Changes in Phosphoinositide Turnover in Isolated Guinea Pig Hearts Stimulated With Isoproterenol

Istvan Edes, R. John Solaro, and Evangelia G. Kranias

The incorporation of $^{32}$P into phospholamban, troponin I, phosphatidylinositol, and inositol trisphosphates was studied in Langendorff-perfused guinea pig hearts stimulated with isoproterenol. Hearts were perfused with Krebs-Henseleit buffer containing $[^{32}P]$P, and freeze-clamped at different times during the positive inotropic response. Exposure of the hearts to 0.1 μM isoproterenol for up to 1 minute was associated with significant (up to threefold) increases in phospholamban and troponin I phosphorylation, but there was no significant increase in $^{32}$P incorporation into phospholipids. However, longer exposure (2 minutes or more) to isoproterenol was associated with increases in the degree of $^{32}$P labeling of phosphatidylinositols and phosphatidic acid. Examination of $^{32}$P labeling of inositol trisphosphates in the same hearts revealed that the radioactivity associated with these compounds decreased with time. The decreases were significant at times of exposure of 2 minutes or longer to β-adrenergic stimulation. The tissue levels of the inositol 1,4,5-trisphosphate isoform were also measured in hearts perfused with isoproterenol for 3 minutes, and they were found to be significantly lower compared with values obtained in control hearts. The effects of isoproterenol on $^{32}$P incorporation into phospholipids and proteins were observed in the presence of prazosin, and they were completely abolished by the β-receptor blocker propranolol. Examination of the phosphoinositide-specific phospholipase C activity in the perfused hearts revealed that isoproterenol stimulation was associated with a decrease in the membrane-associated enzymatic activity at physiological calcium concentrations. These findings indicate that β-adrenergic stimulation of isolated hearts is associated with changes in basal phosphatidylinositol turnover that may be mediated, at least in part, by inhibition of the phospholipase C enzymatic activity specific for phosphatidylinositols. (Circulation Research 1989;65:989-996)
due to $\beta$-agonist stimulation. Thus, the response of heart cells to $\beta$-adrenergic stimulation is likely to involve coordinated changes in the extent of cAMP-dependent phosphorylation of several regulatory proteins. However, besides protein phosphorylation, the phosphorylation of a special class of phospholipids (polyphosphoinositides) has also been shown to occur as a result of activation of cAMP-dependent protein kinase under in vitro\textsuperscript{3-12} and in vivo\textsuperscript{13} conditions. Increased $^{32}$P incorporation into phosphatidylinositol (PI), phosphatidylinositol 4-monophosphate (PIP), and phosphatidylinositol 4,5-diphosphate (PIP$_2$) was found in perfused guinea pig hearts subjected to $\beta$-adrenergic stimulation.\textsuperscript{13} These rapid changes in the amounts of polyphosphoinositides have been proposed to be involved in mediating, at least in part, the effects of $\beta$-adrenergic agents.\textsuperscript{13} However, there are no data on the mechanism by which activation of the $\beta$-receptor is associated with increased formation of polyphosphoinositides in the mammalian myocardium.

In this report, we present evidence that prolonged activation of the $\beta$-receptor in perfused, beating hearts is associated with 1) increased $^{32}$P labeling of phosphatidylinositol; 2) decreased $^{32}$P labeling of inositol trisphosphates; and 3) decreased activity of the membrane-associated phosphoinositide-specific phospholipase C (PI-PLC). These findings suggest that $\beta$-receptor stimulation may be involved in the regulation of the basal phosphoinositide turnover through the inhibition of PI-PLC in the mammalian heart.

