Calcium-independent phospholipase A2 mediates CREB phosphorylation and c-fos expression during ischemia

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Calcium-independent phospholipase A2 (iPLA2) is a predominant phospholipase A2 activity present in myocardium and is selective for arachidonoylated plasmalogen substrates (5, 12). Furthermore, iPLA2 is rapidly activated after global ischemia (5). We (21) recently demonstrated the activation of cAMP-dependent protein kinase (PKA) by lysoplasmenylcholine, an enzymic product of iPLA2-mediated hydrolysis of plasmenylcholine. This activation was found to be specific for lysophospholipids containing a choline polar head group. Thus the activation of PKA by iPLA2 catabolites may represent a key signal transduction mechanism for the phosphorylation of cAMP response element-binding protein (CREB) and the induction of nuclear gene expression in response to myocardial ischemia.

Elevation of intracellular cAMP levels can result in either stimulation or repression of specific gene expression, and most of these genes contain one or more cAMP response elements (CREs) (14). The signal transduction of cAMP is through PKA. The regulatory subunit of PKA binds cAMP and releases the active catalytic subunit (4). This subunit phosphorylates the transactivation domain of CREB at serine-133 as well as CRE modulator and several activating transcription factors, which induces the expression of genes containing CREs (19). The CRE proteins are basic leucine zipper transcription factors and are active as either homo- or heterodimers, which bind to the specific consensus sequence TGACGTCA CRE (10).

In cardiac myocytes, CREB has been shown to be expressed and phosphorylated in response to elevated levels of cAMP (6, 7). Primary embryonic chick heart cultures stimulated with forskolin or isoproterenol resulted in the increased phosphorylation of CREB protein along with a corresponding increase in CREB mRNA (6). More specifically, the CREB protein is present, but not phosphorylated, within the nuclei of myocytes isolated from neonatal rat hearts (7). Nuclear CREB protein was found to be phosphorylated after stimulation of neonatal myocytes with isoproterenol or forskolin (7). Furthermore, skeletal α-actin and α-myosin gene expression were increased after treatment with isoproterenol, suggesting that increased CREB phosphorylation induces nuclear gene expression (7, 8). Finally, CREB phosphorylation and CREB mRNA expression were identified in end-stage failing human hearts and in rat hearts subjected to prolonged β-adrenergic treatment (7, 15).

CREB phosphorylation during myocardial ischemia has not been extensively examined. Several protooncogenes (e.g., c-fos and c-jun) containing CREs have been highly expressed during reperfusion of ischemic myocardium (3). Because PKA is activated by lysoplasmenylcholine (21) and iPLA2 is rapidly activated during myocardial ischemia (5, 12), the hypothesis to be tested in the studies herein is that CREB is phosphorylated during ischemia.

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myocardial ischemia through an iPLA2-dependent pathway. Accordingly, the present study demonstrates for the first time that CREB is phosphorylated in response to myocardial ischemia and that iPLA2 regulates CREB phosphorylation during brief intervals of ischemia, whereas prolonged ischemia leads to cAMP accumulation, which also regulates CREB phosphorylation.

MATERIALS AND METHODS

Preparation of crude nuclear fractions from Langendorff-perfused rat hearts. Male Sprague-Dawley rats were utilized for preparation of Langendorff-perfused hearts as described previously (2, 5), and the protocol was in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. After the hearts were equilibrated, hearts were subjected to control perfusions, ischemia, or ischemia-reperfusion. In selected experiments, inhibitors such as bromoenol lactate (BEL), H-89, Rp-8-bromo-adenosine 3',5'-cyclic monophosphate (Rp-8-Br-cAMP), or the cAMP analog 8-Br-cAMP (Sigma) were included in perfusion buffers after equilibration before ischemia or perfusions with lipids. In other experiments, selected concentrations of lysoplasmalyethylamine (11), lysophosphatidylcholine (Avanti Polar Lipids), or lysoplasmylethanolamine (11) were included in the perfusion buffer.

