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Review

Inositol trisphosphate and calcium signalling mechanisms

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ABSTRACT

Studies on control of fluid secretion by an insect salivary gland led to the discovery of inositol trisphosphate (IP_3) and its role in calcium signalling. Many cell stimuli act on receptors that are coupled to phospholipase C that hydrolyses phosphatidylinosol 4,5-bisphosphate (PIP_2) to release IP_3 to the cytosol. IP_3 receptors located on the endoplasmic reticulum respond to this elevation of IP_3 by releasing Ca^{2+}, which is often organized into characteristic spatial (elementary events and waves) and temporal (Ca^{2+} oscillations) patterns. This IP_3/Ca^{2+} pathway is a remarkably versatile signalling system that has been adapted to control processes as diverse as fertilization, proliferation, contraction, cell metabolism, vesicle and fluid secretion and information processing in neuronal cells.

1. Introduction

Inositol 1,4,5-trisphosphate (IP_3) and calcium (Ca^{2+}) have been my scientific companions over the last 25 years. My relationship with these two messengers has passed through two distinct phases. Firstly, there was the work that led up to the discovery that IP_3 was a Ca^{2+}-mobilizing second messenger. The second phase was characterized by the excitement of finding that this IP_3/Ca^{2+} signalling system was a key regulator of many different cellular control mechanisms.

1.1. Stimulus-secretion coupling in the insect salivary gland

My interest in Ca^{2+} began when I was trying to understand the mechanisms that control fluid secretion using insect salivary glands as a model system. These glands are normally quiescent and are only called into action when stimulated by 5-hydroxytryptamine (5-HT) during feeding [1]. These studies were carried out at Case Western Reserve University where Sutherland and Rall had just discovered cyclic adenosine monophosphate (cyclic AMP) and had developed their novel second messenger concept [2]. I was very excited to find that the stimulatory effect of 5-HT on fluid secretion was exactly duplicated by the addition of cyclic AMP [1,3]. The next obvious step was to find out how cyclic AMP worked. I approached this problem by characterizing the electrophysiological properties of the secretory response by placing glands in a small Perspex perfusion chamber that enabled the *trans*-epithelial potential to be monitored while adding and removing 5-HT or cyclic AMP. When the glands were at rest, the lumen had a slightly positive potential relative to the bathing medium and depolarized rapidly in response to 5-HT but hyperpolarized after addition of cyclic AMP [4]. Subsequent experiments revealed that cyclic AMP activated an electrogenic potassium pump, which was the prime mover for fluid secretion. Chloride followed passively and this transport of KCl created the osmotic gradient to drive the flow of water. Subsequent resistance measurements showed that the passive flux of chloride was facilitated by a 5-HT-dependent increase in chloride conductance that occurred independently of cyclic AMP [5]. It seemed that 5-HT was having two actions, one mediated by cyclic AMP to drive potassium transport and a second mechanism that increased the flux of chloride (Fig. 1).

It soon became apparent that Ca^{2+} might regulate this passive chloride flux. A collaborative study with Howard Rasmussen clearly showed that 5-HT was having a profound effect on Ca^{2+} dynamics and suggested that Ca^{2+} was being released from an internal store [6]. We subsequently used both electrophysiological and pharmacological techniques to demonstrate that the blowfly salivary gland had two 5-HT receptors operating through separate second messengers [7–9]. One receptor used cyclic AMP to drive potassium transport whereas the other employed Ca^{2+} to open chloride channels.

1.2. Inositol lipid hydrolysis and Ca^{2+} signalling

The most puzzling aspect of the putative Ca^{2+} messenger system in the insect blowfly was the source of Ca^{2+} much of which was derived from an internal store [7]. As I began to search for the messenger that connected cell surface receptors to the internal store, I became aware of the work by Hokin and Hokin who discovered that external agonists stimulated the turnover of phosphatidylinositol (PI) [10]. Although this PI response had been measured in many different cells in response to many different stimulants, its function was somewhat mysterious. This began to change in 1975 when Bob Michell proposed that this lipid hydrolysis was responsible for Ca^{2+} signalling [11]. In order to test out this hypothesis, I began to use ^{3}H-

