Stimulation of Phosphatidylinositol Metabolism in the Isolated, Perfused Rat Heart

Elizabeth A. Woodcock, L. Barbara Schmauk White, A. Ian Smith, and Jennifer K. McLeod

Receptor-stimulated phosphatidylinositol turnover has been studied in isolated, perfused, [3H]inositol-labelled rat hearts by measuring accumulation of inositol phosphates in the presence of lithium chloride. Inositol phosphate accumulation was stimulated by norepinephrine (3x10^{-4} M) and carbachol (10^{-3} M), the increases averaging from 931 ± 59 (n = 6, mean ± SEM, cpm/g heart) to 4,165 ± 609 (n = 6, p < 0.01) for norepinephrine and to 1,853 ± 354 (n = 6, p < 0.05) for carbachol. The stimulation by norepinephrine was significantly higher in right atria (837 ± 151 to 6,614 ± 1,210, n = 6, cpm/g tissue) than in other regions of the heart. Both norepinephrine and carbachol stimulated the formation of inositol monophosphate, inositol bisphosphate, and inositol trisphosphate with norepinephrine stimulation being detected as early as 15 seconds. Furthermore, the inositol trisphosphate was identified as the -1,4,5 isomer by anion exchange high-performance liquid chromatography. These data are consistent with the hydrolysis of phosphatidylinositol-(4,5)-bisphosphate yielding inositol-(1,4,5)-trisphosphate. Inositol-(1,3,4)-trisphosphate was not detected in heart preparations, suggesting unusual metabolism of inositol-(1,4,5)-trisphosphate in heart tissue. (Circulation Research 1987;61:625-631)

The intrinsic contractility of the myocardium is subject to modulation via both the sympathetic and parasympathetic nervous systems. The sympathetic stimulation is largely mediated through β-adrenoceptors and is initiated by rises in cytoplasmic cyclic adenosine monophosphate (cAMP). The mechanism by which cAMP produces an increase in contractility has been extensively investigated and has been shown to involve cAMP-dependent phosphorylation of proteins associated with Ca^{2+} channels in the sarcolemma and sarcoplasmic reticulum. These serve to control cytosolic Ca^{2+} concentration and thus modulate the contractile response. It is now clear that the stimulation of α-adrenoceptors causes a positive inotropic response in some species. In addition to direct effects on contractile force, cardiac α-adrenoceptors appear to be involved in modulating the activity of phosphofructokinase, a major rate-limiting step in energy metabolism. Both the contractile effects and the metabolic effects of α-adrenoceptor stimulation are cAMP-independent and, instead, related to changes in cytosolic Ca^{2+}.

In heart and in other tissues, α-adrenoceptors are coupled to stimulation of phosphatidylinositol (PI) turnover that is an initiating event in many Ca^{2+}-dependent responses. As is currently understood, the PI turnover pathway involves agonist-bound receptors that cause activation of a phospholipase C that selectively hydrolyzes phosphatidylinositol-(4,5)-bisphosphate (PI-P_2) yielding inositol-(1,4,5)-trisphosphate (Ins-(1,4,5)-P_3) and s,n-1,2-diacylglycerol. In many tissues, both of these products have been shown to act as second messengers initiating responses within the cell. Ins-(1,4,5)-P_3 releases Ca^{2+} from stores in the endoplasmic reticulum, and diacylglycerol activates the membrane-bound protein kinase C, which also requires phosphatidylserine and Ca^{2+} for maximal activity. Protein kinase C can phosphorylate the same sarcolemmal protein as the cAMP-dependent protein kinase, which suggests a potential role for this enzyme in controlling cytosolic Ca^{2+} and, thereby, contractility.

Muscarinic receptors that mediate cardiac depression are associated with three different second messenger systems in heart. They have been shown to inhibit adenylate cyclase, activate K^+ channels, and stimulate PI turnover. While depression of cAMP formation or acceleration of K^+ efflux might be expected to cause cardiac depression, stimulation of PI turnover would be expected to increase contractility. Thus, two different agents stimulate PI hydrolysis in heart, and these have opposing effects on cardiac function. This suggests complexity in the operation of the PI-turnover pathway in heart. For these reasons, a detailed study of PI turnover in intact hearts has been undertaken to better the understanding of its role in controlling cardiac function.

