Myocardial Stretch Stimulates Phosphatidylinositol Turnover

Rüdiger von Harsdorf, Rudolph E. Lang, Merryl Fullerton, and Elizabeth A. Woodcock

The mammalian myocardium responds to stretch by increasing both contractility and the release of atrial natriuretic peptide. These effects are observed in isolated perfused heart preparations as well as in vivo. That atrial natriuretic peptide release can be stimulated by activation of the phosphatidylinositol turnover pathway suggests a possible mechanism by which stretch might activate a biological response. Accordingly, experiments were performed to examine the effect of dilatation of the right atrium on the phosphatidylinositol turnover pathway measured in isolated perfused hearts. Dilatation of the right atrium caused a stimulation of the phosphatidylinositol turnover pathway as measured by an increase in the accumulation of inositol phosphates. In right atria, increases were detected after 1 minute of dilatation, and maximal increases were observed after 10 minutes. Dilatation for 10 minutes caused an increase in inositol monophosphate, inositol bisphosphate, and inositol trisphosphate from 23.3±0.9, 15.4±0.4, and 9.5±0.3 cpm/mg tissue (mean±SEM, n=7) to 74.6±2.3, 20.2±1.3, and 13.6±1.5 cpm/mg tissue (n=8), respectively (p<0.01 for all inositol phosphates). Smaller increases were observed in the other chambers of the hearts. Perfusion with propranolol, prazosin, and atropine (all 1 μM) did not alter the inositol phosphate response to dilatation, indicating that it was not secondary to release of norepinephrine or acetylcholine. Dilatation of the right ventricle also caused a stimulation of inositol phosphate accumulation, but this was lower than after dilatation of the right atrium. These results show that the myocardium can respond to dilatation by an activation of the phosphatidylinositol turnover pathway. Such a mechanism has implications for the release of atrial natriuretic peptide and also provides a potential mechanism for the enhanced contractility after increased venous return. (Circulation Research 1989;65:494–501)
Phosphatidylinositol Turnover

**Materials and Methods**

*Phosphatidylinositol Turnover in Isolated-Perfused Rat Hearts*

Adult male Sprague-Dawley rats weighing 300–350 g were killed by decapitation. The chest was opened, and the hearts were rapidly cannulated via the aorta and perfused in a recirculating manner according to the method of Langendorff. The methods used to study inositol phosphate accumulation in isolated rat hearts are outlined in Figure 1. Hearts were perfused at 37° C with HEPES-buffered Krebs medium and constantly gassed with 95% O₂-5% CO₂ until the perfusate was free of blood. Then, hearts were perfused for 2 hours with Krebs medium containing 1 μCi [³H]inositol/ml to label inositol phospholipids. After the labeling period, radioactive perfusate was removed and replaced with Krebs medium containing 5 mM inositol and 10 mM LiCl. Perfusion was continued for 10 minutes. Antagonist compounds were also added at this stage. Excess nonradioactive inositol was added to prevent the further synthesis of [³H]inositol phospholipids during the stretch procedure or during agonist stimulation. Lithium chloride was added to inhibit the degradation of inositol phosphates, especially inositol monophosphate. Atria or ventricles were then subjected to dilatation for the times indicated in the figures. In some experiments, hearts were perfused with norepinephrine (3×10⁻⁵ M) for 10 minutes. The stimulus was terminated by rapidly chilling the hearts in ice-cold saline. Chilled hearts were rapidly dissected, and water-soluble, [³H]inositol-labeled products were extracted by a chloroform-methanol extraction method as described previously. [³H]-labeled inositol phosphates were separated from [³H]inositol by Dowex 1 ion-exchange resin (formate form) as described elsewhere or by anion-exchange high-performance liquid chromatography as described below.

**High-Performance Liquid Chromatography of Inositol Phosphates**

A Whatman Partisil SAX column packed by Waters (Milford, Massachusetts) was used in a radial compression system that was run on a Waters Model 441 liquid chromatograph. [³H]inositol-labeled compounds were eluted by a linear gradient of 0–2 M ammonium formate buffered to pH 3.7 with phosphoric acid. The flow rate was 1 ml/min. Ins 1-P, Ins 4-P, Ins 1,4-P₂, and Ins 1,4,5-P₃ were identified using [³H]-labeled standards. Other peaks on the chromatograms were identified as glycerophosphoinositol 4-phosphate and glycerophosphoinositol 4,5-bisphosphate by comparison with [³₂P]-labeled standards prepared as described by Downes et al. [²H]Ins 1,3,4,5-P₄ was prepared from [³H]Ins 1,4,5-P₃ using a crude preparation of Ins 1,4,5-P₃ kinase from rat brain.