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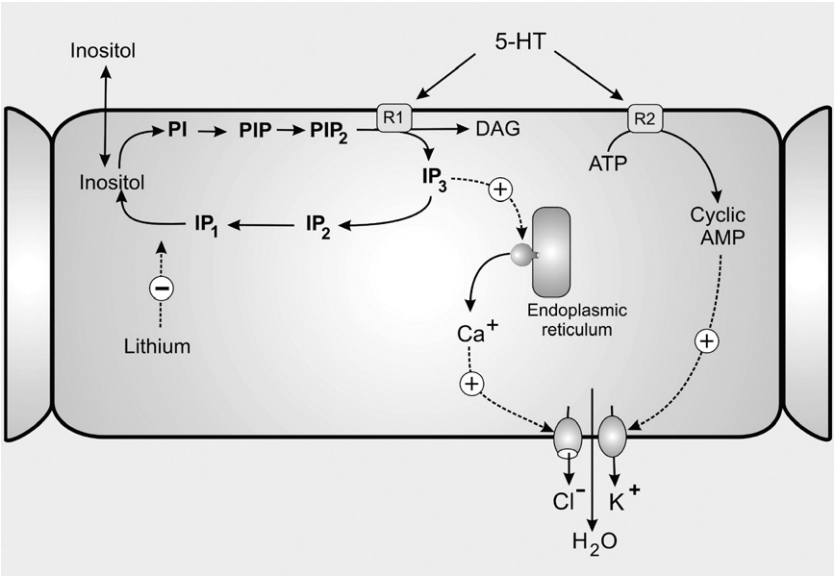

Fig. 1. Stimulus–secretion coupling in the blowfly salivary gland.

inositol to study the PI response in the insect gland. This label was rapidly incorporated into PI and was released from the lipid during agonist stimulation of intact glands and could be collected in the bathing medium. This meant that it was possible to monitor the PI response at regular time intervals in intact glands simply by measuring the efflux of ^{3}H-inositol [12]. This efflux was very small when the glands were at rest, but upon addition of 5-HT there was a dose-dependent increase in the efflux of ^{3}H-inositol.

In order to relate this PI hydrolysis to Ca^{2+} signalling, it was necessary to have some way of measuring intracellular Ca^{2+}. At this stage, Ca^{2+} indicators were not available and Ca^{2+}-sensitive microelectrodes proved to be difficult to prepare and were decidedly unreliable so I devised an indirect method of measuring Ca^{2+} entry into the insect gland by monitoring the *trans*-epithelial flux of ^{45}Ca [13]. Using this method it was possible to show that the dose response curve for inositol efflux lay to the left of that for Ca^{2+} flux, which in turn, lay to the left of the secretory response [12]. This sensitivity sequence was entirely consistent with Michell's notion [11] that the PI response generated the Ca^{2+} signal responsible for cell stimulation. In order to obtain more direct evidence that PI hydrolysis was linked to Ca^{2+} signalling, I tried to find out whether the depletion of inositol could inhibit signalling. When glands were stimulated for 2 h with a high dose of 5-HT and washed repeatedly to remove the inositol that escaped into the medium, there was a dramatic desensitisation of Ca^{2+} signalling [14]. What was particularly significant was the fact that Ca^{2+} signalling could be rescued by incubating the glands in inositol thus enabling them to reconstitute the level of the PI required for signalling [15]. It was clear from these experiments that the PI response was directly linked to Ca^{2+} signalling and the next problem was to find out how the PI response functioned to release internal Ca^{2+}.

1.3. Inositol trisphosphate (IP_3) and Ca^{2+} mobilization

In order to investigate the link between PI hydrolysis and Ca^{2+} signalling, it seemed important to find out more about the biochemical processes underlying the PI response. I began by exploring some interesting work by Allison and his colleagues on Li^{+} and inositol phosphate metabolism [16,17]. They found that Li^{+} was a potent inhibitor of the inositol monophosphatase that hydrolysed inositol 1-phosphate (IP_1) to inositol (Fig. 1). This seemed to be an important observation because it was this Li^{+}-sensitive step that released the free inositol that I had been measuring to monitor PI hydrolysis. This inositol efflux method offered a simple way of testing the inhibitory effect of Li^{+}. Since this inositol was derived from the IP_1 produced by hydrolysing PI, Li^{+} should reduce this efflux by inhibiting the hydrolysis of IP_1 to inositol, which is exactly what happened [18]. When Li^{+} was withdrawn, there was the very large overshoot of inositol efflux that resulted from the inositol monophosphatase suddenly hydrolysing the IP_1 that had built up behind the Li^{+} block.

