtained from Sigma.

**Generation of Transgenic Mice Overexpressing iPLA<sub>2</sub>B in a Cardiac Myocyte-specific Manner**—Cardiac myocyte-specific expression of iPLA<sub>2</sub>B transgenic mice was accomplished by inactivation of the 2.4-kb coding region of the wild-type Chinese hamster iPLA<sub>2</sub>B (from iPLA<sub>2</sub>B-pFAST (19)) into the SalI site of the αMHC vector downstream from the αMHC promoter (35). Transgenic founders were generated by microinjection of a NolI-linearized fragment containing the αMHC promoter-iPLA<sub>2</sub>B DNA sequence directly into the pronuclei of mouse (B6CBAF1/J) zygotes, resulting in integration of the transgene into the mouse germ line. Two founders, identified by PCR analysis of mouse tail DNA, were mated with C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) to establish transgenic lines. Second and third generation heterozygous mice, typically 3–4 months of age, were used for all studies.

**Homogenization and Western Blot Analysis of Control and Transgenic Mouse Hearts**—For preparation of cytosolic and membrane fractions, samples of tissue were homogenized in 25 mm imidazole, pH 8.0, containing 1 mm EGTA and 0.25 mm sucrose using a Polytron tissue homogenizer and centrifuged at 100,000 g for 1 h to separate crude membrane and cytosolic (supernatant) fractions. The membrane fraction was resuspended in a volume of homogenization buffer equal to the cytosolic fraction. Proteins were separated by SDS-PAGE (38) and transferred to polyvinylidene difluoride membranes as described previously (25). Cytosolic and resuspended membrane fractions were probed with antibodies directed against iPLA<sub>2</sub>B peptide corresponding to residues 277–295 (CSYHHKSRPYGRASPLHWA) (25) in conjunction with a protein A-horseradish peroxidase conjugate for visualization by enhanced chemiluminescence. Recombinant iPLA<sub>2</sub>B prepared as described previously was used as a standard (19).

**Extraction and Electrospray Ionization Mass Spectrometry of Lipids from Wild-type and Transgenic Mouse Myocardium**—Lipids were extracted from mouse myocardium (≈50 mg) by a modified Bligh and Dyer technique. Mass spectrometry (ESI/MS) analyses were performed using a Finnigan TSQ 7000 spectrometer as described previously (37–39). For analyses of choline and ethanolamine glycerophospholipids as well as free fatty acids, LiOH in methanol (50 nmol/mg of protein) was added to the diluted tissue extracts. Membrane glycerophospholipids (including lysophosphatidylcholines) and sphingomyelins were directly quantitated in comparison to an internal standard (including lysophosphatidylcholines) after correction for 13C isotope effects in the positive ion mode (37–39).

**Electrophysiologic Studies in ischemic hearts**—Isolated Langendorff-Perfused Mouse Hearts—Ventricular tachyarrhythmias induced by acute ischemia were characterized using an isolated Langendorff-perfused heart preparation as described previously by Lerner et al. (32). Frequencies of spontaneous arrhythmias (premature ventricular contractions (PVCs) and episodes of malignant ventricular tachyarrhythmias (VT)) were counted for 30 min after coronary ligation and tabulated as PVC frequency per 5-min intervals. A run of ventricular tachycardia was defined as 10 or more beats with a cycle length <100 ms. After 30 min, hearts were perfused through the aortic catheter with 1% Evans blue dye to delineate the ischemic zone for subsequent tissue analyses. To determine the effects of BEL on induction of arrhythmias after ischemia, hearts were
perfusion with buffer containing 10 μM BEL beginning 5 min before coronary artery ligation and during the ischemic interval. All studies were randomized and blinded.

**Assay of Calcium-independent Phospholipase A₂ Activity**—Calcium-independent phospholipase A₂ activity was measured by quantitating the release of radiolabeled fatty acid from 1-α-1-palmitoyl-2-[1-14C]arachidonyl phosphatidylcholine in the presence of cytosolic or membrane fractions as described previously (19).