**Dilatation of Right Atria and Right Ventricles**

Right atria and right ventricles were dilated with latex balloons (size 5) attached via a catheter to a 1-ml syringe. Balloons were inserted via the inferior vena cava. Right atria were dilated by a volume of 75 μl and right ventricles by 300 μl.

Right atrial pressure during balloon dilatation was assessed by injecting 50, 75, or 100 μl Krebs medium into the right atrium through a catheter inserted into the lower vena cava. The pressure was monitored by means of a transducer connected to a catheter positioned in the upper vena cava. Immediately before administration of the volume, the perfusion of the heart was stopped, and the tricuspid valve was occluded by inflation of a balloon located in the right ventricle. The completeness of the occlusion
was verified by adding dye to the bolus administered into the atrium.

Under basal conditions, systolic right atrial pressure was 3.9±0.4 mm Hg (n=4). Injection of 50, 75, or 100 μl Krebs medium increased this to 4.7±0.6 (n=6), 5.2±0.5 (n=9), and 5.8±0.4 (n=6), respectively. These values are well within the range of right atrial pressures observed during expansion of blood volume.9

**Assay of Lactate Dehydrogenase Activity**

Lactate dehydrogenase activity was measured in aliquots of the final heart perfusate. The incubations contained 0.1 M glycine-NaOH buffer (pH 10.0), 25 mM Na DL-lactate, 1 mM nicotinamide adenine dinucleotide and 50 μl perfusate. Increases in absorbance at 340 nM were determined over a 10-minute period at room temperature.

**Materials**

Latex balloons were obtained from Hugo Sacks Electronic 7801 (Masch-Hugotetten, FRG). [3H]myo-Inositol (specific activity, 10–20 Ci/mmol) and [3H]inositol phosphate standards were obtained from the Radiochemical Centre (Amersham, UK) except for Ins 4-P, which was obtained from New England Nuclear (Boston, Massachusetts). [32P]Phosphoric acid was purchased from the Australian Atomic Energy Commission (Lucas Heights, New South Wales). Norepinephrine bitartrate was obtained from Sigma Chemical (St. Louis, Missouri). Prazosin was provided by Pfizer Laboratories (New York) and propranolol by Imperial Chemical Industries (Melbourne, Australia). All other chemicals were AR grade.

**Results**

**Right Atrial Dilatation and Phosphatidylinositol Turnover**

We and others have previously reported an increase in inositol phosphate generation in isolated rat hearts perfused with norepinephrine or carbachol.19,25 In the present study, experiments were undertaken to investigate the effect of dilating right atria on inositol phosphate accumulation in the right atrium as well as in the other chambers of the hearts. Isolated hearts were labeled with [3H]inositol, followed by 10 minutes of perfusion with nonradioactive inositol and lithium chloride. Right atria were then dilated by a volume of 75 μl for 1, 10, or 20 minutes. At the end of the stretch period, hearts were chilled rapidly, and water-soluble [3H]inositol-labeled compounds were extracted. These were separated into inositol monophosphate (InsP1), inositol bisphosphate (InsP2), and inositol trisphosphate (InsP3) by column chromatography.

Increases in all of the three inositol phosphate fractions were observed in right atria after 1 minute of dilatation. Larger increases were observed after dilatation for 10 or 20 minutes. The response at 20 minutes was not significantly different from the 10-minute response (Figure 2).

**TABLE 1. Effect of 10-Minute Right Atrial Dilatation on Profile of Inositol Phosphates in Other Heart Chambers**

<table>
<thead>
<tr>
<th></th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
<th>Septum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>InsP1</td>
<td>17.6±1.4</td>
<td>6.1±0.5</td>
<td>9.6±0.7</td>
<td>8.5±0.5</td>
</tr>
<tr>
<td>InsP2</td>
<td>14.1±0.7</td>
<td>11.1±0.8</td>
<td>13.2±0.9</td>
<td>15.6±0.9</td>
</tr>
<tr>
<td>InsP3</td>
<td>8.5±0.4</td>
<td>4.2±0.3</td>
<td>3.9±0.15</td>
<td>5.2±0.4</td>
</tr>
<tr>
<td><strong>Dilated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>InsP1</td>
<td>27.2±1.9*</td>
<td>11.3±0.5*</td>
<td>13.1±0.9*</td>
<td>13.5±0.9*</td>
</tr>
<tr>
<td>InsP2</td>
<td>14.2±0.6</td>
<td>15.9±1.0*</td>
<td>9.9±1.0</td>
<td>14.7±1.4</td>
</tr>
<tr>
<td>InsP3</td>
<td>9.3±0.7</td>
<td>6.6±0.4*</td>
<td>3.9±0.4</td>
<td>6.15±0.4</td>
</tr>
</tbody>
</table>