In order to complete this Li^{+} study, I tried to measure PI metabolites after separating them out on anion-exchange columns [19]. As expected, there was a large accumulation of IP_1 during Li^{+} inhibition, but what was unexpected was the appearance of two additional peaks running after the IP_1. On the basis of standards supplied by Rex Dawson and Robin Irvine, these two peaks turned out to be inositol 1,4-bisphosphate (IP_2) and inositol 1,4,5-trisphosphate (IP_3). On the basis of what was known previously, the simplest hypothesis was that IP_3 was formed first and was then sequentially hydrolysed to IP_2, IP_1 and then inositol (Fig. 1). In order to test this hypothesis, I developed a rapid perfusion system [20] that enabled me to establish the rate at which these inositol phosphates were produced following 5-HT stimulation. These studies on the kinetics of 5-HT-induced inositol phosphate formation revealed that IP_3 and IP_2 increased first with no apparent latency whereas IP_1 followed by inositol appeared much later. I was very excited by this result because it revealed that IP_3 was being generated very quickly. I already knew that there was a 1–2 s latency before the onset of the electrical change caused by the Ca^{2+}-dependent activation of the chloride channels and I now found that IP_3 was being elevated before this physiological response. It was this observation that led me to propose that IP_3 might be the diffusible messenger that coupled receptor activation to the mobilization of internal Ca^{2+} [21].

In order to test whether IP_3 could release Ca^{2+} from internal stores, I set up a collaboration with Irene Schulz and Robin Irvine. The latter provided large amounts of IP_3 that was then tested on a permeabilized pancreatic cell preparation developed by Streb and Schulz. We were all very excited to find that IP_3 did indeed mobilize Ca^{2+} from an internal store that we identified as the endoplasmic reticulum and this was reported in Nature in 1983 [22]. In the following year, there were 7 papers (with 4 published in Nature) that confirmed this initial observation in many other cell types including liver cells [23], Swiss 3T3 cells [24], insulin-secreting cells [25,26], *Limulus* photoreceptors [27,28] and leukocytes [29]. It seemed clear therefore that IP_3 was the long sought after Ca^{2+}-mobilizing second messenger [30–32].

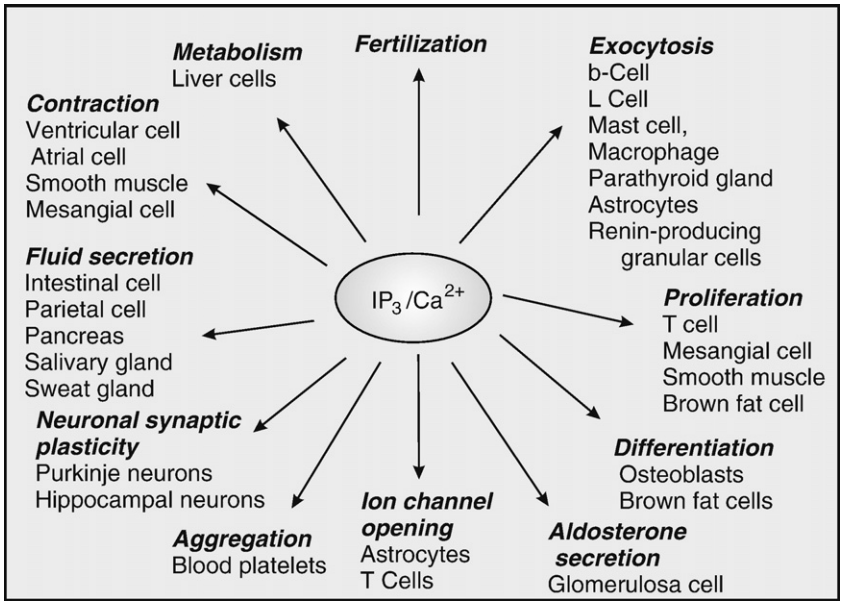

Fig. 2. Regulation of multiple cellular processes by the IP_3/Ca^{2+} signalling pathway.