### Materials and Methods

#### Heart Perfusion

Hearts from anesthetized (30 mg/kg sodium pentobarbital) and heparinized (500 units/kg) Hartley guinea pigs were rapidly excised and immediately cannulated for retrograde aortic perfusion with Krebs' buffer-balanced salt solution containing (mM) NaCl 118, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, NaHCO$_3$ 25, Na$_2$EDTA 0.5, KH$_2$PO$_4$ 0.23, and glucose 5.5 yielding an effective Ca$^{2+}$ concentration of 2 mM. The buffer solution was saturated with 95% O$_2$-5% CO$_2$, pH 7.4, and bath temperature was set at 37°C. The hearts were initially perfused at a constant aortic pressure (65 mm Hg) for 20–25 minutes in a drip-through mode. The perfusion circuit was then switched to a recirculating system containing 120 ml buffer and 2 mCi $^{32}$P orthophosphate ($^{32}$P$_2$P$_4$) for 30 minutes. After the labeling period, the circuit was returned to a nonrecirculating flow with nonradioactive buffer for 1 minute. The drugs of interest (0.1 $\mu$M isoproterenol) were then introduced into the buffer flow line. At different times of the positive inotropic response, the hearts were freeze-clamped with precooled (-196°C) Wollenberger clamps, powdered, and stored under liquid nitrogen, as we previously described.\textsuperscript{14}

To ensure the blockade of the $\alpha_1$-receptor-mediated processes in the perfused hearts, isoproterenol was administered in the presence of 0.1 $\mu$M prazosin.\textsuperscript{15} The heart rate, ventricular pressure, and its first derivative (dP/dt) were continuously monitored through a pressure transducer attached to a multichannel polygraph (Grass Instruments, Quincy, Massachusetts).

#### Preparations of Membrane Vesicles Enriched in Sarcoplasmic Reticulum and Myofibrils

Microsomes enriched in SR membranes were prepared as we previously described\textsuperscript{14} with the following slight modifications. The frozen powdered tissue was simultaneously thawed and homogenized for 15 seconds with a Polytron homogenizer (model PT-10, Brinkmann Instruments, New York, New York) in 10 vol ice-cold buffer containing (mM) KH$_2$PO$_4$ 30 at pH 7.0, NaF 70, EDTA 5, succrose 0.3 (M), phenylmethylsulphonyl fluoride 0.3, and dithiothreitol 0.5.

For the myofibrillar protein preparation, the starting material was the pellet obtained after the first centrifugation of the heart homogenate during SR isolation. The purification of myofibrils was carried out by previously described procedures.\textsuperscript{16,17} The protein content was determined by the amido black technique\textsuperscript{18} with bovine serum albumin as standard.

#### Gel Electrophoresis

Polyacrylamide gel electrophoresis under denaturing conditions was performed by the procedure described by Laemmli\textsuperscript{19} with 5–22.5% gradient slab gels. Samples of $^{32}$P-labeled SR or $^{32}$P-labeled myofibrils were solubilized in electrophoresis sample buffer containing 62.5 mM Tris-HCl at pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, 5% $\beta$-mercaptoethanol, and a trace amount of bromophenol blue. Generally, 150–200 $\mu$g protein was loaded on each lane of the gel. After electrophoresis, the gels were fixed, stained with Coomassie blue, destained, and placed in sealed plastic bags into Kodak Lanex Regular cassettes loaded with Kodak X-Omat films. The radioactive bands, corresponding to phospholamban and troponin I, were identified and cut from the gels for counting in a liquid scintillation counter. Phosphate incorporation was calculated by using the specific activity of $[\gamma^{32}]$P]ATP determined for each individual heart, which was expressed as pmol $[\gamma^{32}]$P/mg protein.

#### Preparation of Phosphatidylinositol Derivatives and Inositol Trisphosphates

Polyphosphoinositide extraction from the powdered tissue was accomplished by the method of Schachat.\textsuperscript{20} The final lipid extracts were dried under vacuum and used immediately for determination of total lipid phosphorous\textsuperscript{21} and for further analysis on thin layer chromatography according to Jolles et al.\textsuperscript{22} Total lipid phosphorous was used to verify that the amounts of phospholipids applied to thin layer chromatography were similar between control and isoproterenol-treated hearts. After thin layer chro-
matography, the plates (Silicagel-G, Fisher, Cincinnati, Ohio) were placed in contact with Kodak X-Omat film in Kodak Lanex Regular cassettes. The identified radioactive spots were scraped from the plates and counted for radioactivity. Identification of phospholipids was accomplished by running standards on the same plate as the extracted lipids. Results were corrected for recoveries, which were monitored with the inclusion of [3H]PI (90%), [3H]PIP (65%), and [3H]PIP2 (55%) in cardiac homogenates.

