Phosphorylation of phospholipids in isolated guinea pig hearts stimulated with isoprenaline

Gyorgyi JAKAB,* Stephen T. RAPUNDALO,* R. John SOLARO† and Evangelia G. KRANIAS*

*Department of Pharmacology and Cell Biophysics and †Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267, U.S.A.

Phosphorylation of phospholipids was studied in Langendorff perfused guinea pig hearts subjected to β -adrenergic stimulation. Hearts were perfused with Krebs–Henseleit buffer containing [^{32}P]P_i and freeze-clamped in a control condition or at the peak of the inotropic response to isoprenaline. ^{32}P incorporation into total phospholipids, individual phospholipids and polyphosphoinositides was analysed in whole tissue homogenates and membranes, enriched in sarcoplasmic reticulum, prepared from the same hearts. Isoprenaline stimulation of the hearts did not result in any significant changes in the levels of phosphate incorporation in the total phospholipid present in cardiac homogenates (11.6 ± 0.4 nmol of ^{32}P /g for control hearts and 12.4 ± 0.5 nmol of ^{32}P /g for isoprenaline-treated hearts; $n = 6$), although there was a significant increase in the degree of phospholipid phosphorylation in sarcoplasmic reticulum (3.5 ± 0.3 nmol of ^{32}P /mg for control hearts and 6.7 ± 0.2 nmol of ^{32}P /mg for isoprenaline-treated hearts; $n = 6$). Analysis of ^{32}P incorporation into individual phospholipids and polyphosphoinositides revealed that isoprenaline stimulation of the hearts was associated with a 2–3-fold increase in the degree of phosphorylation of phosphatidylinositol monophosphate and bisphosphate as well as phosphatidic acid in both cardiac homogenates and sarcoplasmic reticulum membranes. In addition, there was increased phosphate incorporation into phosphatidylinositol in sarcoplasmic reticulum membranes. Thus, perfusion of guinea pig hearts with isoprenaline is associated with increased formation of polyphosphoinositides and these phospholipids may be involved, at least in part, in mediating the effects of β -adrenergic agents in the mammalian heart.

INTRODUCTION

The dynamics of contraction and relaxation in cardiac muscle are modulated by a number of physiological and pharmacological interventions, among which β -adrenergic stimulation appears to represent a key control mechanism. The β -adrenergic actions of catecholamines on the myocardium are mediated by cyclic AMP-dependent phosphorylation of a variety of intracellular proteins leading to functional modification. The phosphoproteins include intermediates of the glycogenolytic cascade, the outer cell membrane or sarcolemma, the myofibrils and the sarcoplasmic reticulum. The protein phosphorylated in sarcolemma, due to β -adrenergic stimulation by isoprenaline, has an M_r of 15000 (Presti *et al.*, 1985) and it may be involved in increasing transsarcolemmal Ca^{2+} flux through the slow inward Ca^{2+} channel (Reuter, 1983). In the case of myofibrils, the sites of phosphorylation are troponin I and C-protein (Solaro *et al.*, 1976; Kranias *et al.*, 1985). Stimulation of phosphate incorporation into troponin I and C-protein by isoprenaline was associated with a decrease in the Ca^{2+} sensitivity of the myofibrillar Mg^{2+} -dependent ATPase activity (Kranias *et al.*, 1985). The site of phosphorylation in SR, due to isoprenaline stimulation of the heart, has been shown to be on phospholamban, an oligomeric proteolipid (Lindemann *et al.*, 1983; Kranias *et al.*, 1985). Phosphorylation of phospholamban *in situ* is associated with increased rates of Ca^{2+} transport by the SR (Kranias *et al.*, 1985) as well as Ca^{2+} -ATPase activity (Lindemann *et al.*, 1983). Thus, the response of

heart cells to β -adrenergic stimulation is likely to involve co-ordinated changes in the extent of cyclic AMP-dependent phosphorylation of several regulatory proteins.