Western blotting. Crude nuclear proteins were quantitated and normalized before Western blot analysis. Anti-CREB (1 μl/ml, rabbit; New England Biolabs), anti-phosphorylated CREB (Serine-133) (1 μl/ml, rabbit; New England Biolabs), and anti-iPLA2 (10 μg/ml, rabbit; Cayman Chemical) were utilized as primary antibodies along with the horseradish peroxidase-conjugated secondary antibody (1:7,000 dilution, goat anti-rabbit horseradish peroxidase; Sigma). Commercially available, positive controls (New England Biolabs) for either CREB or

![Western blot analysis](http://ajpheart.physiology.org/)

**Fig. 1.** cAMP response element-binding protein (CREB) phosphorylation during myocardial ischemia. Soluble nuclear fractions were prepared from isolated, perfused adult rat hearts subjected to the indicated experimental conditions and were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis as described in MATERIALS AND METHODS. **A:** Western blot analysis of phosphorylated CREB utilizing an antibody specific for phosphorylated CREB as the primary antibody. Phosphorylated CREB cell extracts (phospho-CREB) were used as a positive control for CREB phosphorylation. Nonphosphorylated cell extracts (CREB) were used as a negative control for phosphorylated CREB. **B:** Western blot analysis of total cellular CREB utilizing anti-CREB as the primary antibody as described in MATERIALS AND METHODS. **C:** bar graph showing quantitation of phosphorylated CREB. Intensity of each band from multiple analyses with anti-phosphorylated CREB was quantitated utilizing National Institutes of Health (NIH) Image software and expressed as a percentage of the intensity of the control. *P < 0.05 for comparisons between phosphorylated CREB from control-perfused hearts and phosphorylated CREB from hearts rendered globally ischemic for the indicated intervals.
phosphorylated CREB were prepared from cell extracts of human neuroblastoma cells (SK-N-MC cells) that were either untreated or treated, respectively, with fibroblast growth factor, 1-methyl-3-isobutylxanthine, and forskolin. Immunoreactive bands were visualized by chemiluminescence detection (Amersham) on X-ray film (X-OMAT AR). The intensity of each band from multiple analyses was quantitated with the use of NIH Image software and expressed as a percentage of the intensity of the control sample.

**cAMP and PKA assays.** Myocardial cAMP content was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Calbiochem). Results were expressed as picomoles of cAMP per milligram of ventricular protein. PKA activity was measured from reconstituted catalytic and regulatory subunits as previously described (21).

**RNA extraction and Northern blot analysis of c-fos expression.** Total RNA was isolated from 500 mg of powdered tissue from isolated, perfused rat hearts with the use of the RNAzol B reagent (Tel-Test). RNA (10 μg) was subjected to size fractionation on a 1% agarose gel containing formaldehyde and was transferred to a Duralon (Stratagene) membrane. RNA was fixed to the membrane by ultraviolet cross-linking with the use of a Stratalinker (Stratagene), and the membranes were prehybridized at 42°C for 6 h in 5× standard sodium citrate (SSC), 0.2% SDS, 2× Denhardt’s solution, 50% formamide, 50 mM KPO4, and 100 μg/ml denatured salmon sperm DNA, and hybridized for 18–36 h with 1×106 cpm/ml of α-32P-labeled probe, i.e., c-fos (Oncogene) or dlyceraldehyde-3-phosphate dehydrogenase (Clontech). Unbound probe was removed by washing in 0.1× SSC and 0.1% sodium dodecyl sulfate, and hybridized probe was visualized by detection on X-ray film (X-OMAT AR). Hybridized probe was quantitated with the use of NIH Image software and expressed as the degree of induction of c-fos mRNA.

**RESULTS**

**Elevated CREB phosphorylation during ischemia.** Because CREB is expressed and phosphorylated in cardiac myocytes and has been shown to be expressed and phosphorylated in failing human hearts (6, 15), the phosphorylation of CREB was determined in the Langendorff-perfused rat heart. Western blot analysis of nuclear fractions from perfused hearts indicated that...
CREB is rapidly phosphorylated (e.g., CREB is phosphorylated after 2 min of global ischemia) (Fig. 1, A and C). Only minimal levels of phosphorylated CREB were detected in control-perfused hearts. CREB phosphorylation steadily increased through 15 min of global ischemia. The increase in phosphorylated CREB is due to an increase in phosphorylation and not due to an increase in CREB protein because basal levels of CREB, as determined by Western blot analysis by using an antibody specific for CREB, did not change during global ischemia (Fig. 1B). The specificity of the phosphorylated CREB antibody was determined using nonphosphorylated and phosphorylated CREB control cell extracts. Phosphorylated CREB was detected exclusively in the phosphorylated CREB control lane by anti-phosphorylated CREB (Fig. 1A), whereas basal levels of CREB protein were detected in both phosphorylated and nonphosphorylated CREB lanes by anti-CREB (Fig. 1B).