Values shown are inositol phosphate accumulations in (mean±SEM cpm/mg tissue) seven control hearts and eight hearts subjected to right atrial dilatation. The data were derived from the hearts depicted in Figure 1. For clarity, only the responses at 10 minutes are shown. Apart from the right atria, no significant increase in inositol phosphates was observed after 1 minute of dilatation, and responses at 20 minutes were similar to those seen at 10 minutes.

*p<0.01, t<0.05 by Peritz F test.34
was detectable after 1 minute of dilatation. Thus, permanent dilatation of right atria is associated with a degree of tissue damage possibly caused by ischemia. The possibility must be considered that tissue damage due to ischemia contributes to the observed stimulation of inositol phosphate accumulation. To address this possibility, additional experiments were performed in which atria were dilated in a pulsatile manner, producing 30 seconds of dilatation a minute for 10 minutes. This pulsatile dilatation produced stimulation of inositol phosphate accumulation similar to that observed with constant dilatation without causing increased lactate dehydrogenase release (Tables 2 and 3). This indicates that the observed stimulation of PI turnover is not dependent on tissue damage. Furthermore, stimulation of inositol phosphate accumulation was detected in chambers of the heart that were not themselves stretched and, therefore, cannot be ischemic. This shows that ischemia is not required for activation of PI turnover by atrial distension.

Identification of Phosphatidylinositol Turnover Pathway Products During Right Atrial Dilatation

It is now generally accepted that the PI turnover pathway in many tissues is complex and that a number of different inositol phosphate products are formed. Experiments were performed to investigate the products of the PI turnover pathway in dilated right atria. Water-soluble, [3H]inositol-labeled products were examined by anion-exchange high-performance liquid chromatography. An increase in the active calcium-releasing compound Ins1,4,5-P3 was observed together with increases in Ins1,4-P2 and Ins4-P, which are degradation products of Ins1,4,5-P3. Other peaks on the chromatograms were identified as glycerophosphoinositol, glycerophosphoinositol 4-phosphate, and glycerophosphoinositol 4,5-bisphosphate. These compounds were not increased by dilatation (Figure 3).

Chromatographic profiles obtained in right atria were similar to those reported previously in ventricular tissue and different from those reported in many other tissues. In none of the profiles observed in atria was there any peak at the position of Ins1,3,4,5-P4. Also, the breakdown products of Ins

| Table 2. Release of Lactate Dehydrogenase During Right Atrial Dilatation |
|-----------------------------|-------------|-----------------|
| Duration of dilatation (min) | Control     | Dilated (permanent) | Dilated (30 sec/min) |
| 1                           | 0.6±0.2     | 0.7±0.3          |                  |
| 10                          | 0.8±0.2     | 3.3±1.2*         | 0.95±0.3         |
| 20                          | 0.9±0.3     | 11.5±3.2*        |                  |

Values shown are lactate dehydrogenase activity (mean±SEM nmol lactate oxidized/min/ml perfusate) (n=8). Atria were dilated with a volume of 75 μl for the times indicated.

*p<0.01 relative to controls (Peritz F test).

