Published in final edited form as:

Semin Cell Dev Biol. 2006 April ; 17(2): 230–232. doi:10.1016/j.semcdb.2006.02.001.

ITP

Michael Whitaker

Institute of Cell and Molecular Biosciences University of Newcastle upon Tyne Medical School
Framlington Place NE2 4HH UK

Abstract

I describe how we came to microinject inositol trisphosphate into sea urchin eggs and found that it was a very potent activator of calcium release.

Keywords

calcium; inositol trisphosphate; fertilization

When I went back to my lab books I was struck by their quaintness, a quaintness not unusual in things rediscovered after a couple of decades. My experimental summaries were made by hand on paper, not in a spreadsheet; figures for publication were drawn in ink; the manuscript corrections were cut and pasted in. 1984 did not in the event epitomise the continual war and totalitarian repression imagined by Orwell, but it was the year that Indira Ghandi was assassinated, that the Soviets (another quaint item) boycotted the Los Angeles Olympic Games and during which Apple launched the Macintosh. These facts I learned by typing a few words into an internet search engine, an act that will itself seem quaint, I am sure, in twenty years time.

1984 was for me not only the year in which I cut and pasted a manuscript physically for the last time and thereafter did it electronically. It was also the year that for me calcium signalling finally embraced lipid biochemistry. I want to set out briefly the circumstances that led me to microinject ITP into sea urchin eggs.

The calcium legacy-from Tripods to tripod

I owe my interest in calcium signalling to Peter Baker. Peter was a student of Alan Hodgkin's and thus keen on the giant axon of the squid and also my Physiology supervisor in Cambridge. He wrote a startlingly good review summarising calcium transport and compartmentation [1] and I was hooked. At that time there was great interest in calcium and the control of neurotransmitter release [2]. I became interested in measuring the calcium-stimulated kinetics of vesicle-membrane fusion directly by using calcium-buffered solutions in broken cells. This led us eventually through Baker's interest in sea urchin eggs [3] to Vacquier's experiments on the sea urchin cortical granules [4, 5]. Steinhardt had worked with Baker and Hodgkin on the sodium-calcium exchange in squid axon [1] and had gone on to perform the classic experiments with calcium ionophore in sea urchin eggs that he describes in this volume. I was very much taken with fertilization, so it was natural that I spend a year with Steinhardt in Berkeley; at the time he was looking at both calcium and pH changes at fertilization [6-9]. During that year I learned microinjection and fluorescence

methods and, most importantly, the logical tripod essential for demonstrating causal linkages, described by Steinhardt in his article in this volume.

Phorbol ester – an exchanger offers a clue

Back in London in the autumn of 1983, Peter Baker mentioned to me that he had found that phorbol ester (known then as TPA) stimulated protein synthesis in sea urchin eggs. The significance of this experiment was that TPA, a tumour promoter, had been recently shown by Nishizuka to stimulate protein kinase C [10, 11]. I knew from Steinhardt's work that protein synthesis was stimulated after fertilization by an increase in pH and from Epel's earlier studies that the pH increase at fertilization was due to a sodium-dependent extrusion of protons that could be detected as an acidification of the sea water in which eggs were fertilised [12]. So I conjectured that TPA was stimulating this sodium-hydrogen exchange. In early December I found that TPA caused an acidification of the sea water in which unfertilised eggs were suspended. TPA mimicked the natural stimulation of protein kinase C by diacylglycerols [10], products of the hydrolysis of phosphatidylinositol phospholipids. I had followed with great interest the idea that receptor mediated stimulation of phosphoinositide turnover was correlated with calcium mobilisation [13, 14] and had noted Berridge's groundbreaking observation that phosphoinositide turnover lead to the rapid production of inositol 1,4,5-trisphosphate [15]. However, until Baker's chance remarks led me to look at the effect of TPA on sodium-proton exchange, it had not occurred to me that there might be parallels between receptor-mediated calcium mobilisation and calcium mobilisation at fertilisation. I called Michael Berridge who introduced me to Robin Irvine, a very talented biochemist who had isolated and characterised the many inositol polyphosphates. He sent me a panel of inositol polyphosphates. I put them in the freezer.

A Christmas present

When thinking about experiments I am cautious and a sceptic. From experience I was very aware that unfertilised sea urchin eggs were easily activated – it could happen just by inserting an electrode or micropipette. I imagined the need for very many experiments and their controls. The inositol polyphosphates stayed in the freezer. It was Josh Zimmerberg who persuaded me to defrost them. Josh was visiting over the Christmas holidays to collaborate on the idea that exocytosis was driven by osmotic forces, something we later published [16]. Two days before Christmas, we carried out some preliminary experiments with ITP at 100 μM in the pipette and found a very rapid fertilization membrane elevation consistent with a substantial release of calcium. We left to celebrate the holidays in good spirits. My memory has always told me that when I came after Christmas to do controls, the euphoria evaporated. Going back to my lab book, I see that I was not mistaken (Figure 1). I had used the same eppendorf pipette that I used to fill the ITP micropipette to fill the control pipette and thus these control eggs activated when microinjected. What this showed was that ITP was very potent and that we had been injecting it far in excess. Once this was clear, I was content. ITP was so effective as an activator that it was straightforward to distinguish its effects from those of inadvertent activation by microinjection (Figure 2).