The second and continuing phase of my relationship with this signalling system has been the excitement of seeing the IP_3/Ca^{2+} pathway emerge as a highly versatile signalling system capable of regulating many different cellular processes. We now know that much of this remarkable versatility depends on the way this signal system is organized in both time (Ca^{2+} oscillations) and space (elementary events and waves) [33–35].

1.4. IP_3 and Ca^{2+} oscillations

My interest in the spatiotemporal aspects of signalling began very early when we discovered that the *trans*-epithelial potential across the insect salivary gland oscillated in response to 5-HT [4]. An interesting feature of these oscillations is that their frequency varied with agonist concentration [4,36]. Since these changes in frequency occurred over the same range of 5-HT concentrations that caused changes in fluid secretion, we proposed that the signalling system operated through frequency modulation (FM) rather than amplitude modulation (AM) [37]. Since these potential oscillations depended on changes of resistance, which were controlled by Ca^{2+}, we speculated that they resulted from an underlying oscillation of Ca^{2+} [38]. When aequorin was introduced to monitor intracellular Ca^{2+}, particularly striking examples of Ca^{2+} oscillations were revealed in both liver cells [39] and mammalian oocytes [40–42]. Frequency modulation was particularly apparent for the liver cell oscillator. What fascinated me most about such oscillatory activity was its spontaneous nature. In some cases, the level of Ca^{2+} was found to remain constant for up to 60 s and was then interrupted by a spontaneous Ca^{2+} spike that then returned back to the resting level for another 60 s before the next spike occurred and so on. In the case of mammalian oocytes undergoing fertilization, these inter-spike intervals can last for 3–4 min [40–42]. The question that intrigued me and still does today is what happens during this inter-spike interval that results in the appearance of the spontaneous spikes?

When we first became aware of such Ca^{2+} oscillations, we had little information about how intracellular Ca^{2+} was regulated. However, once IP_3 had been discovered it suggested a mechanism for how such cytosolic Ca^{2+} oscillations emerge and how information can be encoded through changes in frequency. An important aspect of these new models was the process of Ca^{2+}-induced Ca^{2+} release (CICR), which provides the positive feedback signal to account for the rapid regenerative component of each Ca^{2+} transient. The ryanodine receptors of muscle cells were already known to display this process of CICR as first described in 1970 by Makoto Endo [43] and it was soon shown that IP_3 receptors were equally sensitive to Ca^{2+} [44–46]. In effect, the IP_3 receptor is a coincident receptor in that it requires both IP_3 and Ca^{2+} before it will open. This Ca^{2+} sensitivity means that the process of CICR applies equally as well to IP_3 receptors as it does to ryanodine receptors. Although this IP_3-dependent process of CICR nicely explained the regenerative process responsible for the upstroke of the Ca^{2+} spike, it did not address the critical question of what initiates this spike in the first place. There already was evidence from work on muscle cells that the spontaneous release of Ca^{2+} often occurred when the internal store became over loaded with Ca^{2+}. IP_3 receptors seem to have a similar sensitivity to store loading. Ludwig Missiaen showed that Ca^{2+} loading resulted in a spontaneous release of Ca^{2+} [47].

This sensitivity of IP_3 receptors to the level of Ca^{2+} within the lumen of the endoplasmic reticulum has led to a store loading model of Ca^{2+} oscillations [32,48,49]. The basis of this model is that the loading of the internal store plays a critical role in the timing mechanism of Ca^{2+} oscillations because it sets the sensitivity of the IP_3 receptors and thus determines when the next Ca^{2+} spike will initiate [32,48,49]. The frequency of Ca^{2+} oscillations will thus depend upon how quickly the store can be loaded and this in turn will depend upon the rate at which it enters across the plasma membrane, which explains why oscillations are so sensitive to variations in the external concentration of Ca^{2+}. It may also explain how frequency can be regulated if one assumes that the rate of Ca^{2+} entry is controlled by agonist concentration.

This store loading Ca^{2+} oscillator model provides an explanation of the rhythmical contractions of vascular, lymphatic, airway and corpus cavernosum smooth muscle cells [50]. Neurotransmitters and hormones modulate the frequency of this cytosolic oscillator by increasing either the level of IP_3 or the influx of Ca^{2+}. A similar oscillatory mechanism may also operate in pacemaker cells such as the Interstitial cells of Cajal (ICCs) that drive the rhythmical contractions found in gastrointestinal and urethral smooth muscle cells.