The inositol phosphates were extracted from the powdered tissue with chloroform-methanol according to Zilberman et al., and the final combined aqueous phases were dried under vacuum. The isoproterenol and Cardiac Phospholipids isolation of inositol trisphosphates (inositol 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate) was performed in two steps. In the first step, partial purification was achieved on a Dowex-1 column (formate form) as previously described. The column was calibrated with various sugar phosphates. Examination of the fraction containing inositol trisphosphates revealed that it was absolutely free of different sugar monophosphates or bisphosphates as well as creatine phosphate, but it was heavily contaminated with 32P-labeled ATP. The presence of heavily labeled ATP in this fraction interfered with analysis of the inositol trisphosphates by high-pressure liquid chromatography. Thus, paper electrophoretic separation was chosen for the final purification of inositol trisphosphates, but this procedure did not allow separation of inositol 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate. Therefore, the results reflect the combined radioactive activity in the two isoforms of inositol trisphosphate.

The electrophoretic mobility of inositol trisphosphates was more than two times higher than that of ATP, and a complete separation could be easily achieved by this method. After electrophoresis, the paper was dried and placed in contact with Kodak X-Omat film. The identified spots were cut from the paper and counted for radioactivity. In all experiments, the isolated inositol trisphosphate fraction contained 500–800 cpm/ aliquot counted (background, 50–70 cpm). The measurements were corrected for recovery (70–80%), which was monitored with [3H]inositol 1,4,5-trisphosphate.

**Phosphoinositide-Specific Phospholipase C Activity Measurements**

Hearts were perfused with nonradioactively labeled Krebs' buffer and freeze-clamped after 3 minutes of stimulation with 0.1 μM isoproterenol. The isolation of sarcolemma-enriched membrane vesicles was carried out as previously described, except that okadaic acid (0.1 μM) was included in all the isolation buffers to inhibit the endogenous phosphatase activities. Fluoride, which we have generally used as a phosphatase inhibitor, was omitted from the isolation buffers since this anion may activate the PI-PLC enzyme. Okadaic acid, at concentrations of 0.1 μM, did not interfere with the PI-PLC enzymatic activity, and it was found to stabilize the 32P-phosphoester bonds obtained in situ (authors' unpublished observations). The yield of the sarcolemma-enriched membrane preparation was 0.5–0.8 mg protein from one guinea pig heart. The pattern and relative proportions of membrane proteins were the same in each preparation from either control or isoproterenol-treated hearts. Various enzyme markers were assayed in the sarcolemma-enriched membrane preparation, and they were found to be similar in the control and isoproterenol-treated hearts. Cytosolic contamination was minimal as judged by the levels of the specific activity of lactate dehydrogenase in the membrane preparation (less than 0.5% of the cell homogenate activity assayed either in the presence or absence of Triton X-100). Mitochondrial contamination was estimated by the levels of the sodium azide–inhibitable ATPase activity, and it was found to be 4–5% of the levels present in the cell homogenates. The levels of the Ca2+-dependent ATPase activity were 8.7±0.5 μmol P/hr/mg sarcolemma-enriched membranes compared with 4.9±0.4 μmol P/hr/mg homogenate protein. Na+,K+-ATPase activity was used as the enzymatic marker for sarcolemma. Patient and latent Na+,K+-ATPase activities were measured as previously described by preincubating the membranes (0.5 mg/ml) in the presence of sodium dodecylsulfate (0.4 mg/ml) for 20 minutes at room temperature. Activity was subsequently measured at 37° C, and it was found to be 18.0±1.9 μmol P/hr/mg sarcolemma-enriched membranes compared with homogenates, which had an activity of 5.9±0.8 μmol P/hr/mg protein. These levels of the Na+,K+-ATPase activity in the sarcolemma-enriched preparation were comparable with previously described levels for guinea pig hearts.