Nineteen eighty four

The prize in 1984 goes to the Macintosh with its icon-based operating system. I doubt in the broad scheme of things that phosphoinositide signalling qualifies even as a runner-up. Nonetheless, in the small world of signalling, it was a fine year for phosphoinositides. It was the year in which Berridge clearly formulated phosphoinositide signalling as a bifurcating pathway with InsP_3 as a calcium-releasing agent and diacylglycerol as a protein kinase C agonist [17, 18]. In the context of fertilization, Berridge's predictions of the control of the pH change by the diacylglycerol arm of the pathway were confirmed by our work with TPA,

begun in 1983 [19]. It was also the year in which it was demonstrated that phosphoinositides turned over at fertilisation [20]. It was natural to think that the analogy between receptor activation and sperm-egg interaction at fertilization was exact [21]. It now seems more likely that the prediction made in 1984 that the sperm introduces InsP_3 or a phospholipase activity when it fuses with the egg [22] is closer to the mark [23, 24].

Epilogue

These experiments with ITP have always been important to me because they linked together those scientists who made the biggest impact on the way I think about things: Peter Baker, Richard Steinhardt and Joshua Zimmerberg.

References

- [1]. Baker PF, Blaustein MP, Hodgkin AL, Steinhardt RA. The influence of calcium on sodium efflux in squid axons. *J Physiol.* 1969; 200:431–58. [PubMed: 5764407]
- [2]. Katz B, Miledi R. The effect of prolonged depolarization on synaptic transfer in the stellate ganglion of the squid. *J Physiol.* 1971; 216:503–12. [PubMed: 4326997]
- [3]. Baker PF, Presley R. Kinetic evidence for an intermediate stage in the fertilization of the sea urchin egg. *Nature.* 1969; 221:488–90. [PubMed: 5814707]
- [4]. Vacquier VD. The isolation of intact cortical granules from sea urchin eggs: calcium ions trigger granule discharge. *Dev Biol.* 1975; 43:62–74. [PubMed: 1171035]
- [5]. Baker PF, Whitaker MJ. Influence of ATP and calcium on the cortical reaction in sea urchin eggs. *Nature.* 1978; 276:513–515. [PubMed: 364317]
- [6]. Shen SS, Steinhardt RA. Intracellular pH and the sodium requirement at fertilisation. *Nature.* 1979; 282:87–9. [PubMed: 41185]
- [7]. Grainger JL, Winkler MM, Shen SS, Steinhardt RA. Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo. *Dev Biol.* 1979; 68:396–406. [PubMed: 35433]
- [8]. Winkler MM, Steinhardt RA, Grainger JL, Minning L. Dual ionic controls for the activation of protein synthesis at fertilization. *Nature.* 1980; 287:558–60. [PubMed: 7422008]
- [9]. Whitaker MJ, Steinhardt RA. Ionic regulation of egg activation. *Q Rev Biophys.* 1982; 15:593–666. [PubMed: 6820160]
- [10]. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem.* 1982; 257:7847–51. [PubMed: 7085651]
- [11]. Kikkawa U, Takai Y, Tanaka Y, Miyake R, Nishizuka Y. Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. *J Biol Chem.* 1983; 258:11442–5. [PubMed: 6311812]
- [12]. Johnson JD, Epel D. Intracellular pH and activation of sea urchin eggs after fertilisation. *Nature.* 1976; 262:661–4. [PubMed: 8729]
- [13]. Michell RH, Kirk CJ, Jones LM, Downes CP, Creba JA. The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions. *Philosophical transactions of the Royal Society of London Series B, Biological sciences.* 1981; 296:123–38.
- [14]. Downes P, Michell RH. Phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: lipids in search of a function. *Cell Calcium.* 1982; 3:467–502. [PubMed: 6297743]
- [15]. Berridge MJ. Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. *Biochem J.* 1983; 212:849–58. [PubMed: 6309155]
- [16]. Zimmerberg J, Whitaker M. Irreversible swelling of secretory granules during exocytosis caused by calcium. *Nature.* 1985; 315:581–4. [PubMed: 3925345]
- [17]. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature.* 1984; 312:315–21. [PubMed: 6095092]

- [18]. Berridge MJ. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J.* 1984; 220:345–60. [PubMed: 6146314]
- [19]. Swann K, Whitaker M. Stimulation of the Na/H exchanger of sea urchin eggs by phorbol ester. *Nature.* 1985; 314:274–7. [PubMed: 2984569]
- [20]. Turner PR, Sheetz MP, Jaffe LA. Fertilization increases the polyphosphoinositide content of sea urchin eggs. *Nature.* 1984; 310:414–5. [PubMed: 6087155]
- [21]. Turner PR, Jaffe LA, Fein A. Regulation of cortical vesicle exocytosis in sea urchin eggs by inositol 1,4,5-trisphosphate and GTP-binding protein. *J Cell Biol.* 1986; 102:70–6. [PubMed: 3001104]
- [22]. Whitaker M, Irvine RF. Inositol 1,4,5-trisphosphate microinjection activates sea urchin eggs. *Nature.* 1984; 312:636–639.
- [23]. Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, et al. PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development.* 2002; 129:3533–44. [PubMed: 12117804]
- [24]. Whitaker MJ. Calcium signalling in eggs and embryos. *Physiol Rev.* 2005 in press.

29/12/83 IP *pyrula*
 Using 0.5M KCl 100mM BSA - pH 7.0.
 so for (i) to generate 100mM BSA Mg no ATP activities.
 (ii) 0.5M KCl 100mM BSA pH 7.0 doesn't.
 (iii) same solution did (but used 300mM - 1M glycine)
 (iv) 100mM IP 2 plus activities KCl.
 (v) 100mM IP 1 plus activities.

Figure 1.

Problems with the controls. A page from the lab book.

Figure 2. Learning the nomenclature. Entries in the lab book use ITP as an abbreviation for inositol 1,4,5-trisphosphate. (a) Summary of experimental data in a spreadsheet-free era; (b) An early draft of a figure (top left) uses ITP, not Ins 1 4 5 P₃; the later draft of the figure and some photographs of InsP₃-activated eggs are also shown.