**iPLA2-mediated CREB phosphorylation during myocardial ischemia.** Because we have recently shown that PKA can be activated by lysoplasmenylcholine (21), and because iPLA2 activity increases rapidly after myocardial ischemia (5, 12), the iPLA2-specific inhibitor BEL (12) was used to determine if CREB phosphorylation during myocardial ischemia was mediated by iPLA2. BEL (10 μM) inhibited CREB phosphorylation.

**Fig. 3.** Translocation of Ca^{2+}-independent phospholipase A2 (iPLA2) during myocardial ischemia. Membrane preparations containing nuclei were prepared from ventricular tissue of isolated, adult rat hearts subjected to either control perfusion or increasing intervals of global ischemia as described in MATERIALS AND METHODS. Membrane proteins were subjected to SDS-PAGE and Western blot analysis using anti-iPLA2 as the primary antibody and quantitated as described in MATERIALS AND METHODS.* P < 0.05 for comparison between control perfused hearts and ischemic conditions.

**Fig. 4.** Lysoplasmenylcholine (Lyso-PlasCho) induces CREB phosphorylation, which is reversible and inhibited by the cAMP-dependent protein kinase (PKA) inhibitor H-89. Isolated, adult rat hearts were either control perfused or perfused with 500 nM LysoPlasCho for the indicated time intervals. In selected experiments, hearts were perfused for 15 or 30 min in the absence of LysoPlasCho after LysoPlasCho treatments or were treated with either 10 μM of the inhibitors BEL (B) or H-89 (H) before perfusion with LysoPlasCho. A and B: soluble nuclear fraction from each experimental condition was subjected to SDS-PAGE, followed by Western blot analysis with anti-phosphorylated CREB as the primary antibody and was quantitated as described in MATERIALS AND METHODS.* P < 0.01 for comparisons between phosphorylated CREB from control-perfused hearts and phosphorylated CREB from hearts perfused with 500 nMol/L LysoPlasCho for the indicated intervals. †P < 0.01 for comparisons between phosphorylated CREB from control-perfused hearts and phosphorylated CREB from hearts perfused with LysoPlasCho and phosphorylated CREB from hearts perfused with LysoPlasCho, followed by perfusion without the addition of LysoPlasCho or hearts treated with H-89, followed by perfusion with LysoPlasCho. C: cAMP content of hearts subjected to the indicated experimental conditions was determined using an ELISA as described in MATERIALS AND METHODS.
iPLA2-specific inhibitor BEL results in a reduction in CREB phosphorylation in ischemic rat hearts. Accordingly, the levels of cAMP in isolated, perfused hearts subjected to 5 or 15 min of global ischemia were examined. The levels of cAMP were elevated twofold after 15 min of global ischemia compared with control perfused hearts (Fig. 2C). In contrast, there was no statistically significant increase in cAMP levels measured in hearts subjected to 5 min of global ischemia (Fig. 2C). These results, in conjunction with the observation that BEL attenuates CREB phosphorylation to a greater extent after 5 min of global ischemia compared with 15 min of global ischemia, suggest that iPLA2 mediates CREB phosphorylation in response to short intervals of myocardial ischemia and that cAMP mediates CREB phosphorylation in response to longer intervals of myocardial ischemia.

Because CREB is a target for phosphorylation by PKA, we next confirmed that CREB phosphorylation during myocardial ischemia is through a PKA-dependent mechanism. The PKA-specific inhibitors H-89 (10 μM) and Rp-8-Br-cAMP (10 μM) significantly reduced CREB phosphorylation in hearts subjected to both 5 and 15 min of global ischemia, with 5-min ischemic hearts treated with H-89 exhibiting no detectable phosphorylated CREB (Fig. 2B). Additionally, it should be appreciated that 15 min of reperfusion after 15 min of global ischemia resulted in a significant reduction in phosphorylated CREB (Fig. 2B).

iPLA2 translocation during myocardial ischemia. Because pretreatment of ischemic rat hearts with the iPLA2-specific inhibitor BEL results in a reduction in CREB phosphorylation, and because iPLA2 has been shown to be activated during myocardial ischemia (5, 12), the presence of iPLA2 in membrane preparations containing nuclei from control and globally ischemic hearts was determined. Western blot analyses demonstrated minimal amounts of iPLA2 present in the membrane preparations isolated from control-perfused hearts. In contrast, iPLA2 was translocated to the membrane fraction within 5 min of global ischemia (Fig. 3). Further increases in iPLA2 in this membrane fraction were observed with prolonged global ischemia (Fig. 3).