Materials and Methods
Preparation of Isolated Ventricular Myocytes

The method of preparation of isolated myocytes was based on that of Powell et al. Adult male Sprague-Dawley rats weighing 200–300 g were killed by...
Table of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PI, PtdIns</td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>PI-P, PtdIns(4,5)-P</td>
<td>Phosphatidylinositol-(4,5)-bisphosphate</td>
</tr>
<tr>
<td>PI-P2, PtdIns(4,5,6)-P</td>
<td>Phosphatidylinositol-(4,5,6)-trisphosphate</td>
</tr>
<tr>
<td>InsP, inositol P</td>
<td>Inositol-1-phosphate</td>
</tr>
<tr>
<td>InsP2, inositol P(4)</td>
<td>Inositol-1-phosphate-(4,5,6)-trisphosphate</td>
</tr>
<tr>
<td>InsP2, inositol P(3,4)</td>
<td>Inositol-1-phosphate-(3,4,5)-trisphosphate</td>
</tr>
<tr>
<td>InsP2, inositol P(1,4)</td>
<td>Inositol-1-phosphate-(1,4,5)-trisphosphate</td>
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<tr>
<td>InsP2, inositol P(1,3)</td>
<td>Inositol-1-phosphate-(1,3,4)-trisphosphate</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid</td>
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Decapitation. The chest was opened immediately and an ice slurry placed over the heart. The chilled hearts were removed, rinsed clear of blood, and then perfused via the dorsal aorta according to the method of Langendorf. The perfusate was Ca²⁺-free Krebs medium buffered to pH 7.4 with HEPES buffer (20 mM) and was constantly gassed with 95% O₂-5% CO₂. Perfusion was continued in a nonrecirculating manner for 15 minutes or until all blood was removed. Subsequently, collagenase (2 mg/ml) was added to the perfusing solution, and perfusion was continued in a recirculating manner for 30 minutes. The flaccid ventricles were then chopped into slices 200 µm size and centrifuged at 500g for 15 minutes. Cells were washed twice with Ca²⁺-free KH and then incubated at 1-5 x 10⁶ cells/ml.

Phosphatidylinositol Turnover in Isolated Myocytes

Freshly isolated myocytes were incubated at 1-5 x 10⁶ cells/ml in Ca²⁺-free KH containing 20 µCi/ml [H]inositol to label phospholipids. After 2 hours incubation at 37°C, nonradioactive inositol (5 mM) was added and incubation continued for another 15 minutes. Labelled cells were then harvested by centrifugation, washed twice with KH containing 0.2% bovine serum albumin and 5 mM inositol.

[¹⁴C]inositol-labelled cells were incubated in KH containing 10 mM LiCl, 5 mM inositol, 0.2% bovine serum albumin, and other additions, as indicated, in a final volume of 0.3 ml. Incubation was at 37°C for 20 minutes and was terminated by adding 0.94 ml chloroform:methanol (1:2). Inositol phosphates were separated on AG-I resin (Bio-Rad Laboratories, Richmond, Calif.) as described elsewhere.12

Phosphatidylinositol Turnover in Isolated, Perfused Hearts

The method used for isolation and perfusion of the hearts was as described above for preparation of myocytes. The perfusing solution was KH, constantly gassed with 95% O₂-5% CO₂ and maintained at 37°C. Hearts were perfused in a nonrecirculating manner for 15 minutes or until the perfusate was free of blood. After this time, hearts were perfused for 2 hours with KH containing 1 µCi/ml [H]inositol to label inositol phospholipids. Following the labelling period, the perfusion was continued for another 15 minutes with medium containing 5 mM nonradioactive inositol. Antagonist compounds were added at this stage. Agonist compounds were then added together with 10 mM LiCl. Perfusion was continued for either 15 seconds or 20 minutes (as indicated) and was terminated by dropping the heart into liquid nitrogen. Where 15-second perfusions were used, the time of perfusion was taken from the time of appearance of agonist in the perfusate leaving the heart. This was estimated using dye perfusion experiments in 6 hearts.