**Statistical Analyses**—One-way repeated measures ANOVA was used to compare the frequencies of spontaneous PVCs in wild-type control and transgenic iPLA₂ (TGIPLA₂) hearts over consecutive 5-min intervals during 30 min of ischemia with or without BEL pretreatment as indicated. Individual comparisons between wild-type control and TGIPLA₂ groups at each time interval studied were made with a two-tailed Student’s t test. The total number of episodes of VT in WT in wild-type and TGIPLA₂ transgenic hearts during 30 min of ischemia in the presence or absence of BEL pretreatment were compared by ANOVA. A value of p < 0.05 was considered significant.

**RESULTS**

Transgenic mice expressing iPLA₂β in a cardiac myocyte-specific fashion were generated by exploiting the specificity inherent in the αMHC promoter. Tissue samples from the major organs of wild-type and TGIPLA₂β mice revealed the presence of an intense band corresponding to iPLA₂β at the expected molecular mass (85 kDa) in transgenic hearts that was not visible in wild-type heart tissue (Fig. 1A). No expression was detected in brain or liver, while a faint band corresponding to iPLA₂β was detected in the kidneys of transgenic mice. Both cytosolic and crude membrane fractions from myocardium of TGIPLA₂β mice displayed robust iPLA₂β catalytic activity, while cardiac iPLA₂β activity was diminutive in wild-type mice (Fig. 1B). It should be noted that the amount of iPLA₂β activity present in transgenic mice is comparable to that naturally present in rabbit (2, 40), canine (4, 8), and human myocardium (10, 41). No differences in cardiac function were detected by echocardiographic analysis of 4-month-old iPLA₂β transgenic animals nor were there any differences in the body weights of animals nor were there any differences in the body weights of

**Fig. 1. iPLA₂β expression in wild-type and transgenic mice.** A, Western analysis of iPLA₂β expression in cytosol from selected tissues including brain, heart, liver, and kidney from WT and transgenic (TG) mice (25 μg of protein/lane). Purified recombinant iPLA₂β was used as standard. B, iPLA₂β activity present in the cytosolic fractions of brain, heart, liver, and kidney of wild-type and TGIPLA₂β mice. Phospholipase A₂ activity assays were performed by measuring arachidonic acid (AA) release (nmol/mg of protein/min) as described under “Experimental Procedures.” Error bars indicate ± S.E. for three separate experiments. n = 3.

**Fig. 2.** Electrospray ionization mass spectroscopy of phospholipids in wild-type and transgenic myocardium. A, ethanolamine glycerophospholipid (PE) molecular species in WT (light bars) versus TGIPLA₂β (dark bars) myocardium. Individual ethanolamine glycerophospholipid molecular species quantified include: 1, 18:0-20:4; 2, 16:0-22:6; 3, 18:1-20:4; 4, D16:0-22:6; 5, D16:0-22:4; 6, 18:0-22:6; 7, D18:0-22:6; 8, 18:1-22:4; 9, D18:0-22:4; 10, D18:1-22:6; 11, D18:0-22:6 where D and P denote diacyl and plasmalogens subclasses, respectively, *, p < 0.01; **, p < 0.001 (n = 3). B, phosphatidylcholine (PC) molecular species in WT (light bars) versus TGIPLA₂β (dark bars) hearts. Individual molecular species quantified include: 1, 16:0-16:0; 2, 16:0-18:2; 3, 16:0-18:1; 4, 16:0-20:4; 5, 18:1-18:1; 6, 18:0-18:1; 7, 16:0-22:6; 8, 16:1-22:4; 9, 18:0-20:4; 10, 18:0-22:6; 11, 18:1-22:4. *, p < 0.01; **, p < 0.001 (n = 3). In each case, other molecular species representing <2% of the total pools were also identified without demonstrable differences between control and transgenic mice.