However, besides protein phosphorylation, phosphorylation of phospholipid may mediate, at least in part, the isoprenaline effects on the mammalian myocardium. Phosphorylation of a special class of phospholipids, the polyphosphoinositides, has been shown to occur *in vitro* in various membrane systems by cyclic AMP-dependent protein kinase, indicating the presence of a phosphorylation cascade (Sarkadi *et al.*, 1983; Enyedi *et al.*, 1984; Farkas *et al.*, 1984). This phosphorylation has been implicated in regulation of the intracellular calcium levels either through changes in membrane permeability (Redman, 1972; Michell, 1982), Ca^{2+} -ATPase activity (Buckley & Hawthorne, 1972; Varsanyi *et al.*, 1983), or membrane calcium binding (Buckley & Hawthorne, 1972). Previous studies on phospholipid phosphorylation in intact cells have shown that incubation of slices from rat hearts (Kiss & Farkas, 1975) or fragments from cat and guinea pig hearts (Lapetina *et al.*, 1976) with isoprenaline was associated with increased phosphorylation of phosphatidic acid, while there was no stimulation in the phosphate incorporation into phosphatidylinositol.

The present study was undertaken to determine whether stimulation by β -adrenergic agents is associated with phosphorylation of polyphosphoinositides in an intact beating heart preparation. Our findings suggest that at the peak of the positive inotropic response to

isoprenaline there was increased phosphorylation of phosphatidylinositol monophosphate and bisphosphate, as well as of phosphatidic acid. Thus, phosphorylation of both proteins and phospholipids may play a role in mediating the effects of catecholamines in the intact myocardium.

MATERIALS AND METHODS

Heart perfusion

Hearts from anaesthetized (30 mg of pentobarbital/kg) and heparinized (5000 U/kg) Hartley guinea pigs (600–700 g) were rapidly excised and immediately perfused in a modified Langendorff type apparatus (Grupp & Grupp, 1984) using a Krebs buffer/balanced salt solution containing, in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25 NaHCO₃, 0.5 NaEDTA, 0.26 KH₂PO₄ and 5.5 glucose, yielding an effective total Ca²⁺ concentration of 2.0 mM and K⁺ concentration of 4.9 mM. The buffer solution was saturated with O₂/CO₂ (19:1) (pH 7.4), and bath temperature was set at 37 ± 0.2 °C. All haemodynamic parameters were measured according to Grupp & Grupp (1984) and recorded using a Grass multichannel polygraph.

The hearts were perfused at a constant aortic pressure of 65 mmHg, initially for 20–30 min in a drip-through flow to allow the preparations to stabilize. The perfusion circuit was switched to a recirculating system (120 ml) to which 1.0 mCi of [³²P]P_i was added for 30 min. Then the circuit was returned to a non-recirculating flow with nonradioactive buffer for 1 min. Isoprenaline (0.1 μM) was then infused and at the peak of the positive inotropic response, the hearts were freeze-clamped with precooled (–196 °C) Wollenberger clamps, powdered, and stored under liquid N₂ (Kranias & Solaro, 1982). The specific radioactivity of [³²P]ATP in hearts perfused with [³²P]P_i was determined from the specific radioactivity of [³²P]-phosphocreatine (Kopp & Barany, 1979) at the end of the perfusion. There was no significant change in the specific activity of [γ-³²P]ATP among hearts perfused on a particular day with the same stock solution of [³²P]P_i (the ratio of the low and high values in a typical group was about 0.8).

Microsomes enriched in SR vesicles

Microsomes enriched in SR vesicles were prepared as previously described by Kranias & Solaro (1982) with the following modifications. The frozen powdered tissue was simultaneously thawed and homogenized in 20 ml of Medium I for 15 s with a Polytron PT-10 (setting 6; Brinkmann Instruments). Medium I consisted of 30 mM-KH₂PO₄ (pH 7.0), 10 mM-NaF, 5 mM-EDTA and 0.3 M-sucrose, with 0.3 mM-phenylmethanesulphonyl fluoride and 0.5 mM-dithiothreitol added prior to homogenization. Protein was determined according to the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard. Enzyme marker assays revealed that mitochondrial contamination was approx. 35% and sarcolemmal contamination was less than 10%, as previously reported for SR preparations from perfused rabbit hearts (Kranias & Solaro, 1982).