1.5. Spatial aspects of Ca^{2+} signalling

As imaging techniques have improved, it has been possible to observe spatial organization of the IP_3/Ca^{2+} signalling system. It is now possible to visualize the activity of the individual building blocks of calcium signals as first described in cardiac cells [51,52], which

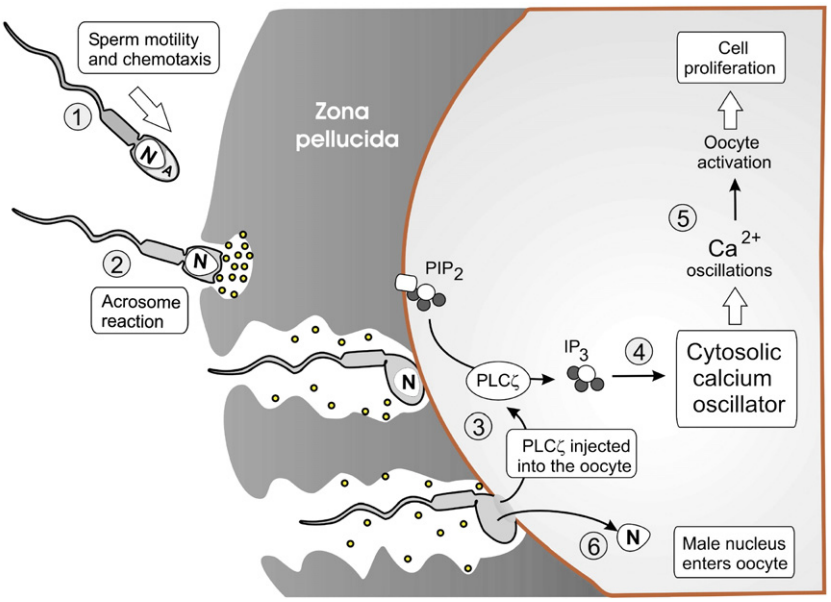

Fig. 3. Operation of the IP_3/Ca^{2+} signalling pathway in the control of mammalian fertilization.

revealed the existence of elementary events that were called sparks. These sparks are small bursts of calcium released from a localized group of ryanodine receptors. The IP_3/Ca^{2+} signalling system might be similarly organized into elementary events [53,54]. The elementary events produced by IP_3 receptors have been called puffs [54]. The puffs were then characterized by Martin Bootman and Peter Lipp who showed how they are recruited to produce global calcium signals in HeLa cells [55,56]. Such studies on the elementary events have greatly increased our understanding of how calcium signals are constructed [33,34].

1.6. The role of IP_3/Ca^{2+} signalling pathways in cellular control mechanisms

A remarkable feature of the IP_3/Ca^{2+} signalling pathway is that it participates in the control of a large number of cellular processes (Fig. 2). In some cases, it plays a direct role in generating Ca^{2+} signals whereas in other cases it functions to modulate the Ca^{2+} signals produced by other signalling pathways. The following examples will illustrate the versatility of this Ca^{2+} signalling mechanism. Control of salivary secretion by the insect gland described earlier is an example where IP_3/Ca^{2+} signalling has a direct role by activating the *trans*-epithelial flux of Cl^- (Fig. 1). In the case of these insect salivary glands and in many other cell types, the Ca^{2+} signal appears as regular oscillations as has been described for liver cells [39], pancreatic cells [57], smooth muscle cells [50,58–60], interstitial cells of Cajal [61] and mammalian oocytes [40–42]. In the last case, there is an unusual activation mechanism that depends upon the sperm fusing with the oocyte to introduce the sperm-specific phospholipase Cζ (PLCζ), which then begins to hydrolyse PtdIns4,5P_2 to generate the IP_3 that initiates the Ca^{2+} oscillations [62] (Fig. 3). These oscillations then control a number of egg responses such as cortical granule release, the