To determine whether myocardial ischemia activates iPLA₂β, resulting in iPLA₂β-catalyzed hydrolysis of lipids in intact myocardium, a Langendorff perfused heart preparation was used. Samples of normally perfused and ischemic tissue from control and TGIPLA₂β hearts were analyzed for fatty acid and lysolipid mass by ESI/MS. Similarly the release of fatty acids into the effluent during control and ischemic conditions was quantified. This strategy provides clear data on the amount of PLA₂ activity manifest in the ischemic milieu in intact myocardium and avoids potential artifacts resulting from intrapreparative alterations in the enzyme (covalent or conformational) or the loss of effects of critical regulators of PLA₂ activity (e.g. ATP and calmodulin) during the homogenization process. After 15 min of coronary occlusion, release of fatty acids into effluents from TGIPLA₂β perfused hearts increased 22-fold compared with WT controls. Similarly a 4-fold increase in fatty acid and lysophosphatidylcholine accumulation in the ischemic zone of hearts was present in TGIPLA₂β mice but not in WT mice (Fig. 3). Moreover the release of fatty acids into the venous effluent and the accumulation of lysolipids and fatty acids in ischemic zones of hearts from transgenic mice were nearly completely ablated by pretreatment of Langendorff perfused hearts with the iPLA₂β mechanism-based inhibitor BEL (Fig. 3). We specifically point out that murine ischemia in wild-type hearts was remarkable for the near absence of fatty acids in ischemic zones of hearts from transgenic mice were nearly completely ablated by pretreatment of Langendorff perfused hearts with the iPLA₂β mechanism-based inhibitor BEL (Fig. 3).
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Fig. 3. Electrospray ionization mass spectrometric determination of fatty acid release and lysosphatidylcholine accumulation in WT and TGiPLA$_{2}\beta$ Langendorff perfused hearts. A, nonesterified fatty acid (NEFA) release obtained from effluent from control or ischemic Langendorff hearts at 15 min. *, $p < 0.0001$. B, nonesterified fatty acid mass in ischemic heart tissue from WT and TGiPLA$_{2}\beta$ hearts. *, $p < 0.01$. C, lysosphatidylcholine (LPC) mass in ischemic tissue from WT and TGiPLA$_{2}\beta$ hearts. *, $p < 0.0001$. Release of nonesterified fatty acid into the effluents (A) and accumulation of nonesterified fatty acid (B) and lysosphatidylcholine (LPC) (C) in ischemic tissue from Langendorff perfused hearts (WT (light bars) and TGiPLA$_{2}\beta$ (dark bars)) were measured in the absence or presence of LAD coronary artery occlusion (ischemia) in the absence (−) or presence (+) of BEL pretreatment as indicated.

acid release into the eluent and the absence of lysosphatidylcholine and fatty acid accumulation in ischemic zones, which contrasts dramatically with every other model of cardiac ischemia previously studied (including humans).

To determine the role of iPLA$_{2}\beta$ in arrhythmogenesis during acute cardiac ischemia, hearts from WT and TGiPLA$_{2}\beta$ mice were examined for PVCs and malignant ventricular arrhythmias after coronary ischemia in a Langendorff perfused heart preparation (32). Virtually no PVCs were observed in hearts from TGiPLA$_{2}\beta$ mice under normal flow conditions. However, ligation of the left anterior descending coronary artery in hearts from TGiPLA$_{2}\beta$ mice resulted in an increased frequency of PVCs and coupled beats within minutes of cardiac ischemia that was accompanied by malignant VT. The frequency of spontaneous PVCs was higher in hearts expressing iPLA$_{2}\beta$ compared with controls at each 5-min time interval examined during the first 20 min of ischemia ($p = 0.0003$ versus WT) with a single episode in one WT heart (1 of 19 hearts) ($p = 0.002$ by ANOVA) (Fig. 4A). The greatest increases in PVC frequency in TGiPLA$_{2}\beta$ hearts occurred in the 10–15- and 15–20–min intervals after coronary ligation. The PVC frequencies in these intervals were an average of 5.5-fold higher than that of the wild-type control hearts. Multiple episodes of nonsustained VT occurred in TGiPLA$_{2}\beta$ hearts (VT occurred in 9 of 24 hearts) compared with a single episode in one WT heart (1 of 19 hearts) ($p = 0.002$ by repeated measures ANOVA compared with hearts without BEL pretreatment) and in WT control hearts ($p = 0.03$ by repeated measures ANOVA compared with hearts without BEL pretreatment) (Fig. 4B). Moreover BEL pretreatment completely abolished VT in TGiPLA$_{2}\beta$ Langendorff perfused hearts (0 of 10 mouse hearts) ($p = 0.002$ by ANOVA comparing TGiPLA$_{2}\beta$ hearts with and without BEL pretreatment).