Gel electrophoresis

Polyacrylamide-gel electrophoresis of ³²P-labelled microsomes enriched in SR vesicles was performed according to the procedure described by Laemmli (1970)

using a 5–18% gradient acrylamide slab gel. Some of the samples were boiled (90 °C, 5 min) prior to electrophoresis. The radioactive bands corresponding to phospholamban were identified on the autoradiogram and then cut from the fixed gel for counting in nonaqueous scintillation fluid. [³²P]P_i incorporation into phospholamban was quantified by dividing the ³²P incorporation in each band by the specific activity of [³²P]ATP determined for that heart, and expressed as nmol or pmol of ³²P/mg of protein.

Preparation of major phospholipid classes

Individual phospholipid extracts were prepared by slight modification of two different methods. In the first method (Bligh & Dyer, 1959), powdered tissues (50–100 mg) and membranes enriched in SR (250 μg) were thoroughly mixed and stirred with 2–4 ml of methanol for about 5 min. Chloroform (1–2 ml) was added, mixed, and the solutions were incubated under shaking at 37 °C for 15 min. The samples were then cooled to room temperature and subsequently mixed with 1–2 ml of chloroform and 1–2 ml of 0.1 M-KCl. At this point, the samples were kept at 4 °C overnight, to obtain phase separation. The organic solvent layer, containing the lipid material, was evaporated. In the second method, 2 ml of hexane/propan-2-ol/conc. HCl (75:50:1 by vol.) was added to the homogenized tissues and sarcoplasmic reticulum systems using the Volpi *et al.* (1983) procedure. The suspension was vortex-mixed and stored overnight at 4 °C. After separation of phases, the top organic phase was removed and the bottom phase was washed twice with 0.4 ml of hexane. The organic phases were pooled and dried in vacuum. The lipids were redissolved and applied to silica gel H plates (500 μm) impregnated with 7.5% magnesium acetate and separation was obtained using the method described by Gentner *et al.* (1981) and Bell *et al.* (1982). Phospholipid spots were visualized by exposure to iodine vapour or sprayed with a char reagent composed of 50% (v/v) H₂SO₄, heated at 180 °C for 30 min and identified by standards run simultaneously. The phospholipid spots were scraped, the phosphorus group of phospholipids was digested and phosphate determination was obtained as described by Rouser *et al.* (1960) and Kahovcova & Odavic (1969). Total phosphorus determination was performed by the method of Hess & Derr (1975). To determine [³²P]P_i incorporation the developed plates were dried at room temperature and exposed to Kodak OG-1 film at –70 °C, for 24–48 h in order to visualize the radioactive lipids. The identified spots were scraped from the plates and radioactivity was determined in a liquid scintillation spectrometer.

Preparation of phosphatidylinositol derivatives from powdered cardiac tissues and microsomes enriched in SR

The extraction is based on partitioning homogenized tissue and SR between an organic and an acidic aqueous phase. Acidic conditions were necessary to remove polyphosphoinositides quantitatively and to prevent their loss into the aqueous phase. Polyphosphoinositide extraction was accomplished by the methods of Schacht (1981) and Volpi *et al.* (1983). The final lipid extracts were dried under a stream of N₂ and used immediately for determination of total lipid phosphorus, of radioactivity and for further analysis on high performance t.l.c. plates. The lipids were redissolved in a small volume of

Table 1. Distribution of individual phospholipids in guinea pig cardiac tissue and in sarcoplasmic reticulum

Phospholipids were extracted as described in the Materials and methods section and they are expressed as % of total phospholipid. Values represent the means $\pm$ S.E.M. of four to six determinations; n.d., not detectable.

Phospholipid	Cardiac homogenate	SR
Phosphatidylcholine	44.8 $\pm$ 3.5	43.2 $\pm$ 4.6
Phosphatidylethanolamine	28.7 $\pm$ 3	16.6 $\pm$ 1.8
Polyglycerolphospholipid (cardiolipin)	11.0 $\pm$ 1.5	4.6 $\pm$ 0.1
Phosphatidylinositol	6.3 $\pm$ 0.6	10.7 $\pm$ 0.5
Sphingomyelin	5.5 $\pm$ 1.8	9.5 $\pm$ 1.6
Phosphatidylserine and lysophosphatidylethanolamine	5.3 $\pm$ 0.3	7.9 $\pm$ 0.3
Lysophosphatidylcholine	n.d.	1.1 $\pm$ 0.1
Unidentified	2.5 $\pm$ 0.5	5.7 $\pm$ 0.4

chloroform/methanol/water (75:25:2, by vol.) and aliquots of redissolved samples were subjected to t.l.c. (Kieselgel high performance thin plates; Merck) according to Gonzalez-Sastre & Folch-Pi (1968). The identified radioactive spots were scraped from the plate and counted for radioactivity. The inositol-containing phospholipids were identified by running standards on the same plate as the extracted lipids.