Frozen hearts were homogenized in 3 ml KH and 9.4 ml chloroform:methanol (1:2) by a Polytron homogenizer followed by sonication. Phases were then separated by using chloroform and methanol, and inositol phosphates were separated from the aqueous phase using AG-I resin (formate form) for separation of total inositol phosphates according to Minneman and Johnson12 and for measuring the different inositol phosphates separately according to Berridge et al.13

Identification of the Isomer of Inositol Trisphosphate

Distinguishing Ins-(1,4,5)-P₃ from Ins-(1,3,4)-P₃ was achieved essentially as described by Irvine et al.14 The chromatography column was a Whatman partisil SAX column (Clifton, N.J.) packed by Waters (Milford, Mass.) for use in a radial compression system. The inositol phosphates were eluted using a linear gradient of 0–1 M ammonium formate buffered to pH 3.7 with orthophosphoric acid. Flow rate was 1 ml/min, and the gradient had eluted from the column in 47 minutes. Under these conditions, ATP eluted at 43 minutes and Ins-(1,4,5)-P₃, eluted after the gradient at 47–49 minutes. The nature of the [³²P]inositol labelled material was checked by including [³²P]-labelled Ins-(1,4,5)-P₃ prepared from erythrocytes.15 The identity of the [³²P]-labelled standard was validated using commercially available [³²P]Ins-(1,4,5)-P₃.

Chromatography was performed on a Waters Model 441 liquid chromatography system (Rochester, Minn.).

Preparation of Inositol Trisphosphate From Adrenal Glomerulosa Cells

Rat adrenal glomerulosa cells were prepared as described elsewhere.16,17 Inositol phospholipids were labelled by incubating cells for 18 hours with [³²P]inositol (20 µCi/ml) in HEPES-buffered medium 199 at 37°C. Subsequently, 5 mM nonradioactive inositol was added, and the cells were incubated for another 10 minutes before being harvested by centrifugation and washed twice in medium 199 containing 5 mM inositol. [³²P]-labelled cells (3–5 x 10⁶/ml) were incubated in medium 199 containing 10 mM LiCl and 10⁻⁷ M angiotensin II for 15 seconds. Inositol phosphates were extracted as described above.

Analytical Methods

Statistical significance was evaluated with a Perutz F test for comparison between multiple groups.18
Chemicals

\[^{3\text{H}}\text{-myo-Inositol, specific activity 10–20 Ci/mmol, was purchased from the Radiochemical Centre, Amersham, U.K.}^{2\text{P}}\text{-Phosphoric acid was purchased from the Australian Atomic Energy Commission, Lucas Heights, New South Wales.}^{3\text{H}}\text{-myo-Inositol-(1,4,5)-trisphosphate (Amersham) was a gift from Dr. M.E. Dunlop, Royal Melbourne Hospital, Victoria. Nor-}

epinephrine bitartrate, carbachol (carbamylcholine), compound 48/80, atropine sulfate, and dibutyryl cAMP were obtained from the Sigma Chemical Co., St. Louis, Mo. Prazosin was provided by Pfizer Laboratories, New York; propranolol was provided by Imperial Chemical Industries, Melbourne, Australia. All other chemicals used were AR grade.

Results

Stimulation of Phosphatidylinositol Turnover in Isolated Ventricular Myocytes

Initial experiments were carried out to investigate PI turnover in isolated ventricular myocytes. Freshly isolated myocytes were labelled for 2 hours with \[^{3\text{H}}\text{-inositol and subsequently incubated with stimulatory agonists in the presence of 10 mM LiCl and 5 mM nonradioactive inositol. Accumulation of inositol phosphates was linear for 30 minutes and plateaued thereafter. A 20-minute incubation was used in subsequent experiments. As has been described previ-

ously, both norepinephrine and carbachol produced dose-dependent stimulations of PI turnover. Norepinephrine was more potent than carbachol, but the two agents produced similar maximum stimulations (Figure 1).