The concurrent activation of iPLA$_{2}\beta$ during ischemia in conjunction with the generation of malignant ventricular arrhythmias and their rescue by inhibition of the expressed iPLA$_{2}\beta$ transgene formally fulfills traditional criteria for proof of a cause and effect relationship between two phenomena (i.e., malignant arrhythmias are rare when diminutive amounts of iPLA$_{2}\beta$ activity are present (WT mice), malignant arrhythmias are manifest during activation of the expressed iPLA$_{2}\beta$ transgene by ischemia, and arrhythmias are ablated by mechanism-based inhibition of the expressed iPLA$_{2}\beta$ enzymic activity).

DISCUSSION

The results of the present study identify the rapid and dynamic activation of iPLA$_{2}\beta$ activity in response to ischemia in intact myocardium and its role in ischemic electrophysiologic dysfunction. Since the initial discovery of intracellular calcium-independent phospholipases A$_{2}$, a substantial controversy has evolved regarding their role as dynamic enzymes responding to pathophysiologic perturbations or whether iPLA$_{2}\beta$ activities represent static "housekeeping" enzymes responsible for phospholipid remodeling. Prior results utilizing a variety of cell culture systems, tissue homogenates, purified enzymes, and pharmacologic inhibitors have all strongly suggested an important role for iPLA$_{2}\beta$ in mediating dynamic cellular responses to a variety of physiologic or pathophysiologic stimuli. For example, prior work has demonstrated that iPLA$_{2}\beta$ 1) associates with and is modulated by ATP and calmodulin (23, 41, 2) mediates ligand-induced arachidonic acid release in many cell types (43–47), 3) is a sensor of metabolic alterations modulating insulin release (40, 41) and mitochondrial function (22, 48), and 4) modulates ion channel kinetics (13, 49). The results of the present study provide unambiguous evidence for the dynamic and rapid response of iPLA$_{2}\beta$ to the pathologic perturbation of ischemia in intact myocardium and underscore its role as a biochemical mediator of arrhythmogenesis in ischemic myocardium.

Death from ventricular tachyarrhythmias during acute myocardial ischemia is the major cause of mortality from atherosclerotic heart disease. Comprehensive epidemiologic and electrophysiologic studies have documented that the majority of human victims of acute myocardial ischemia die from ventricular tachyarrhythmias. Due to the relative ease of genetic manipulation of mouse embryos, the mouse has become the standard model for examining the role of specific proteins in prominent human diseases. For example, important insights into the mechanisms underlying atherosclerosis, diabetes, and cardiac diseases have been gleaned from murine models, although unquestionably substantial differences in mouse and
VT episodes in transgenic hearts as determined by ANOVA (p/H11005, open bars) ligation. Repeated measures ANOVA showed a significant difference between the PVC frequencies for wild-type controls and TGiPLA2 Langendorff hearts. The mean PVC frequency per interval (PVC frequency/5-min interval) was then plotted 40). The fact that the observed arrhythmias were due to the tachyarrhythmias in TGiPLA2 substantiated by the rescue of ischemia-induced ventricular (30 min) results in cell death (1, 32). However, during acute murine ischemia, spontaneous VT occur infrequently (31, 32) in contrast to the 35–60% incidence of malignant ventricular tachyarrhythmias present in most other species after LAD coronary artery occlusion (e.g., rabbit, rat, pig, dog, and human) (1, 24, 31–33, 50, 51).

