INOSITOL PHOSPHATE GENERATION IN THE HEART: MECHANISMS AND FUNCTIONAL RELEVANCE

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The studies described in this thesis have used principally the rat neonatal cardiomyocyte (NCM) model to investigate previously unresolved questions regarding inositol phosphate signalling in the heart. Inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃) is known to be an arrhythmogenic molecule in the setting of cardiac ischaemia and subsequent reperfusion, but the mechanisms responsible for its enhanced generation in pathological circumstances, as well as those suppressing its generation during phospholipase C (PLC)-coupled receptor stimulation under physiological conditions, have not been characterised. [³H]InsP generation in response to norepinephrine (NE) was largely insensitive to the PtdIns(4,5)P₂-binding compound neomycin. In contrast, the Ca²⁺ ionophore A23187 stimulated [³H]InsP generation in a manner which was inhibited by neomycin. Further studies in permeabilised NCM showed that elevation of Ca²⁺ generated a larger proportion of [³H]Ins(1,4,5)P₃ than direct G protein stimulation, and introduction of excess unlabelled Ins(1,4,5)P₃ protected more [³H]Ins(1,4,5)P₃ in the setting of Ca²⁺ elevation than of GTPγS stimulation. Analysis of these effects demonstrated that while elevated intracellular Ca²⁺ is required to generate substantial amounts of Ins(1,4,5)P₃, G protein activation of NCM leads to an InsP response which is largely independent of Ins(1,4,5)P₃. As the major InsP isomer which accumulated in NCM is Ins(4)P, which can only be formed from Ins(1,4)P₂, these studies provided
strong evidence that the InsPs formed in response to G protein activation derive from \( \text{Ins}(1,4)P_2 \).

Thus, InsP responses in heart may involve generation of principally \( \text{Ins}(1,4)P_2 \) or \( \text{Ins}(1,4,5)P_3 \). It was hypothesised that alternate PLC isoforms may be involved in mediating these two different responses, and that the highly \( \text{Ca}^{2+} \)-sensitive isoform PLC-\( \delta_1 \) may mediate \( \text{Ca}^{2+} \)-stimulated InsP responses. Adenoviral manipulation of PLC-\( \delta_1 \) content in NCM was performed in order to establish a role or otherwise for PLC-\( \delta_1 \) in the InsP response to elevated \( \text{Ca}^{2+} \). Despite marked effects on PLC-\( \delta_1 \) expression, antisense PLC-\( \delta_1 \) adenovirus did not reduce, and sense PLC-\( \delta_1 \) adenovirus did not enhance, InsP generation in response to A23187, providing no evidence for PLC-\( \delta_1 \) as the mediator of the response to elevated \( \text{Ca}^{2+} \). Surprisingly, PLC-\( \delta_1 \) overexpression decreased NE-stimulated responses in intact NCM. Further experiments showed reduced GTP\( \gamma \)S responses in permeabilised NCM, suggesting diminished activity of either the G protein or PLC involved in NE responses. To address this, the cellular content of G\( \alpha_1 \), PLC-\( \beta_1 \) and PLC-\( \beta_3 \) was examined. PLC-\( \delta_1 \) overexpression affected the content of PLC-\( \beta_1 \) but not of G\( \alpha_1 \) or PLC-\( \beta_3 \), demonstrating specific coupling of G\( \alpha_1 \) to PLC-\( \beta_1 \) in NCM. Thus, these data show that G protein (presumably G\( \alpha_1 \)) stimulation of \( \text{Ins}(1,4)P_2 \) generation in NCM is mediated by PLC-\( \beta_1 \). The identity of the PLC responsible for \( \text{Ca}^{2+} \)-activated InsP generation in NCM remains unknown.

A transgenic mouse line with inhibited G\( \alpha_1 \) signalling was studied in an attempt to further confirm the role of G\( \alpha_1 \) in \( \alpha_1 \)-adrenergic receptor-mediated InsP generation in the heart. Although hypertrophy in response to pressure overload in the hearts of these mice was reduced, no changes were evident in InsP generation. However, the
low magnitude of agonist-stimulated InsP responses in mouse heart means that these studies remained inconclusive.

As the heart responds to PLC stimulation by generation of either Ins(1,4)P$_2$ or Ins(1,4,5)P$_3$, preferential Ins(1,4)P$_2$ generation in the heart could represent a mechanism for DAG production without concomitant Ins(1,4,5)P$_3$ generation, or could implicate Ins(1,4)P$_2$ itself in an aspect of cardiac function. Transfection studies using hypertrophied NCM outlined a novel antihypertrophic role for the enzyme responsible for metabolism of Ins(1,4)P$_2$, inositol polyphosphate 1-phosphatase (INPP). Thus, it appears that the generation of Ins(1,4)P$_2$ in the heart may not only act to prevent Ins(1,4,5)P$_3$ generation, but may also have a signalling function of its own.
Declaration

This is to certify that:

(i) the thesis comprises only my original work;

(ii) due acknowledgment has been made in the text to all other material used;

(iii) and the thesis is less than 100,000 words in length, exclusive of tables, figures and bibliographies.

Scot J Matkovich

September 2000
Publications and communications

Peer-reviewed journals:


Abstracts and posters:


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‘...our complicated experiments have no longer anything to do
with nature in her own right, but with nature changed and transformed
by our own cognitive activity.’

Werner Heisenberg (1901-1976)
**Commonly used abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ANP</td>
<td>atrial natriuretic peptide (atrial natriuretic factor)</td>
</tr>
<tr>
<td>cpm</td>
<td>counts per minute</td>
</tr>
<tr>
<td>DAG</td>
<td>sn-1,2-diacylglycerol</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>N,N,N',N'-ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ET-1</td>
<td>endothelin-1</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>InsP</td>
<td>inositol phosphate</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MLC-2v</td>
<td>myosin light chain-2, ventricular isoform</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>NCM</td>
<td>neonatal rat cardiomyocytes</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine (noradrenaline)</td>
</tr>
<tr>
<td>PE</td>
<td>phenylephrine</td>
</tr>
<tr>
<td>PLC</td>
<td>phospholipase C</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PtdIns</td>
<td>phosphatidylinositol</td>
</tr>
<tr>
<td>PTX</td>
<td>pertussis toxin</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>sec</td>
<td>second(s)</td>
</tr>
<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
</tr>
</tbody>
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