Phosphatidylinositol Turnover in the Intact Heart

To gain further insight into PI turnover and its importance in cardiac function, experiments were carried out in isolated, perfused rat hearts. \[^{3\text{H}}\text{-inositol-labelled hearts were perfused for 20 minutes with different adrenergic and cholinergic agonist and antagonist compounds, and the effects of these on inositol phosphate accumulation were determined. Antagonists we were added 15 minutes before addition of agonists and lithium chloride. Concentrations of norepinephrine and carbachol were chosen to produce maximum stimulation as demonstrated in experiments with isolated myocytes (Figure 1). Both norepinephrine } (3 \times 10^{-3} \text{ M}) \text{ and carbachol } (10^{-3} \text{ M}) \text{ stimulated accumulation of inositol phosphates. The stimulation by norepinephrine, approximately 4 times, was higher than that produced by carbachol, approximately 2 times (Figure 2). The stimulation by norepinephrine in the isolated heart preparation was also higher than that obtained with isolated myocytes. Stimulation by norepinephrine was inhibited by prazosin } (10^{-7} \text{ M}) \text{ but not by propranolol } (10^{-7} \text{ M}), \text{ indicating that it was mediated by } \alpha_1 \text{-adrenoceptors. The stimulation by carbachol was inhibited by atropine } (10^{-7} \text{ M}) \text{ as expected for mediation via muscarinic receptors.}

In neutrophils and platelets, cAMP has been shown to modulate receptor-mediated PI turnover. Since cardiac contractility is modulated by cAMP, effects of cAMP on PI turnover stimulated by norepinephrine were investigated. As is shown in Figure 2, dibutyryl cAMP \ (10^{-4} \text{ M}) \text{ did not alter the PI-turnover response to norepinephrine. The lack of interaction with cAMP is further shown by the lack of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Stimulation of inositol phosphate accumulation in perfused, \[^{3\text{H}}\text{-inositol-labelled hearts. Labelled hearts were perfused with the indicated adrenergic and cholinergic agonist and antagonist compounds for 20 minutes. Zero time experiments represent hearts labelled with \[^{3\text{H}}\text{-inositol, then frozen, and extracted without lithium chloride perfusion. ATR, atropine } 10^{-7} \text{ M; PRO, propranolol, } 10^{-7} \text{ M; PRAZ, prazosin, } 10^{-7} \text{ M; NOR, norepinephrine. } 3 \times 10^{-3} \text{ M; cAMP, } 10^{-4} \text{ M dibutyryl,3',5'-cAMP; CCH, carbachol } 10^{-4} \text{ M. Values shown are mean } \pm \text{ SEM. Numbers indicate number of experiments.}}}
\end{figure}
effect of propranolol that would prevent norepinephrine stimulation of cAMP accumulation mediated by β-adrenoceptors.

**Contributions of Cardiac Mast Cells to the Observed Phosphatidylinositol-Turnover Response**

Rat heart contains large numbers of mast cells that respond to muscarinic cholinergic but not α-adrenergic agonists. To test for possible contribution of cardiac mast cells to the observed PI-turnover response, experiments were performed using hearts perfused with compound 48/80 (a selective mast cell activator that stimulates PI turnover) at 10 μg/ml, a concentration sufficient to maximally stimulate isolated mast cells. Compound 48/80 did not increase inositol phosphate accumulation in the isolated perfused hearts. The total inositol phosphates accumulated in 20 minutes was 821 ± 133 (n = 6) compared with 937 ± 59 (cpm/g heart) (n = 5) for control hearts. Thus, mast cells do not appear to make an important contribution to the PI turnover observed in the isolated hearts, and it is unlikely that they contribute greatly to the observed muscarinic stimulation.

**Localization of the Phosphatidylinositol Turnover Response**

Experiments were performed to determine whether the PI-turnover response to α-adrenergic and cholinergic stimulations was observed in all compartments of the heart. Hearts were perfused with either norepinephrine or carbachol for 20 minutes and then dissected into right and left atria, right and left ventricle, and septum. Individual regions of the heart were analyzed for inositol phosphates as described above. As is shown in Figure 3, accumulation of inositol phosphates in response to norepinephrine was higher in right atria but was similar in all other regions. Stimulation by carbachol was not higher in right atria but was similar in all dissected regions.

**Effect of Perfusate Ca^2+ Concentration**

The importance of the extracellular Ca^2+ concentration was investigated by perfusing hearts with Krebs medium containing 0, 1, and 10 mM Ca^2+ ion. To avoid precipitation at high Ca^2+ concentrations, a 1 M solution of CaCl₂ was infused into the perfusate, above the heart, to give a final concentration of 10 mM. Control experiments were performed as well as experiments with infused norepinephrine (3 × 10⁻⁵ M). Inositol phosphate accumulation per gram of heart, in the presence of norepinephrine, averaged 3,883 ± 225, 4,164 ± 554, and 4,051 ± 257 for 0, 1, and 10 mM Ca^2+ perfusates, respectively. Thus, there were no significant differences in the norepinephrine–PI-turnover responses at these three Ca^2+ concentrations.