In all cases of which we are aware, acute ischemia-induced arrhythmogenesis is accompanied by phospholipolysis (as assessed by release of fatty acids and accumulation of lysolipids). The present results unequivocally demonstrate that murine ischemia in wild-type mice is not accompanied by fatty acid release as is present in every other animal model of ischemia previously studied of which we are aware. We have exploited the natural species-specific knockdown of iPLA2 in the mouse (which does not possess substantial iPLA2 activity and does not release fatty acids or accumulate lysolipids during ischemia) to recapitulate complex ventricular tachyarrhythmias during murine myocardial ischemia by expressing amounts of iPLA2 activity in transgenic mice that are comparable to those present in wild-type rat, rabbit, dog, and human myocardium (6, 8, 10, 40). The fact that the observed arrhythmias were due to the catalytic competency of the expressed iPLA2 beta transgene was substantiated by the rescue of ischemia-induculated ventricular tachyarrhythmias in TGiPLA2 beta ischemic hearts through mechanism-based inhibition by BEL. Moreover rescue of malignant ventricular tachyarrhythmias in transgenic animals by BEL pretreatment just minutes prior to ischemia demonstrates that no preexisting abnormality was present in transgenic hearts predisposing them to arrhythmogenesis that was not immediately reversible by BEL. For example, the reversibility by BEL rules out developmental alterations in the conduction system of transgenic mice predisposing them to arrhythmias or anatomical developmental abnormalities as a cause of arrhythmias in this study. The demonstration that amounts of fatty acids released in TGiPLA2 beta hearts during ischemia are similar to amounts present in venous eluents in other animal and human paradigms of cardiac ischemia suggests that the amount of phospholipolysis occurring in this transgenic model has physiologic relevance.

Since cardiac electrophysiologic characteristics are dependent on the lipid constituents surrounding ion channels, it seems likely that ischemia induces activation of iPLA2beta in a way in that it can effectively access and hydrolyze sarcolemmal phospholipids. Indeed, in a cell culture model of cardiac ischemia, we have demonstrated the rapid and selective hydrolysis of sarcolemmal membrane lipids in response to metabolic deprivation by quantitative electron microscopic autoradiography (52). Many factors contribute to arrhythmogenesis in humans including the heterogeneity of ischemic damage, the metabolic history of the compromised myocyte, and the magnitude and duration of the ischemic insult. The present results show that in early ischemic injury prior to cell death (5–15 min) accelerated phospholipolysis can precipitate electrophysiologic dysfunction, while at later time points after irreversible injury and cell death (25–30 min), the frequency of arrhythmias in dying tissue is similar to that manifest in both WT and transgenic animals.

Although it was not known when initially discovered in the 1980s (2–10), this system is among the most ancestral of signaling systems, being extensively utilized in plants and insects. For example, a calcium-independent phospholipase A2 is the enzymatic mediator of the “elicitor”-evoked acidification of plant cytosol that is utilized to protect plants from invasion by bacteria or fungi (53). The elicitor system in plants utilizes R_{Kc} and R_{G} receptor-mediated activation of a calcium-independent phospholipase A2 in the plasma membrane leading to lysophosphatidylcholine generation and the subsequent activation of the vacuolar proton transporter resulting in cytosolic acidification (53). Similarly, in Periplanta americana, exposure to hypertrehalose factor II, or alternatively depletion of internal calcium stores by thapsigargin, results in iPLA2 activation and ryanodine receptor-mediated increases in calcium ion flux (54). Indeed we have demonstrated the calcium-dependent calmodulin-regulated modulation of myocardial iPLA2 activity (23) and have identified IQ and 1-9-14 motifs near the C terminus that mediate the interaction between iPLA2beta and calmodulin (25). Recent studies by Bolotina and co-workers (49) implicate iPLA2 as the enzyme system mediating altered ISOC and ICRAC currents in a variety of different cell types. Collectively these studies identify an ancestral signaling pathway hundreds of millions of years old that, in ischemic myocardium, leads to
electrical instability in ischemic zones in Homo sapiens. The precise electrical and ionic mechanisms that result from dysfunctional iPLA₂ activation during ischemia are complex with contributions from multiple signaling cascades likely initiated by lysolipids and fatty acids generated by iPLA₂ catalysis. Alterations in membrane molecular dynamics and aliphatic chain and polar head group conformational space likely contribute to the observed effects. Whatever the precise biochemical mechanisms underlying iPLA₂-mediated electrophysiologic dysfunction in ischemic zones, these results unambiguously demonstrate that iPLA₂β is activated in ischemic zones and hydrolyzes phospholipid substrate (accumulation of lysophosphatidylcholine) in ischemic zones and that iPLA₂ activation is sufficient to induce electrophysiologic dysfunction during cardiac ischemia. We specifically point out that these experiments do not mean that other factors do not contribute to ischemia-induced arrhythmogenesism but rather that ischemia-induced activation of iPLA₂β-mediated hydrolysis is sufficient to induce ventricular tachyarrhythmias with a time course, pattern, and frequency in the ischemic mouse heart that is strikingly similar to that present in humans (1, 32). These studies, in conjunction with the high iPLA₂β activity present in human myocardium (10, 41), strongly support the notion that iPLA₂β-mediated hydrolysis is a prominent, and perhaps a major factor, in ventricular electrical dysfunction and sudden death during human myocardial ischemia.