PI-PLC activity was assayed in a reaction mixture (200 μl) containing 10 μg sarcolemma-enriched membrane preparation in the presence of either [3H]PIP or [3H]PIP2 as substrates (20 μM, 3,000–3,500 cpm/nmol). We have recently characterized the PI-PLC activity in guinea pig hearts (I. Edes, E.G. Kranias, submitted manuscript), and we have determined optimal assay conditions for this enzymatic activity, which were used in the present study. Optimal hydrolysis of either PIP or PIP2 occurred in the presence of 50 mM bis-Tris buffer, pH 7.0, containing (mM) MgCl2 2.5, NaCl 100, free Ca2+ 1, and 0.07% (wt/vol) deoxycholate. The phospholipid substrates (PIP and PIP2) were dried under N2, deoxycholate was added, and the solutions were vortexed and sonicated before their use. Reactions were incubated in duplicates at 37° C for 3 minutes, and they were terminated by adding 1 ml chloroform-methanol-HCl (50:30:0.2, v:v:v). Samples were then mixed with 0.3 ml of 1 M HCl containing 5 mM EGTA and centrifuged to obtain a phase separation. An aliquot of the aqueous layer was counted to determine formation of the labeled reaction prod-
ucts, inositol bisphosphate or inositol trisphosphate. Results were corrected for the nonenzymatic hydrolysis, which occurred in the absence of added proteins. Under these assay conditions, the PI-PLC activity was linear with time (3 minutes); either PIP or PIP$_2$ was used as substrate. The enzymatic activity was dependent on calcium and was found to be specific for PIP and PIP$_2$. No other phospholipids (phosphatidylinositol, phosphatidylcholine, or phosphatidylethanolamine) were hydrolyzed under our assay conditions, up to 20 minutes at 37° C with various detergent (deoxycholate) and calcium concentrations. Analysis of the end products of the reaction revealed that more than 95% of the radioactivity was associated with the inositol 1,4,5-trisphosphate fraction when PIP$_2$ was used as substrate.

**Other Procedures**

Tissue levels of cAMP and of inositol 1,4,5-trisphosphate were determined with specific assay kits ($[^3H]$cAMP assay kit and $[^3H]$inositol 1,4,5-trisphosphate assay system, Amersham, Arlington Heights, Illinois). Hearts were perfused as described above, except that nonlabeled phosphate buffer was used. The cAMP and inositol 1,4,5-trisphosphate determinations were carried out within 1 week after the perfusions. Results were corrected for recoveries, which were monitored with $[^3H]$cAMP (70–80%) or $[^3H]$inositol 1,4,5-trisphosphate (75–85%) included in separate portions of the cardiac homogenates.

The specific radioactivity of $[^3P]$ATP of each perfused heart was determined from the specific activity of $[^3P]$phosphocreatine at the end of perfusion. There was no significant change in the measured specific activity of $[^3P]$ATP among hearts perfused on a particular day with the same stock solution of $[^3P]$Pi. The specific radioactivity (mean±SD) determined in cpm/pmol ATP was 11.3±2.2 (n=4) for controls and 11.1±1.4 (n=3), 10.9±1.0 (n=4), 11.6±2.2 (n=4), and 12.0±2.0 (n=4) for hearts frozen after 0.5, 1, 2, and 3 minutes of perfusion with isoproterenol, respectively. Equilibrium between intracellular $[^3P]$Pi, $[^3P]$ATP, and $[^3P]$phosphocreatine was reached within 20 minutes of perfusion with $[^3P]$-labeled buffer.

Statistical analysis was performed using the Student's $t$ test for unpaired observations, and values with $p<0.05$ were regarded as statistically significant.