RESULTS

The contractile effects of isoprenaline were examined in all hearts perfused with [32 P]P_i. Isoprenaline, at a final effective dose of 0.1 μ M, produced a 237 $\pm$ 9 % (n = 6) increase in myocardial contractility (as measured by $+dP/dt$ of left ventricular pressure) compared to control hearts (100 %). This positive inotropic effect occurred rapidly and reached a peak response after about 1 min of drug exposure. The rate of myocardial relaxation (as measured by $-dP/dt$ of left ventricular pressure) was found to be increased 212 $\pm$ 26 % (n = 6) upon exposure to 0.1 μ M-isoprenaline in the same hearts.

Isoprenaline stimulation of the beating hearts was associated with a significant (2–3-fold; n = 6) increase in the phosphate incorporation into phospholamban in SR (results not shown). Phospholamban exhibited the characteristic mobility shift upon phosphorylation *in situ* and these results are in agreement with our previous observations on rabbit hearts (Kranias & Solaro, 1982) and with those by Lindemann *et al.* (1983) on guinea pig hearts.

In guinea pig hearts the major phospholipids identified were phosphatidylcholine and phosphatidylethanolamine, as shown in Table 1. The amounts of the total phospholipid phosphate were determined in three separate pairs of control and isoprenaline-stimulated perfused hearts. The total phospholipid was 236 $\pm$ 7.5 nmol of P_i/mg of homogenate protein for control hearts and 268 $\pm$ 28 nmol of P_i/mg of homogenate protein for isoprenaline-stimulated hearts. These amounts were similar to those present in untreated (non-perfused) hearts which were 198 $\pm$ 22 nmol of P_i/mg of homogenate protein. In membrane fractions enriched in sarcoplasmic reticulum, isolated from the same perfused hearts, the major phospholipids were phosphatidylcholine and phosphatidylethanolamine, followed by phosphatidylinositol (Table 1). The amount of total phospholipid phosphate was 1.47 $\pm$ 0.09 μ mol of P_i/mg of SR membranes from perfused control heart, 1.39 $\pm$ 0.06 μ mol of P_i/mg of SR from isoprenaline-stimulated hearts and 1.58 $\pm$ 0.08 μ mol of P_i/mg of SR from untreated (non-perfused) hearts (n = 8).

Isoprenaline stimulation of the hearts did not significantly alter the overall level of phosphorylation of the total phospholipids present in cardiac homogenates. The amount of 32 P incorporation into total phospholipid was 138 $\pm$ 22 pmol of 32 P/mg of homogenate protein of control hearts, and 142 $\pm$ 12 pmol of 32 P/mg of homogenate protein of isoprenaline-stimulated hearts (n = 6). In contrast, there was increased phosphorylation of the total phospholipids in SR membranes. The degree of phosphorylation of the total phospholipid was 3.5 $\pm$ 0.26 nmol of 32 P/mg of SR isolated from perfused control hearts and 6.7 $\pm$ 0.2 nmol of 32 P/mg of SR isolated from isoprenaline-stimulated hearts (n = 6).

Analysis of the 32 P incorporation into individual phospholipids in cardiac homogenates revealed that

Table 2. Effect of isoprenaline on 32 P incorporation into phospholipids in perfused guinea pig hearts and in SR

Phospholipids were extracted from homogenates or from SR isolated from hearts frozen after perfusion with Krebs–Henseleit buffer (control) or 0.1 μ M-isoprenaline for 1 min (isoprenaline), as described under 'Materials and methods'. Values represent the means $\pm$ S.E.M. of four determinations. * P < 0.05 (t test).