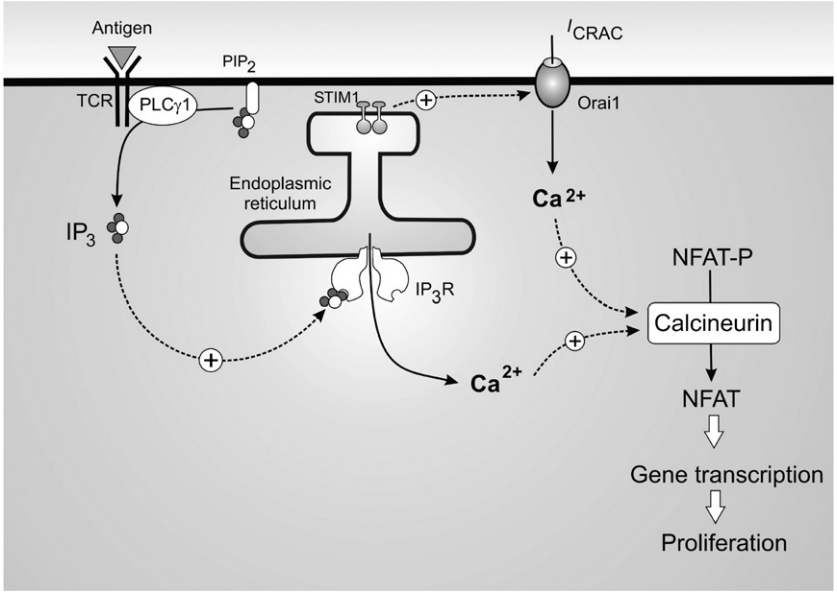

Fig. 4. The role of the IP_3/Ca^{2+} signalling pathway in regulating gene transcription and proliferation in T cells.

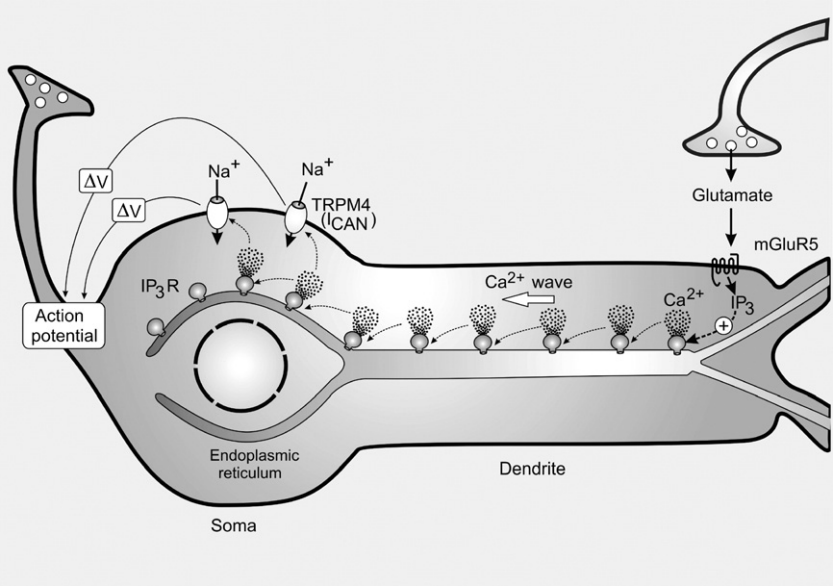

Fig. 5. Pacemaker activity in respiratory neurons depends on the generation of a dendritic Ca^{2+} wave that is triggered by IP_3. The wave is transmitted by IP_3 receptors that are activated by a process of Ca^{2+}-induced Ca^{2+} release (CICR). When the wave invades the soma, it activates the TRPM4 channels responsible for the pacemaker depolarization (ΔV) that triggers the action potentials that control breathing.

block to polyspermy, the completion of meiosis and the initiation of the cell cycle programme leading to embryonic development. The Ca^{2+} pulses thus activate the oocyte to begin the multiple rounds of cell proliferation that occur at the beginning of development.