**Results**

Isoproterenol, at a concentration of 0.1 μM, produced a rapid time-dependent increase in myocardial contractility (as measured by $+dP/dt$ of left ventricular pressure). This positive inotropic effect reached a peak (240% of control) at 30–45 seconds; subsequently, it slowly declined (Figure 1). The rate of myocardial relaxation (as measured by $-dP/dt$ of left ventricular pressure) also increased on exposure to isoproterenol. However, the time to reach the maximal level of $-dP/dt$ was slightly longer (60 seconds) than for $+dP/dt$, and this level was subsequently maintained for up to 3 minutes. Isoproterenol elicited a positive chronotropic response, producing a gradual and continuous elevation in the heart rate (Figure 1).

The time course of the effect of isoproterenol on $^{32}$P incorporation into phospholamban and the inhibitory subunit of troponin (troponin I) is shown in Figure 2. Increases in phosphorylation of these two proteins occurred in a similar manner. There was an initial rapid increase in $^{32}$P incorporation into both proteins on exposure to isoproterenol, which is in agreement with previous observations. Prolonged (≥2 minutes) β-adrenergic stimulation resulted in a further gradual increase in $^{32}$P incorporation in phospholamban and troponin I. A similar time course was observed for the intracellular cAMP content after β-adrenergic stimulation. In the isoproterenol-stimulated hearts, there was a close correlation between elevation of cAMP levels and in $^{32}$P incorporation into phospholamban (r=0.95) and troponin I (r=0.92). Both correlations, calculated with a computer program using the Fisher $z$ transformation, were significant ($p<0.025$).

Analysis of the $^{32}$P incorporation into individual phospholipids in cardiac homogenates (Figure 3) revealed that prolonged (≥2 minutes) isoproterenol stimulation was associated with considerable increases in the degree of $^{32}$P labeling of phosphatidylinositol and phosphatidic acid. The time courses of $^{32}$P incorporation into the individual phosphati-
dyinositol (PI, PIP, and PIP2) were similar (Figure 4); they showed a slow elevation in the degree of labeling, which was insignificant for the first minute of exposure to isoproterenol. However, prolonged (≥2 minute) exposure was associated with significant (p<0.05) increases in 32P labeling of the phospholipids. In hearts, perfused with isoproterenol for 2 or 3 minutes, the increase in 32P incorporation in PIP2 corresponded to increases in the amount of this compound. Thin layer chromatography of the phospholipid fraction (0.6–0.7 μmol lipid phosphorous), which was extracted from these hearts, revealed PIP2 as a faint band that followed the charring reaction (with 50% sulphuric acid). Furthermore, the P, content of this compound could be measured (data not shown). However, under similar experimental conditions, the PIP2 band, which was from control hearts or hearts stimulated with isoproterenol for 1 minute or less, could not be detected with either the charring reagent or iodine vapor. The 32P incorporation into phosphatidic acid increased in

**Figure 2.** Time course of cyclic AMP levels (■) and phosphorylation of phospholamban (●) and troponin I (○) by isoproterenol stimulation. Hearts were perfused and freeze-clamped as described in the legend of Figure 1. Assays for cyclic AMP content, 32P incorporation into phospholamban, and troponin I were performed as described under "Materials and Methods."

**Figure 3.** Autoradiogram of phospholipids after lipid extraction and thin layer chromatography. Control (C) and isoproterenol (Iso)-stimulated (for 0.5, 1, 2, and 3 minutes) hearts were perfused and freeze-clamped as described in the legend of Figure 1. Lipid extraction and chromatography were carried out as described under "Materials and Methods." PA, phosphatidic acid; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-monophosphate; PIP2, phosphatidylinositol 4,5-diphosphate.