REFERENCES

1. Braunwald, E., Zipes, D. P., and Libby, P. (2001) *Heart Disease: A Textbook of Cardiovascular Medicine*, W. B. Saunders Company, Philadelphia, PA.
2. Gross, R. W., and Sobel, B. E. (1979) Trans. Assoc. Am. Physicians 92, 136–147.
3. Gross, R. W., and Sobel, B. E. (1982) J. Mol. Cell. Cardiol. 14, 619–626.
4. Gross, R. W. (1984) *Biochemistry* 23, 158–165.
5. Wolf, R. A., and Gross, R. W. (1985) *J. Biol. Chem.* 260, 7285–7303.
6. Haney, S. L., Stuppy, R. J., and Gross, R. W. (1990) *J. Biol. Chem.* 265, 10622–10630.
7. Ford, D. A., Hazen, S. L., Saffitz, J. E., and Gross, R. W. (1991) *J. Clin. Investig.* 88, 331–335.
8. Gross, R. W. (1992) Trends Cardiovasc. Med. 2, 115–121.
9. Hazen, S. L., and Gross, R. W. (1992) *Circ. Res.* 70, 486–495.
10. Hazen, S. L., and Gross, R. W. (1993) *J. Biol. Chem.* 268, 9892–9900.
11. Kim, D., and Clapham, DE. (1988) *Science* 244, 1174–1176.
12. Ordway, R. W., Walsh, J. V., Jr., and Singer, J. J. (1989) *Science* 244, 1176–1179.
13. Gubitosi-Klug, R., Yu, S. P., Choi, D. W., and Gross, R. W. (1995) *J. Biol. Chem.* 270, 2885–2888.
14. Tang, J., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) *J. Biol. Chem.* 272, 8567–8575.
15. Steward, A., Ghosh, M., Spencer, D. M., and Leslie, C. C. (2002) *J. Biol. Chem.* 277, 29526–29536.
16. Mancuso, D. J., Jenkins, C. M., and Gross, R. W. (2000) *J. Biol. Chem.* 275, 9937–9945.
17. Hazen, S. L., Zupan, L. A., Weiss, R. H., Getman, D. P., and Gross, R. W. (1991) *J. Biol. Chem.* 266, 7277–7232.
18. Hazen, S. L., Hall, C. R., Ford, D. A., and Gross, R. W. (1993) *J. Clin. Investig.* 92, 2513–2522.
19. Wolf, M. J., and Gross, R. W. (1996) *J. Biol. Chem.* 271, 30879–30885.
20. Larsen Forsell, P. K. A., Kennedy, B. P., and Claesson, H.-E. (1999) *Eur. J. Biochem.* 262, 575–585.
21. Liu, S. J., and McHowat, J. (1998) *Am. J. Physiol.* 34, H1162–H1142.
22. Broekemeier, K. M., Iben, J. R., LeVan, E. G., Crouser, E. D., and Pfeiffer, D. R. (2002) *Biochemistry* 41, 1522–1526.
23. Wolf, M. J., and Gross, R. W. (1996) *J. Biol. Chem.* 271, 29989–29992.
24. Wolf, M. J., Wang, J., Turk, J., and Gross, R. W. (1997) *J. Biol. Chem.* 272, 1522–1526.
25. Jenkins, C. M., Wolf, M. J., Mancuso, D. J., and Gross, R. W. (2001) *J. Biol. Chem.* 276, 7129–7135.
26. Radda, G. K. (1999) *Biochem. Biophys. Res. Commun.* 266, 723–728.