| Table 3. Inositol Phosphate Accumulation in Hearts Perfused for 10 Minutes With 30 μM Norepinephrine or Subjected to 10 Minutes of Right Atrial Dilatation or 10 Minutes of Right Atrial Dilatation in the Presence of Blockers Propranolol, Prazosin, And Atropine (all 1 μM), or Dilated 30 sec/min for 10 Minutes (Pulsatile Dilatation) |
|-----------------------------|-------------|-----------------|-----------------|-------------------|
|                            | Right atrium | Left atrium | Right ventricle | Left ventricle |
| Control                     | 22.3±0.8    | 17.6±1.4     | 6.1±0.5        | 9.6±0.7          | 8.5±0.5          |
| InsP1                       | 103.8±1.9*  | 95.9±1.9*    | 42.3±5.9*      | 32.0±7.2*        | 34.2±8.4*        |
| InsP2                       | 42.3±2.1*   | 45.9±3*      | 32.6±4.9*      | 17.4±4.9*        | 19.3±5.7         |
| Norepinephrine              | 15.5±1.5*   | 22.3±1.5    | 14.4±0.5*      | 3.7±0.7          | 2.5±0.0          | 4.8±0.6         |
| Dilatation                  | 74.6±2.3    | 272±1.9      | 11.3±0.5       | 13.1±0.9         | 13.5±0.9         |
| InsP1                       | 68.9±5.5    | 26.5±2.7     | 8.8±2.5        | 13.8±2.5         | 14.6±3.2         |
| InsP2                       | 18.5±2      | 16.9±1.5     | 17.8±1.9       | 9.6±1.8          | 15.9±2.4         |
| InsP3                       | 13.5±1.5    | 9.3±0.7      | 6.6±0.4        | 3.9±0.4          | 6.15±0.4         |
| Dilatation and blockers     | 68.9±5.5    | 26.5±2.7     | 8.8±2.5        | 13.8±2.5         | 14.6±3.2         |
| InsP1                       | 18.5±2      | 16.9±1.5     | 17.8±1.9       | 9.6±1.8          | 15.9±2.4         |
| InsP2                       | 13.5±1.5    | 9.3±0.7      | 6.6±0.4        | 3.9±0.4          | 6.15±0.4         |
| Pulsatile dilatation        | 68.9±5.5    | 26.5±2.7     | 8.8±2.5        | 13.8±2.5         | 14.6±3.2         |
| InsP1                       | 68.9±5.5    | 26.5±2.7     | 8.8±2.5        | 13.8±2.5         | 14.6±3.2         |
| InsP2                       | 18.5±2      | 16.9±1.5     | 17.8±1.9       | 9.6±1.8          | 15.9±2.4         |
| InsP3                       | 13.5±1.5    | 9.3±0.7      | 6.6±0.4        | 3.9±0.4          | 6.15±0.4         |

Values shown are inositol phosphate accumulation (mean±SEM cpm/mg tissue) eight experiments. There were no significant differences in inositol phosphate accumulations among the three experiments studying 10 minutes of right atrial dilatation (dilatation, dilatation and blockers, and pulsatile dilatation).

*p<0.01, tp<0.05 compared with control hearts by Peritz F test.
FIGURE 2. Bar graphs of accumulation of inositol monophosphate (open bars), inositol bisphosphate (dotted bars), and inositol trisphosphate (hatched bars) in right atria subjected to balloon stretching. Atria were dilated for 1, 10, and 20 minutes. Upper panel: control atria; lower panel: dilated atria. Values shown are mean ± SEM of eight different heart preparations. **p < 0.01, *p < 0.05 by Peritz F test.

Effects of Adrenergic and Cholinergic Antagonists

PtdIns turnover in heart is stimulated by both norepinephrine and acetylcholine acting through α-adrenoceptors and muscarinic acetylcholine receptors, respectively. Therefore, it is possible that dilatation releases either one or both of these neurotransmitters. The observed stimulation of PtdIns turnover then could be secondary to such release.

To address this possibility, experiments were performed in which right atrial dilatation was carried out in hearts perfused with propranolol, prazosin, and atropine (all 1 mM). Prazosin and atropine at 1 mM have previously been shown to fully block the stimulation of PtdIns turnover during perfusion with 30 μM norepinephrine and 1 mM carbachol, respectively. Such blockade of adrenergic and cholinergic receptors did not significantly alter the inositol phosphate response to right atrial stretch in either the right atrium or the other heart chambers (Table 3). Thus, not only is the inositol phosphate response of the right atrium to dilatation independent of release of neurotransmitters, but the transmission of the signal to other parts of the heart is also independent of release of norepinephrine or acetylcholine.

Comparison of Effects of Right Atrial Dilatation With Those of Norepinephrine Perfusion

Right atria showed a threefold or fourfold stimulation of inositol phosphate accumulation when subjected to 10–20 minutes of dilatation. The stimulation in the other chambers of the heart was somewhat lower. Such stimulation is low compared with responses reported in other tissues but comparable with responses to norepinephrine and carbachol previously reported in heart. Effects of perfusion with maximally effective doses of norepinephrine (30 μM) were examined under conditions identical to those used in the dilatation studies. As is shown in Table 3, the inositol phosphate response to norepinephrine was slightly higher than that to right atrial dilatation with a maximum increase of some fivefold or sixfold in InsP₃. These data show that the maximum stimulation of inositol phosphate accumulation achieved with right atrial dilatation is not markedly different from the response to the most effective stimulant of cardiac PtdIns turnover.