Phospholipid	Incorporation (pmol of P _i /mg of protein)			
	Cardiac homogenate		SR	
	Control	Isoprenaline	Control	Isoprenaline
Phosphatidylcholine	13.5 $\pm$ 2.6	13.2 $\pm$ 0.9	774 $\pm$ 92	799 $\pm$ 95
Phosphatidylethanolamine	5.6 $\pm$ 0.2	4.1 $\pm$ 1.3	25 $\pm$ 5	55 $\pm$ 7*
Phosphatidylinositol	7.9 $\pm$ 1.5	6.6 $\pm$ 0.5	213 $\pm$ 27	381 $\pm$ 45*
Sphingomyelin	8.1 $\pm$ 1.7	5.7 $\pm$ 1.2	697 $\pm$ 91	1028 $\pm$ 54*
Phosphatidylserine and lysophosphatidylethanolamine	65 $\pm$ 11.6	91.3 $\pm$ 16.7	432 $\pm$ 37	442 $\pm$ 6
Phosphatidic acid	13.3 $\pm$ 6.9	49.3 $\pm$ 7*	1101 $\pm$ 59	3813 $\pm$ 98*

Fig. 1. Effect of isoprenaline on ^{32}P incorporation into phosphatidylinositol derivatives in sarcoplasmic reticulum from perfused guinea pig hearts

Lipids were extracted from membrane preparations enriched in sarcoplasmic reticulum (SR) from hearts frozen after perfusion with Krebs-Henseleit buffer (C) or $0.1\ \mu\text{M}$ -isoprenaline for 1 min (Iso). Samples ($10\ \mu\text{g}$) were subjected to high-performance t.l.c. followed by autoradiography to detect ^{32}P -labelled lipids. Phosphatidic acid (PA); phosphatidylinositol (PI); phosphatidylinositol 4-monophosphate (PIP); phosphatidylinositol 4,5-bisphosphate (PIP_2).

isoprenaline stimulation was primarily associated with alterations (3–4-fold increase) in the degree of phosphorylation of phosphatidic acid (Table 2). When ^{32}P incorporation into individual phospholipids in SR membranes was analysed, it was found that isoprenaline increased primarily the phosphorylation of phosphatidic acid, and the magnitude of the stimulation was similar to that observed in cardiac homogenates. ^{32}P incorporation was also increased in sphingomyelin, phosphatidylinositol and phosphatidylethanolamine (although the amount of phosphate incorporation was very low) in SR isolated from isoprenaline-stimulated hearts compared with control hearts.

To determine the degree of phosphorylation of individual phosphoinositides, incorporation was quantified after high-performance t.l.c. (Fig. 1). Six sets of control and isoprenaline-stimulated perfused hearts were used and ^{32}P incorporation into individual phospholipids in cardiac homogenates and SR membranes was analysed. Isoprenaline was associated with a 3-fold increase in the phosphorylation of phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 4-monophosphate, phosphatidic acid and phosphatidylinositol in cardiac homogenates compared with control hearts (Table 3). Analysis of ^{32}P incorporation in polyphosphoinositides of sarcoplasmic reticulum membranes revealed that phosphorylation of phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 4-monophosphate, phosphatidic acid and phosphatidylinositol was also increased 2–3-fold upon isoprenaline stimulation of the hearts (Table 3).

DISCUSSION

Evidence from studies *in vivo* indicates that cyclic AMP-dependent phosphorylation of a $15000\text{-}M_r$ protein in sarcolemma, phospholamban in SR, and troponin I and C-protein in the myofibrils, may be responsible for at least part of the catecholamine effects on the mammalian myocardium (Presti *et al.*, 1985; Kranias *et al.*, 1985; Lindemann *et al.*, 1983; Hartzell, 1984). In the present study, we report that polyphosphoinositides were additionally phosphorylated *in situ* upon isoprenaline stimulation of perfused beating hearts. It was previously reported that addition of isoprenaline to

Table 3. Effect of isoprenaline on ^{32}P incorporation into phosphatidylinositol derivatives in perfused guinea pig hearts

Lipids were extracted from homogenates and SR-enriched membrane preparations isolated from hearts frozen after perfusion with Krebs-Henseleit buffer (control) or $0.1\ \mu\text{M}$ -isoprenaline for 1 min, as described under 'Materials and methods'. Values represent the means $\pm$ S.E.M. of six and eight individually perfused hearts for homogenates and SR, respectively. * $P \leq 0.05$ (*t* test).