**Figure 4.** Time course of 32P incorporation into phosphatidylinositols in isoproterenol-perfused hearts. Hearts were perfused as described in the legend of Figure 1. The extracted lipids were separated by thin layer chromatography (see Figure 3), and the 32P incorporation into phosphatidylinositol (○, PI), phosphatidylinositol 4-monophosphate (●, PIP), phosphatidylinositol 4,5-diphosphate (□, PIP2), and phosphatidic acid (■, PA) was determined. Values represent the mean±SD of at least three different hearts. *p<0.05 when compared with control (0 minute) values.
the first 2 minutes as a result of isoproterenol treatment; subsequently, it appeared to slightly, but not significantly (Student’s t test), decrease (Figure 4).

Examination of the [\(^{32}\)P]phosphate associated with inositol trisphosphates in the same hearts revealed that the labeling of these metabolites, derived from PIP\(_2\), declined with time on isoproterenol stimulation (Figure 5). To investigate selective changes in the inositol 1,4,5-trisphosphate fraction, the absolute tissue level of this compound was also determined from hearts perfused with nonradioactive buffer. After 3-minute perfusion with isoproterenol, a significant (p<0.05) decrease was found in the inositol 1,4,5-trisphosphate level (32.8±3.1 pmol/mg protein) as compared with controls (44.0±1.9 pmol/mg protein). Thus, there appeared to be an inverse relation between the time courses of \(^{32}\)P incorporation into inositol trisphosphates (\(\psi\)) in isoproterenol-perfused hearts. Hearts were perfused as described in the legend of Figure 1. Inositol trisphosphates were extracted and isolated as described under “Materials and Methods.” Values represent the mean±SD of at least three different hearts. *p<0.05 when compared with control (0 minute) value.

Figure 5. Time course of \(^{32}\)P incorporation into inositol trisphosphates (\(\psi\)) in isoproterenol-perfused hearts. Hearts were perfused as described in the legend of Figure 1. Inositol trisphosphates were extracted and isolated as described under “Materials and Methods.” Values represent the mean±SD of at least three different hearts. *p<0.05 when compared with control (0 minute) value.

In the present study, we report that prolonged (2 minutes or longer) stimulation of \(\beta\)-adrenergceptors by isoproterenol resulted in increases in the \(^{32}\)P labeling of phosphatidylinositolos and a decline in the levels of \(^{32}\)Pinositol trisphosphates in perfused guinea pig hearts. These changes on phosphatidylinositol turnover by isoproterenol were observed in the presence of the \(\alpha\)-blocker propranolol, and they were completely abolished by the \(\beta\)-receptor blocker timolol; these findings suggest that they were mediated through \(\beta\)-receptor activation.

TABLE 1. Effect of Isoproterenol on Membrane-Associated PI-PLC Activity in Perfused Beating Hearts

<table>
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<tr>
<th>PIP or PIP(_2)</th>
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<td>(nmol/min/mg)</td>
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</tr>
<tr>
<td>Control</td>
<td>Isoproterenol</td>
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<tr>
<td>Control</td>
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Results are expressed as the mean±SD of six hearts. PIP, phosphatidylinositol 4-monophosphate; PIP\(_2\), phosphatidylinositol 4,5-diphosphate. Hearts were perfused with 0.1 \(\mu\)M isoproterenol and freeze-clamped after 3 minutes, as described in the “Materials and Methods” section. The phosphoinositide-specific phospholipase C activity was measured from the sarcolemma-enriched membrane fraction with [\(^{3}\)H]PIP or [\(^{3}\)H]PIP\(_2\) used as substrates.

\(*p<0.05\) when compared with control values.

Discussion

In the present study, we report that prolonged (2 minutes or longer) stimulation of \(\beta\)-adrenergceptors by isoproterenol resulted in increases in the \(^{32}\)P labeling of phosphatidylinositolos and a decline in the levels of \(^{32}\)Pinositol trisphosphates in perfused guinea pig hearts. These changes on phosphatidylinositol turnover by isoproterenol were observed in the presence of the \(\alpha\)-blocker prazosin, and they were completely abolished by the \(\beta\)-receptor blocker propranolol; these findings suggest that they were mediated through \(\beta\)-receptor activation.