Induction of CREB phosphorylation by lysophosphatidylcholine. Because our present studies utilizing BEL have suggested that iPLA2 may mediate, in part, CREB phosphorylation during myocardial ischemia through a PKA-dependent mechanism, the ability of the product of iPLA2, lysoplasmenylcholine, to elicit CREB phosphorylation was assessed. Western blot analysis of crude nuclear proteins indicated that CREB phosphorylation is elevated after only 2 min of heart perfusion with 500 nmol/l lysoplasmenylcholine (Fig. 4A). The phosphorylation of CREB increased with increasing time intervals of perfusion with lysoplasmenylcholine. Perfusion of hearts with lysoplasmenylcholine for 15 min, followed by perfusion with lysoplasmenylcholine-free buffer resulted in a timedependent reduction in CREB phosphorylation (Fig. 4B). Additionally, H-89 pretreatment of lysoplasmenylcholine-treated hearts resulted in a significant decrease in phosphorylated CREB compared with the CREB phosphorylation obtained by lysoplasmenylcholine perfusion alone (Fig. 4B). In contrast, BEL pretreatment of lysoplasmenylcholine-treated hearts did not reduce phosphorylated CREB (Fig. 4B). Additionally, neither lysoplasmenylcholine nor BEL treatments of isolated adult rat hearts resulted in changes in measured cAMP compared with control-perfused hearts (Fig. 4C).

Because the in vitro activation of PKA by lysophospholipids containing a choline polar head group has been demonstrated (21), the effects of other lysophospholipids on CREB phosphorylation in isolated, perfused adult rat hearts was examined. Perfusion of hearts with either lysoplasmenylcholine or lysophosphatidylcholine at both concentrations tested (50 and 500 nM) resulted in CREB phosphorylation compared with that in control perfused hearts (Fig. 5). Lysoplasmeylcholine perfusions resulted in more CREB phosphorylation compared with lysophosphatidylcholine. In contrast, lysoplasmenylethanolamine did not elicit...
CREB phosphorylation above the levels observed in control perfused hearts (Fig. 5).

**CREB phosphorylation induced by 8-Br-cAMP treatment and lysoplasmenylcholine.** Because CREB is phosphorylated through a PKA-dependent mechanism, and because PKA can be activated by both cAMP and products of iPLA$_2$ (i.e., lysophospholipids) (21), we examined the effects of the exogenously added cAMP analog on CREB phosphorylation. Although 0.1 μM 8-Br-cAMP did not result in a significant increase in phosphorylated CREB compared with control perfused hearts (Fig. 6A), 1 μM of this cAMP analog elicited CREB phosphorylation (Fig. 6A). Simultaneous perfusion of hearts with both 50 nM lysoplasmenylcholine and 1 μM 8-Br-cAMP resulted in levels of phosphorylated CREB that were approximately the sum of CREB phosphorylation from perfusion with 50 nM of lysoplasmenylcholine and 1 μM 8-Br-cAMP independently (Fig. 6B). These data suggest that cAMP and lysoplasmenylcholine activate PKA through a common site on the PKA regulatory subunit. Further support for this common mechanism of PKA activation is provided by the findings that both cAMP and lysoplasmenylcholine activation of PKA, in an in vitro assay system, are attenuated by treatment with the PKA regulatory site inhibitor Rp-8-Br-cAMP (Fig. 6C). Additionally, Rp-8-Br-cAMP inhibited CREB phosphorylation after both 5 and 15 min of global ischemia (Fig. 2, A and B).

**Inhibition of ischemia-induced c-fos expression by BEL.** Because c-fos expression has been demonstrated to be highly induced during ischemia in porcine myocardium (3), and because c-fos expression in myocardium is regulated, in part, by the cAMP pathway (16), we determined if BEL treatment could attenuate c-fos expression during myocardial ischemia. The levels of c-fos mRNA expression were determined by Northern blot analysis (Fig. 7A). Global ischemia, as well as perfusion of hearts with lysoplasmenylcholine, significantly induced c-fos expression compared with the levels of c-fos mRNA present in control perfused hearts (Fig. 7B). Furthermore, treatment of hearts with BEL followed by global ischemia significantly reduced the levels of c-fos mRNA detected (Fig. 7B).