The IP_3/Ca^{2+} signalling pathway also has a role to play in the control of cell proliferation in many other cell types (Fig. 2). This role is well-established in the lymphocyte activation [63], which is initiated by antigen binding to the T cell receptor (TCR) that then recruits phospholipase Cγ1 (PLCγ1) to produce IP_3 (Fig. 4). This IP_3 acts through the IP_3 receptor (IP_3R) to release Ca^{2+} from the internal store. As the ER empties, it sends a signal to the Ca^{2+} release-activated Ca^{2+} (CRAC) channel responsible for maintaining the Ca^{2+} entry current I_{CRAC} for the 2 h period required to induce lymphocytes to grow. The Orai1 protein has been identified as the channel responsible for carrying I_{CRAC} [64]. The coupling between store emptying and activation of the CRAC channel is still unclear. A stromal interaction molecule 1 (STIM1) in the ER may function as the sensor of store emptying [64,65]. Both the release and entry processes create the Ca^{2+} signal to stimulate calcineurin (CaN) to dephosphorylate the nuclear factor of activated T cells (NFAT), which enters the nucleus where it initiates the transcriptional events that lead to cell proliferation [63].

The brain is particularly rich in the components that function in the IP_3/Ca^{2+} signalling pathway, which may play an important role in contributing to various neuronal processes such as the changes in synaptic plasticity that underlie learning and memory [66]. In the case of Purkinje neurons, the IP_3 receptors are located on the endoplasmic reticulum that extends into the multitude of spines that decorate the dendritic tree [67]. The metabotropic receptors, which are also located on the spines, are responsible for generating IP_3 that contributes to the process of long-term depression (LTD) that is responsible for motor learning. Like many learning mechanisms, LTD occurs when the Purkinje neuron detects the near simultaneous arrival of two synaptic inputs, one coming from the climbing fibres that innervate the base of the dendritic tree and the other arriving through the parallel fibres that innervate the spines. In 1993, I proposed that the IP_3 receptor might be that coincident detector that enables the Purkinje cell to associate the information arriving from these two separate inputs [68]. As noted earlier, opening of the IP_3 receptor depends upon the simultaneous presence of both IP_3 and calcium [44–46] and these are exactly the two messengers being supplied by the two inputs to the Purkinje neurons. The climbing fibre results in depolarization of the dendrites to generate a calcium signal whereas the parallel fibres activate the metabotropic receptors to produce IP_3. When these two messengers appear at approximately the same time, there is a large release of calcium within the spine and this may then be responsible for inducing LTD. Although there are other contenders for coincident detectors in these neurons, there has been considerable experimental evidence to support the notion that the IP_3/Ca^{2+} signalling pathway may be responsible for LTD [69,70].

A number of neuronal cells display dendritic Ca^{2+} waves that initiate at synaptic regions and then progress down to the soma through a process of Ca^{2+}-induced Ca^{2+} release (CICR) driven by $InsP_3$ receptors [71–74]. The function of such waves is largely unknown. I have speculated that they may be responsible for carrying Ca^{2+} signals into the nucleus to activate gene transcription [66], but they may also regulate other processes. For example, such waves located in the pyramidal neurons in the prefrontal cortex may regulate the firing patterns that occur during working memory [75]. Another example is found in the respiratory neurons in the pre-Bötzinger complex (pre-BötC) where such an $InsP_3$-dependent dendritic Ca^{2+} wave (Fig. 5) appears to be a central feature of the pacemaker mechanisms that drives breathing [76]. A feature of this rhythm generator is that the pacemaker neurons communicate with each other through glutamatergic synapses, which have a strong positive feedback component that operates periodically to ensure that all the participating neurons fire together to produce a synchronous and robust output signal. The feedback mechanism that serves to amplify the normally weak synaptic signalling process seems to depend on the generation of a dendritic Ca^{2+} wave that is triggered by the formation of $InsP_3$ by mGluR5 receptors (Fig. 5). The Ca^{2+} wave that travels to the soma is responsible for activating TRPM4 channels that provide the inward I_{CAN} current responsible for the membrane depolarization (ΔV) that triggers the burst of action potentials. The increase in the level of Ca^{2+} within the soma precedes the pacemaker depolarization that triggers the action potentials that activate the motor neurons that control breathing [76].