	Incorporation (pmol of P_i /mg of protein)			
	Phosphatidylinositol 4,5-bisphosphate	Phosphatidylinositol 4-phosphate	Phosphatidylinositol	Phosphatidic acid
Homogenates				
Control	15 ± 2	8 ± 1	7 ± 1	15 ± 3
Isoprenaline	$55 \pm 3^*$	$26 \pm 3^*$	8 ± 1	$52 \pm 2^*$
SR				
Control	236 ± 18	116 ± 12	182 ± 19	1210 ± 42
Isoprenaline	$615 \pm 52^*$	$240 \pm 41^*$	$286 \pm 39^*$	$3385 \pm 148^*$

slices from rat heart (Kiss & Farkas, 1975) or fragments from cat and guinea pig heart (Lapetina *et al.*, 1976) was associated with increased phosphorylation of phosphatidic acid while there were no changes in the phosphorylation of phosphatidylinositol. However, these studies used long incubation periods (50–180 min) and failed to associate changes in phospholipid phosphorylation with functional alteration of the cardiac cells due to isoprenaline. Thus, it becomes important to show evidence for changes in phospholipid phosphorylation upon isoprenaline stimulation of the heart. For this reason, we determined the levels of individual phospholipid phosphorylation in tissue homogenates and SR membranes prepared from control and isoprenaline-stimulated hearts.

Our findings indicate that, in both tissue homogenates and microsomes enriched in SR vesicles, ^{32}P labelling of phosphatidylinositol mono- and bisphosphate was about 2–3 times higher in samples from isoprenaline-stimulated hearts than in those from controls. Recent evidence indicated that the isoprenaline stimulation of $[\text{P}^{32}]\text{P}_i$ incorporation into phosphatidylinositol derivatives could be prevented by the β -blocker sotalol, while the α -blocker prazosin had no effect (our unpublished work). The mechanism for the observed increases in the formation of polyphosphoinositides is not presently known. It is possible that increased ^{32}P labelling of phosphatidylinositol derivatives may be catalysed directly by the cyclic AMP-dependent protein kinase. Studies *in vitro* have previously demonstrated that pure phosphatidylinositol and phosphatidylinositol monophosphate may become phosphorylated by the catalytic subunit of the cyclic AMP-dependent protein kinase (Sugimoto *et al.*, 1984; our unpublished work). On the other hand, increased phosphorylation of phosphatidylinositol monophosphate and phosphatidylinositol bisphosphate may be catalysed by membrane-bound phosphoinositide kinases (Varsanyi *et al.*, 1986), which may be regulated by the cyclic AMP-dependent protein kinase. Previous studies have suggested that the increased phosphorylation of polyphosphoinositides obtained upon addition of the catalytic subunit of cyclic AMP-dependent protein kinase to various membrane systems was due to the presence of a phosphorylation cascade (Sarkadi *et al.*, 1983; Enyedi *et al.*, 1984; Farkas *et al.*, 1984).

Formation of polyphosphoinositides appeared to correlate with changes in the Ca^{2+} -ATPase activity in various membrane systems, such as erythrocytes (Buckley & Hawthorne, 1972), skeletal muscle SR (Varsanyi *et al.*, 1983) and reconstituted membrane vesicles (Carafoli & Zurini, 1982). Thus, it is possible that changes in polyphosphoinositide levels in tissue homogenates and SR associated with isoprenaline stimulation of the hearts may reflect changes in the Ca^{2+} -ATPase activities of sarcoplasmic reticulum and plasma membranes. The polyphosphoinositides may affect the Ca^{2+} -ATPase activity through direct interaction with the enzyme or through regulation of important phosphoproteins, such as phospholamban in SR. Indeed, Suzuki & Wang (1987) have recently reported that phosphatidylinositol may regulate the degree of purified phospholamban phosphorylation by cyclic AMP-dependent protein kinase.

Previously, studies *in situ* have shown that perfusion of hearts with isoprenaline was associated with increased

phosphorylation of various intracellular proteins and a role for each of these phosphoproteins in mediating some of the effects of catecholamines on cardiac function has been suggested (Presti *et al.*, 1985; Reuter, 1983; Solaro *et al.*, 1976; Kranias *et al.*, 1985; Lindemann *et al.*, 1983). The present study shows that formation of polyphosphoinositides was also increased at the peak of the inotropic response to isoprenaline *in situ*. The mechanism by which this increased phosphorylation of phosphatidylinositol is related to the altered mechanical properties of the heart during catecholamine stimulation is not presently known. However, it appears that the effects of β -adrenergic stimulation in the mammalian heart may be mediated by complex inter-relations between protein phosphorylation and phosphoinositide phosphorylation.