**Separation of Inositol Phosphates**

To further investigate the stimulation of PI turnover by muscarinic receptors and α₁-adrenoceptors, experiments were performed in which InsP₁, InsP₂, and InsP₃ were measured separately. Hearts were labelled as described above and incubated with stimulatory agonists and lithium chloride for either 15 seconds or 20 minutes. Inositol phosphates were separated as described in “Materials and Methods.” Accumulations of all three inositol phosphates were stimulated by norepinephrine at 20 minutes. At 15 seconds, only the InsP₁ and InsP₂ levels were significantly elevated above control (p < 0.05) although InsP₃ values were higher than control. Stimulation by carbachol could not be detected at 15 seconds, probably reflecting the relatively poor stimulation produced by this agent together with the variation between different hearts (Table 1).

**Identification of the Isomer of Inositol Trisphosphate**

The nature of the material eluting as InsP₃, was examined by high-performance liquid chromatography as described in “Materials and Methods.” Hearts were perfused with norepinephrine (3 × 10⁻⁵ M) for 15 seconds, and the InsP₃ was extracted. Figure 4 shows

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**Table 1. Effects of Norepinephrine and Carbachol on the Accumulation of InsP₁, InsP₂, and InsP₃**

<table>
<thead>
<tr>
<th>Additions</th>
<th>InsP₁ (cpm/g heart)</th>
<th>InsP₂ (cpm/g heart)</th>
<th>InsP₃ (cpm/g heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 seconds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>atr + praz</td>
<td>204 ± 17</td>
<td>250 ± 25</td>
<td>62 ± 12</td>
</tr>
<tr>
<td>nor + atr</td>
<td>261 ± 22*</td>
<td>289 ± 20*</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>cch + praz</td>
<td>211 ± 26</td>
<td>188 ± 13</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>20 minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>atr + praz</td>
<td>375 ± 30</td>
<td>272 ± 19</td>
<td>74 ± 7</td>
</tr>
<tr>
<td>nor + atr</td>
<td>2,487 ± 463*</td>
<td>574 ± 136*</td>
<td>103 ± 17*</td>
</tr>
<tr>
<td>cch + praz</td>
<td>969 ± 128*</td>
<td>368 ± 57*</td>
<td>93 ± 11</td>
</tr>
</tbody>
</table>

Values shown are inositol phosphate accumulation in cpm/g heart, mean ± SEM of 6 experiments. ATR, atropine 10⁻⁴ M; PRO, propranolol, 10⁻⁴ M; PRAZ, prazosin, 10⁻⁷ M; NOR, norepinephrine, 3 × 10⁻⁵ M; CCH, carbachol 10⁻³ M. *p < 0.05 relative to control.
the region of the gradient where InsP₃ elutes and also shows the chromatographic positions of Ins-(1,4,5)-P₃ and ATP. As is shown in the figure, InsP₃ formed in heart after 15 minutes of norepinephrine perfusion and was identified as Ins-(1,4,5)-P₃. There was no appearance of a peak travelling slightly ahead of Ins-(1,4,5)-P₃ close to the ATP peak. This peak is believed to be Ins-(1,3,4)-P₃, which is formed from the -(1,4,5) isomer by successive phosphorylation and dephosphorylation reactions. The expected 2 peaks of InsP₃ were detected in preparations of [³H]inositol-labelled adrenal glomerulosa cells stimulated with angiotensin II for either 15 seconds or 20 minutes (Figure 4). Thus, failure to detect the presence of this isomer in heart was not due to a failure of the chromatographic system to separate the isomers. This experiment was performed at each of the following norepinephrine perfusion times: 5, 15, 30 seconds, 2, 5, 20 minutes. In none of these experiments was there any appearance of Ins-(1,3,4)-P₃, Ins-(1,4,5)-P₃ was detected at each time. A peak of Ins-(1,4,5)-P₃, but not Ins-(1,3,4)-P₃, was present in hearts not perfused with norepinephrine, but this was smaller than peaks obtained in norepinephrine perfused hearts (Table 1).