The increased \(^{32}\)P labeling of phosphatidylinositolos (PI, PIP, and PIP\(_2\)) on isoproterenol stimulation may be either due to increases in their rate of synthesis (by stimulation of PI synthase/PI kinase/PIP kinase) or to decreases in their rate of hydrolysis. Formation of phosphatidylinositolos has been previously shown to be stimulated by cAMP-dependent protein kinase in membrane preparations from different tissues.5–12 However, the mechanism of this stimulatory effect is not known. In the isoproterenol-perfused hearts, increases in the \(^{32}\)P labeling of phospholipids did not correlate well with increases either in protein phosphorylation or cAMP levels. This apparent lack of correlation suggests that the mechanism underlying increases in the \(^{32}\)P labeling of phosphatidylinositolos may be, at least partially, different than the one-step protein kinase
A-mediated phosphorylation of phospholamban and troponin I in situ. Concomitant with increases in the levels of radioactivity associated with phosphatidylinositol, there was a decrease in the degree of 32P labeling of inositol trisphosphates, and this was associated with decreases in the tissue level of inositol 1,4,5-trisphosphate. Thus, the changes in the phosphatidylinositol cycle intermediates, on isoproterenol stimulation, appear to be mediated through a decrease in the PI-PLC enzymatic activity. Indeed, examination of the membrane-bound PI-PLC activity in the isoproterenol-perfused hearts revealed that the enzymatic activity significantly decreased. However, the changes in phosphatidic acid labeling observed on isoproterenol perfusion cannot be explained only on the basis of inhibition of the PI-PLC enzyme. It is possible that additional pathways, which contribute to the synthesis of this phospholipid, were also stimulated by isoproterenol in these hearts.

Elevated cAMP levels were also previously shown to inhibit the agonist-stimulated PI-PLC activity in human thrombocytes and neutrophils. However, it is not clear whether these inhibitory effects on the PI-PLC activity involve modification (phosphorylation) of the enzyme or whether they may be mediated through its regulatory membrane protein. In the sarcotubular membrane preparation, isolated from 32P-labeled perfused beating hearts in this study, there was increased phosphorylation of only the phospholamban and the 15 kDa sarcoplasmic protein after isoproterenol stimulation; these observations were in agreement with previous observations. However, it is possible that phosphorylation of PI-PLC had occurred in situ, but this phosphoprotein was not detectable under our experimental conditions.

It has been previously suggested that activation of the PI-PLC enzyme may be involved in mediating the effects of the α1- and the muscarinic-receptor agonists in the mammalian heart. However, activation of the PI-PLC through the muscarinic receptor system usually requires a high agonist concentration and may not be of physiological significance. Short stimulation of the PI-PLC enzyme by α1-agonists was shown to be associated with an initial rapid loss of phosphoinositides from the labeled PIP and PIP2 pools whereas the label associated with the inositol trisphosphate fraction increased. Based on these previous findings and our own findings with the isoproterenol-perfused hearts, it appears that α1-receptor activation may initially have opposite effects than β-receptor activation on the PI cycle intermediates. In pharmacological studies on rat and rabbit papillary muscles, a functional competition was actually observed between the two adrenergic (α1 and β) systems. Stimulation of the β-adrenergic system attenuated the expression of the α1-receptor–related functional effects. Furthermore, attenuation of the β-receptor system by β-blockers or by muscarinic agonists (carbachol) resulted in a better expression (reinforcement) of the α1-receptor–mediated inotropic responses.

Our findings with beating guinea pig hearts indicate that prolonged stimulation of the β-receptor is associated with changes in the PI cycle intermediates and with attenuation of the membrane-associated PI-PLC basal activity. It is interesting to propose that the PI-PLC enzyme, which has been suggested to mediate the α1-receptor–related responses, may be attenuated by β-adrenergic agents in cardiac muscle. Further studies on the regulation of the PI-PLC enzyme and its interrelation with the different receptor systems will elucidate the role of this enzyme in the mammalian myocardium.

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**KEY WORDS**
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- inositol trisphosphates
- phospholipase C
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