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)

The mammalian myocardium is capable of responding to a variety of stimuli, both external and internal. External control of cardiac function is achieved most importantly through the autonomic nervous system, which modulates both the rate and force of contraction. The mechanisms whereby the sympathetic and parasympathetic nervous systems modulate contractility have been extensively investigated. The increased contractility elicited by sympathetic stimulation is largely mediated via β-adrenoceptors and is dependent on an initial rise in intracellular cyclic cAMP, which subsequently elevates cytosolic calcium.1 Stimulation of cardiac α1-adrenoceptors also causes a positive inotropic response under some conditions.2 This response is independent of cAMP3 and is possibly initiated via the phosphatidylinositol (PtdIns) turnover pathway that mediates α1-adrenoceptor responses in a number of different tissues.4 As currently understood, the PtdIns turnover pathway involves receptor stimulation of a phospholipase C, which selectively cleaves a plasma membrane lipid, phosphatidylinositol-4,5(bis)phosphate (PtdIns 4,5-P2). This cleavage generates two biologically active intracellular messengers, sn-1,2-diacylglycerol (DAG) and inositol-1,4,5(tris)phosphate (Ins 1,4,5-P3). DAG stimulates the membrane-bound, phospholipid-dependent, calcium-dependent protein kinase C, and Ins 1,4,5-P3 can release calcium from stores in the sarcoplasmic reticulum.5 The released calcium acts in concert with protein kinase C to activate a wide range of cellular responses. The isolated heart freed from any influence of the nervous system can respond to stimuli, such as dilatation, caused by an increased volume load. Dilatation of the heart has long been known to cause an increase in contractile function by the Frank-Starling mechanism. More recently, dilata-
tion of the left atrium has been shown to inhibit vasopressin release from the posterior pituitary and dilatation of the right atrium to cause release of atrial natriuretic peptide (ANP) into the circulation. Extensive studies have been performed to investigate mechanisms of release of ANP in vivo and in vitro. In vivo, ANP is released after volume or sodium loading and in vitro by dilating the right atrium or by catecholamines acting at $\alpha_1$-adrenoceptors. In other tissues, responses to stimulation of $\alpha_1$-adrenoceptors are thought to be mediated by the Ptlns turnover pathway. In addition, by directly activating the Ptlns turnover pathway by adding DAG analogues (phorbol esters) and Ca$^{2+}$ ionophores (to replace Ins 1,4,5-P$_3$), it has been possible to stimulate release of ANP in vitro. Thus, it is likely that stimulation of ANP release via $\alpha_1$-adrenoceptors is associated with an activation of the Ptlns turnover pathway. The mechanisms by which right atrial dilatation increases ANP release are unknown. Atrial stretch has been reported to increase calcium influx by causing depolarization of the sarcolemma. Ultrastructural changes also have been demonstrated, at least in murine left atria, but how these relate to function is unclear.

In the present study, we have investigated the effect of dilating the myocardium on the activity of the Ptlns turnover pathway. We show that distension of the right atrium stimulates the generation of Ins 1,4,5-P$_3$ and its degradation products. These results raise the possibility that the responses of the heart to dilatation are, in part, mediated by components of the Ptlns turnover pathway.

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% O$_2$-5% CO$_2$ until the perfusate was free of blood. Then, hearts were perfused for 2 hours with Krebs medium containing 1 $\mu$Ci [H]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 [H]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 noradrenaline ($3 \times 10^{-5}$ 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, [H]inositol-labeled products were extracted by a chloroform-methanol extraction method as described previously. [H]-Labeled inositol phosphates were separated from [H]inositol 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. [H]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-P$_2$, and Ins 1,4,5-P$_3$ were identified using [H]-labeled standards. Other peaks on the chromatograms were identified as glycerophosphoinositol 4-phosphate and glycerophosphoinositol 4,5-bisphosphate by comparison with [3P]-labeled standards prepared as described by Downes et al. Standard [H]Ins 1,3,4,5-P$_4$ was prepared from [H]Ins 1,4,5-P$_3$ using a crude preparation of Ins 1,4,5-P$_3$ kinase from rat brain.
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>Control</td>
<td>17.6±1.4</td>
<td>6.1±0.5</td>
<td>9.6±0.7</td>
<td>8.1±0.5</td>
</tr>
<tr>
<td>InsP₁</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>InsP₂</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>Dilated</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, †p<0.05 by Peritz F test.

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±0.3 (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.

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 ΔL-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.18-23 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 significantly different from the 10-minute response (Figure 2).