27. Das, D. K., Dillmann, W. H., Y-S, Lin, K. M., and Gross, B. R. (2002) Methods Enzymol. 353, 346–365.
28. Tian, R., and Abel, E. D. (2001) *Circulation* 103, 2961–2966.
29. Headrick, J. P., Feurt, J., Hack, B., Flood, A., and Matherne, G. P. (2001) *Exp. Physiol.* 86, 703–716.
30. Verdouw, P. D., van den Doel, M. A., de Zeeuw, S., and Duncker, D. J. (1998) *Cardiovasc. Res.* 39, 121–135.
31. Curtis, M. J. (1998) *Cardiovasc. Res.* 39, 194–215.
32. Lerner, D. L., Yamada, K. A., Schuessler, R. B., and Saffitz, J. E. (2000) *Circulation* 101, 547–552.
33. Landon, B. (2001) *J. Cardiovasc. Electrophysiol.* 12, 1089–1091.
34. Wit, A. L., and Janse, M. J. (1992) *Circulation* 85, 102–141.
35. Robbins, J., Subramaniam, A., and Glick, J. (1989) *Gene (Amst.)* 85, 541–544.
36. Laemmli, U. K. (1970) *Nature* 227, 680–685.
37. Han, X., and Gross, R. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10635–10639.
38. Han, X., and Gross, R. W. (1996) *Am. J. Physiol.* 271, C2695–C2709.
39. Hazen, S. L., Zupan, L. A., Weiss, R. H., Getman, D. P., and Gross, R. W. (1993) *J. Biol. Chem.* 268, 30713–30716.
40. Murakami, M., Kambe, T., Shimbara, S., and Kudo, I. (1999) *J. Biol. Chem.* 274, 3103–3115.
41. Kanai, M., Murakami, K., Kojima, K., Hadano, A., Tajima, M., and Kudo, I. (2000) *J. Biol. Chem.* 275, 18248–18258.
42. Gross, R. W., Ramanadham, S., Kruzska, K., Han, X., and Turk, J. (1993) *Biochemistry* 32, 327–336.
43. Ramanadham, S., Hsu, F.-F., Bohrer, A., Ma, Z., and Turk, J. (1999) *J. Biol. Chem.* 274, 13915–13927.
44. Gottlieb, R., and Williams, S. D. (2002) *Biochem. J.* 362, 23–32.
45. Siess, M., Zalkin, B., Lene, R., Cauduro, P., Trepakova, E., and Bobotina, V. M. (2003) *J. Biol. Chem.* 278, 11909–11915.
46. Janse, M. J., Opthof, T., and Kleber, A. G. (1998) *Cardiovasc. Res.* 39, 165–177.
47. Wit, A. L., and Janse, M. J. (1995) The Ventricular Arrhythmias of Ischaemia and Infarction. Electrophysiological Mechanisms, Futura Publishing, Mount Kisco, NY.
48. Saffitz, J. E., Corr, P. B., Lee, B. I., Gross, R. W., Williamson, E. K., and Sobel, B. E. (1984) *Lab. Invest.* 50, 278–286.
49. Viehweger, K., Dorschbahl, B., and Roos, W. (2002) *Plant Cell* 14, 1509–1525.
50. Sun, D., and Steele, J. E. (2002) *Insect Biochem. Mol. Biol.* 32, 1133–1142.
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