**Fig. 6.** Effects of lysoplasmenylcholine and 8-Br-cAMP perfusion on CREB phosphorylation. A and B: isolated, perfused adult rat hearts were either control perfused or perfused for 15 min with the indicated concentrations of the membrane-permeable cAMP analog 8-Br-cAMP and/or 50 nM LysoPlasCho. The soluble nuclear fractions from perfused hearts subjected to each indicated experimental condition were subjected to SDS-PAGE and Western blot analysis with anti-phosphorylated CREB as the primary antibody and quantitated as described in MATERIALS AND METHODS.*$P,$ 0.01 for comparisons between phosphorylated CREB from control perfused hearts and hearts perfused with either 50 nM lysoplasmenylcholine and 1 μM 8-Br-cAMP. †$P,$ 0.01 for comparisons between CREB phosphorylation from hearts perfused with 50 nM lysoplasmenylcholine and hearts perfused simultaneously with 50 nM lysoplasmenylcholine and 1 μM 8-Br-cAMP. C: in vitro kinase assay of reconstituted PKA regulatory and catalytic subunits using cAMP or LysoPlasCho as activators as described in MATERIALS AND METHODS. The PKA regulatory subunit inhibitor Rp-8-Br-cAMP and the PKA catalytic site inhibitor H-89 were utilized in selected experiments. †$P,$ 0.01 for comparisons between protein kinase activity measured using no stimulant and activity in the presence of cAMP or LysoPlasCho. **$P,$ 0.01 for comparisons between protein kinase activity measured using cAMP or lysoplasmenylcholine respectively, and activity in the presence of cAMP or lysoplasmenylcholine and either Rp-8-Br-cAMP or H-89.
DISCUSSION

The phosphorylation of CREB has been implicated in the transcriptional activation of many genes (e.g., c-fos, c-jun) induced by various stimuli (1, 18). Phosphorylated CREB binds to the distinct consensus sequences (CRE) in the promoter regions of genes regulated by the cAMP-signaling pathway (14). In the heart, it has been shown that CREB is phosphorylated in response to agents that increase intracellular cAMP, including forskolin and isoproterenol (6–8, 15). Although CREB phosphorylation has also been observed in end-stage heart failure, CREB phosphorylation has not been demonstrated in response to myocardial ischemia. The present study demonstrates for the first time that CREB is rapidly phosphorylated in response to brief intervals of myocardial ischemia.

Because PKA has been recently shown to be activated by lysoplasmenylcholine (21) and because iPLA₂ is activated by myocardial ischemia (5, 12), CREB phosphorylation mediated by iPLA₂ during myocardial ischemia was explored. The data presented herein support Fig. 8, which shows a likely mechanism through which PKA is modulated during myocardial ischemia ultimately leading to the phosphorylation of CREB and the expression of c-fos. PKA is initially activated during myocardial ischemia through the actions of iPLA₂-mediated production of lysophospholipids possessing a choline polar head group (see Fig. 8). Furthermore, the prolonged activation of PKA in response to extended intervals of myocardial ischemia is mediated by elevations in cAMP (see Fig. 8). Through either signal, PKA phosphorylates CREB which then leads to c-fos production. The early phase of activation shown in Fig. 8 (i.e., iPLA₂-mediated CREB phosphorylation via activation of PKA by lysophospholipids) is supported by the following four results from hearts rendered globally ischemic for 5 min. First, the iPLA₂ inhibitor BEL almost completely ablates CREB phosphorylation after 5

Fig. 7. Attenuation of c-fos expression by BEL treatment of ischemic myocardium. A: isolated, perfused adult rat hearts were either control perfused, subjected to global ischemia, treated with BEL followed by global ischemia, or perfused with LysoPlasCho as indicated. Northern blot analyses were performed using 3²P-labeled c-fos and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probes as described in MATERIALS AND METHODS. B: bar graph showing quantitation of c-fos mRNA expression from multiple analyses as described in MATERIALS AND METHODS. *P < 0.005 for comparison between induction of c-fos in ischemic hearts and controlperfused hearts. **P < 0.05 for comparison between induction of c-fos in lysoplasmemylcholine perfused hearts and control perfused hearts. †P < 0.005 for comparisons between c-fos mRNA after BEL treatment of ischemic hearts with c-fos mRNA detected after hearts subjected to ischemia without the addition of BEL.