Discussion

It is now well established that the mechanism of action of many different “Ca²⁺-dependent” hormones and neurotransmitters involves the hydrolysis of plasma membrane PIs. The so-called PI cycle has been studied in considerable detail in many different cell types with respect to interactions with Ca²⁺ and to importance in cellular function. However, while cardiac function is heavily regulated by Ca²⁺, the role of PI turnover has been relatively little investigated. Most previous studies have involved the use of isolated myocytes and have demonstrated stimulation of PI turnover by both α₁-adrenergic and muscarinic cholinergic agonists. The present study investigated PI turnover in whole perfused hearts. Such a system has a number of advantages. First, the tissue has not been subjected to collagenase digestion and dispersion and probably more closely resembles an in vivo situation. Second, stimulation by norepinephrine is higher in the intact heart. Third, the use of the intact heart enables studies to be performed using electrical stimulation of the cardiac nerves. Last, comparisons with contractility measurements can be made.

While catecholamine stimulation of cardiac contractility has traditionally been regarded as mediated by β-adrenoceptors, it is now known that there is also a contribution from α₁-adrenoceptors in some species. Unlike the β-receptor-mediated pathway, the α₁-adrenergic mechanism is independent of cAMP. In addition, the metabolic actions of catecholamines, in particular the stimulation of cardiac energy metabolism, are also now known to be mediated by both α₁- and β-adrenoceptors. As with the contractile effects, β-adrenoceptor stimulation apparently depends on elevations of cAMP, whereas α₁-adrenoceptor stimulation is Ca²⁺-mediated. Elevation by cytosolic Ca²⁺ is often associated with, and dependent on, stimulation of plasma membrane PI turnover. Thus, it is possible that stimulation of PI turnover is the initiating event for the contractile effects and the metabolic effects of catecholamines mediated through α₁-adrenoceptors. The difficulty in such an assumption is that stimulation of cardiac muscarinic cholinergic receptors depresses contractility and has no effect on energy metabolism while producing a stimulation of PI turnover. The question of muscarinic receptors and contractility may be explained by the existence of alternative second messengers described in heart. Cardiac muscarinic receptors are also coupled to adenylate cyclase inhibition and activation of K⁺ channels. Both of these latter pathways would be expected to produce cardiac depression. These latter two second messenger systems are uncoupled from muscarinic receptor stimulation by treatment with Bordetella pertussis toxin but coupling to PI turnover is not. Such toxin treatment prevents cholinergic depression of cardiac contractility. These observations indicate that PI turnover is not
important in the cardiodepressive actions of muscarinic agonists. This conclusion is further substantiated by the recent observations that, following pertussis-toxin treatment, muscarinic receptor stimulation can actually increase contractility.28 Such a finding suggests that, in untreated hearts, the tendency of muscarinic receptors to increase contraction via PI-turnover-initiated responses is overridden by the simultaneous effects on K+, cAMP, or both. Furthermore, this finding supports the concept that α,-receptor stimulation of contractility is mediated via the PI-turnover pathway. However, the question of any possible physiologic role for muscarinic receptors coupled to PI turnover remains.

While relatively poorly studied in heart, in other tissues hydrolysis of plasma-membrane PIs yields two second messengers, diacylglycerol and Ins-(1,4,5)-P3. Diacylglycerol stimulates the membrane-bound, phospholipid-dependent, Ca2+-dependent protein kinase C, but Ins-(1,4,5)-P3 releases Ca2+ from specific stores in the endoplasmic reticulum.67 In the studies reported here, we have demonstrated that norepinephrine infusion increases InsP3 levels (Table 1). Detailed examination of the InsP3 fraction indicated it is the Ins-(1,4,5)-P3 isomer. These results are in agreement with the pathway in heart involving the usual cleavage of PI(4,5)-P2, rather than PIP or PI. However, in the heart, Ins-(1,4,5)-P3 appeared not to be converted to the -(1,3,4) isomer as described in parotid glands,29 cultured pancreatic islet cells,30 brain,31 and liver.32 No peak corresponding to Ins-(1,3,4)-P3 could be detected in profiles from hearts perfused with norepinephrine for times ranging from 5 seconds to 20 minutes. Furthermore, only the -(1,4,5) isomer was detected in hearts not perfused with norepinephrine. In parallel experiments, Ins-(1,4,5)-P3 and Ins-(1,3,4)-P3 were both detected in preparations from angiotensin II-stimulated adrenal glomerulosa cells (Figure 4). The function of Ins-(1,3,4)-P3 is not known in any of the tissues where its presence has been described. The fact that the heart does not produce this isomer is of interest in that it indicates that cardiac metabolism of inositol phosphates differs from that in other tissues. Furthermore, this finding may provide further insight into the role, if any, of Ins-(1,3,4)-P3. Cardiac metabolism of inositol phosphates is being investigated further. While Ins-(1,4,5)-P3 is clearly formed in heart, its physiologic significance is not as obvious. Ins-(1,4,5)-P3, has been reported as active,33 inactive,34 and weak35 in releasing Ca2+ from cardiac sarcoplasmic reticulum. Thus, Ins-(1,4,5)-P3 may not be an important factor in raising myocardial Ca2+ concentrations. This is, perhaps, not surprising given the capacity of the heart to control Ca2+ entry across the sarcolemma. This leads to the question of a possible alternative role for Ins-(1,4,5)-P3 in heart.