Effects of Dilating Right Ventricles

It was of interest to determine whether the PtdIns turnover response to dilatation was restricted to the right atrium. Accordingly, experiments were performed in which right ventricles were dilated by a volume of 300 μl. As shown in Table 4, dilatation of
the right ventricle also produced a stimulation of PtIns turnover. This stimulation was lower than that observed after right atrial dilatation and was greatest in the right ventricle itself. High-performance liquid chromatographic analysis confirmed increases in inositol phosphates in dilated right ventricles. Thus, the PtIns turnover response after distension is not restricted to the right atrium and may be a general feature of myocardial tissue.

**Discussion**

In the present study, we have demonstrated a relation between distension of the myocardium and activation of the PtIns turnover pathway. To our knowledge, this is the first report of a mechanical stimulus initiating such an activation. The most obvious explanation for such a finding is that distension elicits the release of either norepinephrine or acetylcholine which subsequently stimulates PtIns turnover. However, addition of adrenergic and muscarinic blockers did not influence the PtIns turnover response to dilatation, indicating that it was not mediated by either of these neurotransmitters. The stimulation of PtIns turnover also is unlikely to be secondary to an increase in cytosolic calcium, which occurs during dilatation.\(^14\)\(^15\) We have previously shown a lack of effect of perfusate calcium (0-10 mM) on the PtIns turnover response in heart,\(^19\)\(^20\) and others have shown a lack of effect of calcium ionophores on the PtIns turnover response in isolated ventricular myocytes.\(^26\)

It is also possible that tissue damage due to ischemia caused by the balloon dilatation was responsible for the stimulation of PtIns turnover. Certainly, permanent dilatation for 10 or 20 minutes was associated with ischemia as shown by release of lactate dehydrogenase. However, dilatation for 1 minute caused a stimulation of PtIns turnover without detectable rises in lactate dehydrogenase in the perfusate. Furthermore, ischemia cannot explain the stimulation of PtIns turnover seen in other chambers of the hearts. Stimulation of PtIns turnover in dilated right atria was higher than in the other chambers of the heart. To further rule out any possible involvement of ischemia, experiments were performed with pulsatile dilatation, which did not produce detectable increase in lactate dehydrogenase release (Tables 2 and 3). Stimulation of PtIns turnover was similar after pulsatile or permanent dilatation. Perfusion with angiotensin II (10^{-7} M), which also caused release of lactate dehydrogenase, did not produce significant stimulation of PtIns turnover. Furthermore, recent studies that also used isolated, perfused rat hearts demonstrated that 30 minutes of ischemia failed to increase inositol phosphate generation.\(^27\) Thus, ischemia is an unlikely mediator of "stretch-activated" PtIns turnover. Taken together, the results suggest that either dilatation directly activates PtIns turnover in cardiac muscle via a "stretch receptor" or that some other as yet unknown factor is released that subsequently stimulates via a receptor coupled to PtIns turnover.

PtIns turnover now is known to be a signaling system for a wide range of hormones, neurotransmitters, and autocrine factors that act at cell surface receptors. It is thought to mediate a large range of responses, including cell growth and mitosis,\(^28\) hormone secretion,\(^29\) contraction,\(^4\) and chemoattraction.\(^30\) However, the role of PtIns turnover in heart is not well understood. The finding that addition of phorbol esters and calcium ionophores can stimulate release of atrial natriuretic peptide\(^22\) indicates one potential function for the pathway, at least in atria. The relation of the PtIns turnover pathway to calcium suggests a possible role in controlling contractility. Although strong experimental evidence for such a relation is lacking, circumstantial evidence is suggestive. First, protein kinase C can phosphorylate the same sarcolemmal protein as the cAMP-dependent protein kinase.\(^31\) This protein is thought to be involved in calcium gating. Second, phospholamban, which is thought to control removal of cytosolic calcium into the endoplasmic reticulum, is a poor substrate for protein kinase C compared with the cAMP-dependent kinase.\(^31\) This could explain why \(\alpha_1\)-adrenoceptor-mediated increases in contractile force are not associated with an acceleration of the relaxation phase.