This research was supported in part by U.S. Public Health Service Grants RO1 HL26057 and PO1 HL22619.

REFERENCES

- Bell, M. E., Peterson, R. G. & Eichberg, J. (1982) *J. Neurochem.* **39**, 192–198
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–916
- Buckley, J. T. & Hawthorne, J. N. (1972) *J. Biol. Chem.* **247**, 7218–7223
- Carafoli, E. & Zurini, M. (1982) *Biochim. Biophys. Acta* **683**, 279–301
- Enyedi, A., Farago, A., Sarkadi, B. & Gardos, G. (1984) *FEBS Lett.* **176**, 235–238
- Farkas, G., Enyedi, A., Sarkadi, B., Gardos, G., Nagy, Z. & Farago, A. (1984) *Biochem. Biophys. Res. Commun.* **124**, 871–876
- Gentner, P., Bauer, M. & Dieterich, I. (1981) *J. Chromatogr.* **206**, 200–204
- Gonzalez-Sastre, F. & Folch-Pi, J. (1968) *J. Lipid Res.* **9**, 532–533
- Grupp, I. & Grupp, G. (1984) *Methods Pharmacol.* **5**, 111–128
- Hartzell, C. F. (1984) *J. Gen. Physiol.* **83**, 563–588
- Hess, H. H. & Derr, J. E. (1975) *Anal. Biochem.* **63**, 607–613
- Kahovcova, J. & Odavic, R. (1969) *J. Chromatogr.* **40**, 90–96
- Kiss, Z. & Farkas, T. (1975) *Biochem. Pharmacol.* **24**, 999–1002
- Kopp, S. L. & Barany, M. (1979) *J. Biol. Chem.* **254**, 12007–12012
- Kranias, E. G. & Solaro, R. J. (1982) *Nature (London)* **298**, 182–184
- Kranias, E. G., Garvey, J. L., Srivastava, R. D. & Solaro, R. J. (1985) *Biochem. J.* **226**, 113–121
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lapetina, E. G., Briley, P. A. & De Robertis, E. (1976) *Biochim. Biophys. Acta* **431**, 624–630
- Lindemann, J. P., Jones, L. R., Hathaway, D. R., Henry, B. G. & Watanabe, A. M. (1983) *J. Biol. Chem.* **258**, 464–471
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Michell, R. H. (1982) *Neurosci. Res. Progr. Bull.* **20**, 338–350
- Presti, C. F., Jones, L. R. & Lindemann, J. P. (1985) *J. Biol. Chem.* **260**, 3860–3867
- Redman, C. M. (1972) *Biochim. Biophys. Acta* **282**, 123–134
- Reuter, H. (1983) *Nature (London)* **301**, 569–574
- Rouser, G., Siakotos, A. N. & Fleischer, S. (1960) *Lipids* **1**, 85–86
- Sarkadi, B., Enyedi, A., Farago, A., Meszaros, G., Kremmer, T. & Gardos, G. (1983) *FEBS Lett.* **152**, 195–198

- Schacht, J. (1981) *Methods Enzymol.* **72**, 626–631
- Solaro, R. J., Moir, A. J. G. & Perry, S. V. (1976) *Nature (London)* **262**, 615–616
- Sugimoto, Y., Whitman, M., Cantley, L. C. & Erikson, R. L. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2117–2121
- Suzuki, T. & Wang, J. H. (1987) *J. Biol. Chem.* **262**, 3880–3885
- Varsanyi, M., Tolle, H. G., Heilmeyer, L. M. G., Dawson, R. M. C. & Irvine, R. F. (1983) *EMBO J.* **2**, 1543–1548
- Varsanyi, M., Messer, M., Brandt, N. R. & Heilmeyer, L. M., Jr. (1986) *Biochem. Biophys. Res. Commun.* **138**, 1395–1404
- Volpi, M., Yassin, R., Naccache, P. H. & Sha'afi, R. I. (1983) *Biochem. Biophys. Res. Commun.* **112**, 957–964

Received 18 June 1987/1 October 1987; accepted 20 November 1987