The other second messenger, diacylglycerol, may be directly involved in increasing contractility. Protein kinase C has been shown to phosphorylate the same sarcolemmal protein as the cAMP-dependent protein kinase A.36 This protein is likely involved in Ca2+-gating. This phosphorylation may thus be important in mediating the contractile effects of α,-adrenoceptor stimulation. Protein kinase C has been reported as relatively weak in phosphorylating phospholamban, which is an excellent substrate for the cAMP-dependent protein kinase.1 Phosphorylation of phospholamban is thought to be important in sarcoplasmic reticulum Ca2+ uptake and in the relaxation phase of contraction. Thus, differences in the substrate specificities of protein kinase C and the cAMP-dependent protein kinase may explain, in part, the differences in the nature of the contraction stimulated through α,- and β-receptors.35

The possible relation between α,-receptor stimulation of PI turnover and stimulation of cardiac energy metabolism has not yet been investigated in detail. The ability of cardiac α,-adrenoceptors to stimulate carbohydrate metabolism is heavily dependent on extracellular Ca2+, requiring levels in the range of 1 mM or above for detectable stimulation.3 In contrast, the stimulation of PI turnover by norepinephrine was insensitive to perfuse Ca2+ concentration in the range 0–10 mM. This means that if α,-adrenoceptor stimulation of carbohydrate metabolism is initiated through the PI-turnover pathway, then its Ca2+ requirement is distal to this initiating step. Possibly, the PI-turnover pathway itself controls extracellular Ca2+ entry via protein kinase C regulation of sarcolemmal Ca2+ channels. Our finding of no effect of perfusate Ca2+ concentration is apparently at variance with previous reports of depressed PI turnover in myocytes and atria in the absence of extracellular Ca2+.39 These latter experiments, however, were performed in the presence of the Ca2+ chelator ethylene-glycol-bis(oxyethylene-nitrile)-tetraacetic acid (EGTA) under conditions of total elimination of bound and free Ca2+. Our experiments were carried out in the absence of added Ca2+ under conditions where the actual perfusate Ca2+ was 5–10 μM. It is possible that very low levels of Ca2+ are required for receptor stimulation of PI turnover, and further increases in Ca2+ have no effect. It is also possible that addition of EGTA causes some generalized damage to the sarcolemma that is reflected in reduced PI-turnover response. Of interest, Brown et al9 found no effect of the Ca2+ ionophore A23187 in ventricular myocytes, which agrees with our findings of Ca2+ independence.

Stimulation of PI turnover by both carbachol and norepinephrine was observed in all chambers of the heart. Similar activity was observed in all regions, with the exception of the right atrium where significantly higher stimulation norepinephrine was observed. Basal- and carbachol-stimulated turnover were not significantly higher, and the reason for the high α,-adrenoceptor activity in right atria is unknown but warrants further investigation.

The results of experiments reported here show that PI turnover can be readily measured in perfused whole heart preparations. The pathway observed in heart appeared to be similar to that observed in other tissues in that Ins-(1,4,5)P3, as well as InsP3 and InsP, was formed rapidly. The functional importance of these products has yet to be established in heart, and the use
of experiments in whole perfused hearts should enable the relation between this signal transduction pathway and cardiac function to be explored in greater detail.

Acknowledgment
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Keywords • rat heart • phosphatidylinositol • inositol trisphosphate • α-adrenergic receptors • muscarinic cholinergic receptors
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