Recent studies have shown that PtIns turnover has wider implications than the generation of Ins 1,4,5-P_3, which releases calcium, and the generation of

<p>| TABLE 4. Effect of 10-Minute Dilatation of the RV on the Accumulation of Inositol Phosphates in Different Chambers of the Heart |
|-----------------------------------|------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>InsP_1</td>
<td>25.5±3.5</td>
<td>17.8±1.3</td>
<td>5.5±0.5</td>
<td>8.0±1.3</td>
</tr>
<tr>
<td>InsP_2</td>
<td>17.6±2.9</td>
<td>13.3±0.1</td>
<td>12.7±1.1</td>
<td>13.7±2.1</td>
</tr>
<tr>
<td>InsP_3</td>
<td>8.9±0.8</td>
<td>6.4±0.1</td>
<td>4.9±0.5</td>
<td>4.4±0.8</td>
</tr>
<tr>
<td>Dilated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>InsP_1</td>
<td>30.5±1.3</td>
<td>25.8±2.2*</td>
<td>8.8±0.8*</td>
<td>10.2±0.9*</td>
</tr>
<tr>
<td>InsP_2</td>
<td>17.9±1.0</td>
<td>18.6±2.7*</td>
<td>16.0±1.2*</td>
<td>13.7±2.2*</td>
</tr>
<tr>
<td>InsP_3</td>
<td>10.1±1.4</td>
<td>7.3±1.7</td>
<td>5.8±1.2</td>
<td>4.9±0.9</td>
</tr>
</tbody>
</table>

Values shown are inositol phosphate accumulation in cpm/mg tissue, mean±SEM of four experiments.

\(^*p<0.05, (Peritz' F test)\(^33\).
DAG to stimulate protein kinase C. In a wide range of cells and tissues, Ins 1,4,5-P_3 has been shown to undergo phosphorylation to Ins 1,3,4,5-P_4.\textsuperscript{2,22} The function of this compound is not fully understood, but it may be involved in calcium gating, at least in oocytes.\textsuperscript{34} In adrenal glomerulosa cells, other iso-

mers of InsP_4 appear to be formed by further phosph-

orylation-dephosphorylation cycles.\textsuperscript{35} In heart, the major inositol monophosphate was Ins 4-P, which can only be formed by dephosphorylation of Ins 1,4-P_2. Thus, while the major inositol phosphate increase seen in our studies is in InsP_4, this must have been formed from more highly phosphorylated species. We cannot rule out direct hydrolysis of Ptlns 4-P in our studies, but, clearly, breakdown of Ptlns itself is not important in heart. Studies reported here and elsewhere\textsuperscript{18,22} have shown that either per-

fusion with norepinephrine or right atrial dilatation causes a stimulation of Ins 1,4,5-P_3 accumulation. Thus, cleavage of Ptlns 4,5-P_2 is involved, as has been described in other tissues. However, unlike data reported in many other cell types, in heart preparations no peak was observed at the position of InsP_4 in either control hearts or dilated hearts or in either atria or ventricles. Also, none of the dephosph-

orylation products of InsP_4 (Ins 1,3,4-P_3, Ins 3,4-
P_2, and Ins 3-P) were observed. These results show that the Ins 1,4,5-P_3 kinase pathway is not operative in heart at detectable levels, at least under the conditions of our perfusion experiments. What this might mean in functional terms cannot be suggested until the full implications of the InsP_4 pathway are understood. Our findings of a lack of detectable InsP_4 and its degradation products are in agreement with a recent report showing similar profiles in BC3H-1 muscle cells in culture.\textsuperscript{36} Furthermore, the cult-

ured muscle cells produced glycerophosphoinosi-
tol, glycophosphoinositol 4-phosphate, and gly-

cerophosphoinositol 4,5-bisphosphate as were observed in heart tissue. Taken together, these findings suggest that the Ins 1,4,5-P_3 kinase pathway may be less important in muscle than in other cell types. However, different results have been reported by others who reported finding Ins 1,3,4-
P_3, a breakdown product of Ins 1,3,4,5-P_4, but not Ins 1,3,4,5-P_4 itself in rat ventricle.\textsuperscript{23} The reason for these differences is unclear, but the chromato-

graphic methods used in the latter study were different from those used in the present study and those used by Ambler et al.\textsuperscript{36}

Results of the present study have shown that a mechanical stimulus (dilatation of the right atrium or the right ventricle) stimulates Ptlns turnover. Stimulation of Ptlns turnover pathway can also release ANP. These findings suggest that the Ptlns turnover pathway may mediate, at least in part, the stretch-induced release of ANP. Stimulation of Ptlns turnover was also observed in other chambers of the heart after right atrial dilatation and dilatation of the right ventricle also caused a stimulation. It is tempting to speculate that Ptlns turnover might be associated with responses of the heart to increased venous return.

Acknowledgment

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