Stimulation of P1Ins turnover during right atrial distension was not confined to the right atrium. Smaller increases were observed in the other chambers of the hearts after right atrial distension for 10 or 20 minutes (Table 1). No increases were detected after 1 minute of dilatation. These results suggest that P1Ins turnover in the right atrium itself responds immediately to dilatation, whereas time is required for transmission of the signal to other chambers of the hearts. Alternatively, it is possible that the smaller stimulations seen in the other chambers mean that increased activity, although present, is undetectable at 1 minute. Increases in inositol phosphates in the other chambers of the hearts were largely confined to the InsP₁ fraction (Table 1). This might reflect preferential breakdown of P1Ins rather than P1Ins 4,5-P₂. However, analysis by high-performance liquid chromatography of the InsP₁ fraction showed that it was composed largely of Ins 4-P, which can only be derived from Ins 1,4,5-P₃ or, possibly, Ins 1,4,5-P₃.
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 Ins 1,4,5-P_3 was observed together with increases in Ins 1,4-P_2 and Ins 4-P, which are degradation products of Ins 1,4,5-P_3. 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 Ins 1,3,4,5-P_4. Also, the breakdown products of Ins

<table>
<thead>
<tr>
<th>Duration of dilatation (min)</th>
<th>Control</th>
<th>Dilated (permanent)</th>
<th>Dilated (30 sec/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6±0.2</td>
<td>0.7±0.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.8±0.2</td>
<td>3.3±1.2</td>
<td>0.95±0.3</td>
</tr>
<tr>
<td>20</td>
<td>0.9±0.3</td>
<td>11.5±3.2</td>
<td></td>
</tr>
</tbody>
</table>

Values shown are lactate dehydrogenase activity (mean±SEM nmoles 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 | Septum     |
| Control         |             |             |               |               |            |
| InsP_1          | 22.3±0.8    | 17.6±1.4    | 6.1±0.5      | 9.6±0.7      | 8.5±0.5    |
| InsP_2          | 15.4±0.4    | 14.1±0.7    | 11.1±0.8     | 13.2±0.9     | 15.6±0.9   |
| InsP_3          | 9.5±0.3     | 8.5±0.4     | 4.2±0.3      | 3.9±0.15     | 5.2±0.4    |
| Norepinephrine  |             |             |               |               |            |
| InsP_1          | 103.8±1.9   | 95.9±1.9    | 42.3±5.9     | 32.0±7.2     | 34.2±8.4   |
| InsP_2          | 42.3±2.1    | 45.9±3     | 32.6±4.9     | 17.4±4.9†    | 19.3±5.7   |
| InsP_3          | 15.5±1.5    | 14.4±0.5    | 3.7±0.7      | 2.5±0.5      | 4.8±0.6    |
| Dilatation      |             |             |               |               |            |
| InsP_1          | 74.6±2.3    | 27.2±1.9    | 11.3±0.5     | 13.1±0.9     | 13.5±0.9   |
| InsP_2          | 20.2±1.3    | 14.2±0.6    | 15.9±1       | 9.9±1.0      | 14.7±1.4   |
| InsP_3          | 13.5±1.5    | 9.3±0.7     | 6.6±0.4      | 3.9±0.4      | 6.15±0.4   |
| Dilatation and blockers |     |             |               |               |            |
| InsP_1          | 68.9±5.5    | 26.5±2.7    | 8.8±2.5      | 13.8±2.5     | 14.6±3.2   |
| InsP_2          | 18.5±2      | 16.9±1.5    | 17.8±1.9     | 9.6±1.8      | 15.9±2.4   |
| InsP_3          | 15.3±1.6    | 10.8±1.3    | 4.7±1.6      | 4.4±1.7      | 6.4±1.9    |
| Pulsatile dilatation |      |             |               |               |            |
| InsP_1          | 63.7±7.2    | 25.9±3.6    | 11.1±3.1     | 13.6±3.1     | 13.8±2.7   |
| InsP_2          | 17.1±2.7    | 14.2±1.8    | 14.8±2.2     | 8.4±1.8      | 15.3±1.3   |
| InsP_3          | 12.8±1.3    | 8.2±1.3     | 5.8±1.3      | 3.8±0.4      | 6.6±1.3    |

Values shown are inositol phosphate accumulation in (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 and blockers, and pulsatile dilatation.