Fig. 8. Proposed mechanisms mediating CREB phosphorylation in response to myocardial ischemia. LPC, choline-containing lysoglycerophospholipids (i.e., lysoplasmenylcholine and lysophosphatidyicholine).
min of global myocardial ischemia. Second, the PKA inhibitor H-89 inhibits CREB phosphorylation after 5 min of global myocardial ischemia. Third, cAMP levels do not increase in the first 5 min of global ischemia. Fourth, the product of iPLA₂, lysoplastenylcholine, elicits CREB phosphorylation in isolated, perfused hearts that are not subjected to ischemia. The second prolonged (or delayed) phase of activation is shown in Fig. 8 (i.e., CREB phosphorylation via activation of PKA by cAMP) and is supported by the following three results from hearts rendered globally ischemic for 15 min. First, the iPLA₂ inhibitor BEL only partially inhibited CREB phosphorylation after 15 min of global myocardial ischemia. Second, the PKA inhibitor H-89 inhibits CREB phosphorylation after 15 min of global myocardial ischemia. Third, cAMP levels increased twofold after 15 min of global ischemia. The downstream targeting of CREB phosphorylation mediating c-fos expression was also supported by the findings that c-fos is induced in ischemic myocardium, as well as myocardium treated with lysoplastenylcholine, and that BEL partially inhibits c-fos expression in ischemic myocardium. Thus the results herein strongly suggest that both lysophospholipid production mediated by iPLA₂ activity and cAMP production share an integral role in signal transduction during ischemia resulting in the phosphorylation of CREB and subsequent induction of immediate, early gene expression.

In tissues other than myocardium, the phosphorylation of CREB occurs after the translocation of the catalytic subunit of PKA to the nucleus (19). Nuclear phosphorylation of CREB by PKA occurs at a single site (serine-133), which then activates transcription (10). We have recently shown that lysoplastenylcholine, a product of iPLA₂ enzymatic action on plasmenylcholine, as well as other lysophospholipids containing a choline polar head group (e.g., lysophosphatidylcholine and lyso platelet activating factor), activate PKA in vitro through a cAMP-independent mechanism that likely involves dissociation of the catalytic subunit from the regulatory subunit (21). These previous studies, in conjunction with the present results, suggest that myocardial ischemia-induced activation of iPLA₂ may result in the formation of lysophospholipid second messengers that activate PKA, allowing the catalytic subunit of PKA to translocate to the nucleus and mediate the phosphorylation of CREB and the subsequent induction of immediate early gene transcription. Alternatively, iPLA₂ that is activated during myocardial ischemia may reside in the nucleus, resulting in production of the PKA activator, lysophospholipid, that would activate PKA locally at the nucleus.

It should also be appreciated that cAMP-independent CREB phosphorylation during early stages of ischemia potentially may be mediated by calmodulin-dependent kinase (17, 20). A role for iPLA₂ in this pathway could be mediated through alterations in intracellular calcium during myocardial ischemia mediated by the production of arachidonic acid and lysoplasmenylcholine (or lysophosphatidylcholine) (9). Although the present studies do not rule out this potential mechanism for ischemia-elicted CREB phosphorylation through the activation of calmodulin-dependent kinase, the present findings suggest that the majority of CREB phosphorylation during the ischemic intervals studied is mediated through PKA because PKA-specific inhibitors blocked CREB phosphorylation elicited by either ischemia or perfusions with lysoplasmenylcholine. Additionally, lysoplasmenylcholine directly activates PKA in kinase assays with purified PKA (21).

It has previously been shown that c-fos is rapidly expressed after myocardial ischemia (3). Immediate early genes, such as c-fos, are regulators of transcription, and it is hypothesized that the induction of immediate early genes (i.e., c-fos) in response to myocardial ischemia may lead to the activation of genes involved in the repair of reversible damage to the myocardium (3). The expression of c-fos during myocardial ischemia likely occurs through the activation of PKA and subsequent CREB phosphorylation because c-fos contains a CRE and is regulated by PKA in the adult rat heart (16). Furthermore, the present results suggest that c-fos is induced through biochemical mechanisms involving the bimodal regulation of PKA by lysoplastenylcholine and cAMP during myocardial ischemia (Fig. 8).

In summary, the results herein support a signaling role of iPLA₂ activation during myocardial ischemia that includes the sequential downstream activation of PKA, CREB phosphorylation, and early gene induction. Additionally, a bimodal regulatory mechanism is proposed for this signal transduction pathway that is mediated by iPLA₂ during the early stages of ischemia, whereas cAMP levels activate this pathway during prolonged ischemia. Thus the present results demonstrate a novel signaling pathway mediated by iPLA₂, which represents the first time a signaling pathway has been shown to be mediated by iPLA₂. Furthermore, these studies demonstrate that iPLA₂ activated during myocardial ischemia likely plays an important role in myocardial ischemia through this pathway that results in the production of early gene products.

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