* p<0.01, †p<0.05 compared with control hearts by Peritz F test.
Effects of Adrenergic and Cholinergic Antagonists

PtdIns turnover in heart is stimulated by both norepinephrine and acetylcholine acting through \( \alpha \)-adrenoceptors and muscarinic acetylcholine receptors, respectively.\(^1^8\) 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 \( 1 \mu M \)). Prazosin and atropine at \( 1 \mu M \) have previously been shown to fully block the stimulation of PtdIns turnover during perfusion with \( 30 \mu M \) norepinephrine and \( 1 \mu M \) carbachol, respectively.\(^1^8\) 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 hearts 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.\(^1^8\) Effects of perfusion with maximally effective doses of norepinephrine (30 \( \mu M \)) 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 InSP\(_4\). 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 \( \mu l \). As shown in Table 4, dilatation of
the right ventricle also produced a stimulation of PtdIns 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 PtdIns 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 dilatation of the myocardium and activation of the PtdIns 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 PtdIns turnover. However, addition of adrenergic and muscarinic blockers did not influence the PtdIns turnover response to dilatation, indicating that it was not mediated by either of these neurotransmitters. The stimulation of PtdIns turnover also is unlikely to be secondary to an increase in cytosolic calcium, which occurs during dilatation. 

It is also possible that tissue damage due to ischemia caused by the balloon dilatation was responsible for the stimulation of PtdIns 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 PtdIns turnover without detectable rises in lactate dehydrogenase in the perfusate. Furthermore, ischemia cannot explain the stimulation of PtdIns turnover seen in other chambers of the heart. Stimulation of PtdIns 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 PtdIns 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 PtdIns turnover. Furthermore, recent studies that also used isolated, perfused rat hearts demonstrated that 30 minutes of ischemia failed to increase inositol phosphate generation. Thus, ischemia is an unlikely mediator of "stretch-activated" PtdIns turnover. Taken together, the results suggest that either dilatation directly activates PtdIns 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 PtdIns turnover.

PtdIns turnover is now 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, hormone secretion, contraction, and chemotraction. However, the role of PtdIns turnover in heart is not well understood. The finding that addition of phorbol esters and calcium ionophores can stimulate release of atrial natriuretic peptide indicates one potential function for the pathway, at least in atria. The relation of the PtdIns 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 sarcoplasmic reticulum as the cAMP-dependent protein kinase.

<table>
<thead>
<tr>
<th>Chamber</th>
<th>Right atrium</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>
<td></td>
</tr>
<tr>
<td>InoP₁</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>
<td>7.1±0.6</td>
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<tr>
<td>InoP₂</td>
<td>17.6±2.9</td>
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<tr>
<td>InoP₃</td>
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<tr>
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<td>30.5±1.3</td>
<td>25.8±2.2</td>
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<td>5.1±1.2</td>
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</tbody>
</table>

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

*p<0.05, (Peritz' F test).
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$_4$. The function of this compound is not fully understood, but it may be involved in calcium gating, at least in oocytes. In adrenal glomerulosa cells, other iso- 

mers of InsP$_4$ appear to be formed by further phosphorylation-dephosphorylation cycles. 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 PtdIns 4-P in our studies, but, clearly, breakdown of PtdIns itself is not important in heart. Studies reported here and elsewhere have shown that either fusion with norepinephrine or right atrial dilatation causes a stimulation of Ins 1,4,5-P$_3$ accumulation. Thus, cleavage of PtdIns 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 dephosphorylation 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. Furthermore, the cultured muscle cells produced glycerophosphoinositol, glycerophosphoinositol 4-phosphate, and glycerophosphoinositol 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. The reason for these differences is unclear, but the chromatographic methods used in the latter study were different from those used in the present study and those used by Ambler et al.

Results of the present study have shown that a mechanical stimulus (dilatation of the right atrium or the right ventricle) stimulates PtdIns turnover. Stimulation of PtdIns turnover pathway can also release ANP. These findings suggest that the PtdIns turnover pathway may mediate, at least in part, the stretch-induced release of ANP. Stimulation of PtdIns 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 PtdIns turnover might be associated with responses of the heart to increased venous return.

**Acknowledgment**

The assistance of Dr. J.A. Angus (Baker Medical Research Institute, Melbourne) in providing equipment and advice is gratefully acknowledged.

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KEY WORDS • phosphatidylinositol • myocardium
Myocardial stretch stimulates phosphatidylinositol turnover.
R von Harsdorf, R E Lang, M Fullerton and E A Woodcock

Circ Res. 1989;65:494-501
doi: 10.1161/01.RES.65.2.494

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