Cardiac cells provide another example of how the IP_3/Ca^{2+} signalling pathway can modify the ongoing Ca^{2+} signalling system that drive contraction [77]. In both atrial and ventricular cells, microdomains of Ca^{2+} create the global signal that drives contraction,

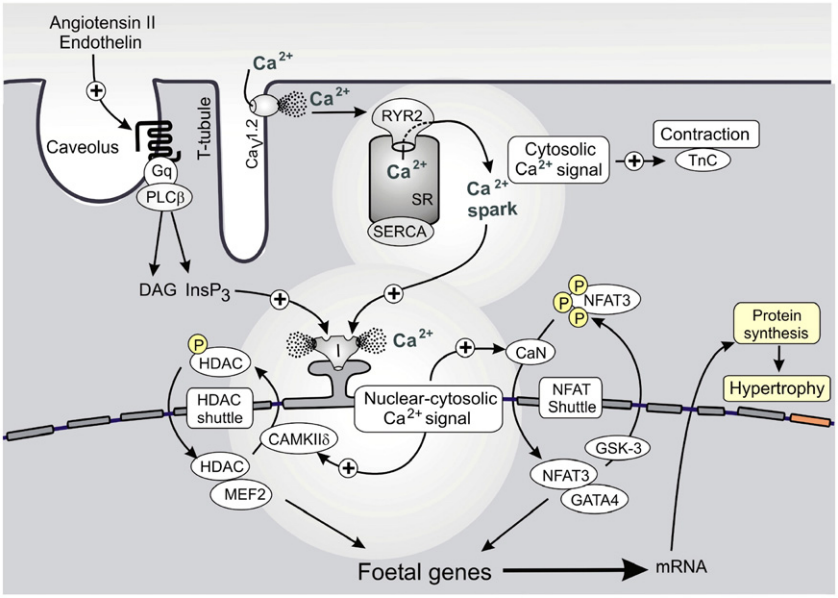

Fig. 6. The role of the IP_3/Ca^{2+} signalling pathway in cardiac hypertrophy. It is proposed that the Ca^{2+} operates in two functional domains. During normal conditions, the Ca^{2+} released by the RYR2 forms the sparks that are predominantly cytosolic signals that induce contraction. In the presence of agonists that generate IP_3, these sparks are amplified by IP_3 receptors near the nucleus to produce a larger nuclear-cytosolic signal capable of inducing the transcriptional events that result in cardiac hypertrophy.

but the mode of recruitment is different. In the case of atrial cells, Ca^{2+} sparks are generated at special zones where the junctional sarcoplasmic reticulum (jSR) lies close to the sarcolemma [78]. This atrial jSR has both type 2 RYRs (RYR2s) and type 2 IP_3 receptors (IP_3R2s). Atrial cells use two distinct mechanisms of Ca^{2+} release. The junctional zones generate Ca^{2+} sparks using a mechanism similar to that described for ventricular cells (see below). $Ca_V1.2$ L-type Ca^{2+} channels in the sarcolemma produce a sparklet that then triggers a spark in the junctional zone at the cell surface. These sparks at the surface then activate the second mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) that spreads the Ca^{2+} signal into the cell in the form of Ca^{2+} wave [78]. Globalization is achieved by the regenerative process of CICR that forms a wave to activate contraction as it spreads into the cell from the plasma membrane.

Under normal conditions, this atrial spark at the surface fails to breach a mitochondrial firewall because the Ca^{2+} is rapidly removed by uptake into the mitochondria and by uptake into the SR by the SERCA pumps. In effect, this microdomain of Ca^{2+} is contained near the surface because not enough Ca^{2+} gets across the firewall to activate the internal RYR2s [78]. However, stronger contractions are induced following stimulation of the atrial cells by either β-adrenergic agonist or by endothelin. The large positive inotropic response induced by endothelin may depend on the ability of IP_3 to enhance the cell surface sparks so that they breach the mitochondrial firewall to stimulate the non-junctional RYR2s to ignite the Ca^{2+} wave that produces the larger global signals. Excessive activation of the IP_3/Ca^{2+} signalling pathway might be the cause of atrial arrhythmias [77,79].

In the case of ventricular cells, the IP_3/Ca^{2+} signalling pathway may also contribute to positive inotropic responses to agonists such as endothelin by enhancing the sparks that are responsible for excitation–contraction coupling [80]. The spatial organization of the enhanced Ca^{2+} signal responsible for this positive inotropic response may be particularly important for understanding the processes responsible for cardiac hypertrophy and heart disease.

A number of signalling pathways have been implicated in the activation of cardiac hypertrophy. One of the characteristics of this hypertrophy is the re-appearance of foetal cardiac genes suggesting that hypertrophic stimuli induce a process of de-differentiation. Many of the extrinsic factors that drive this hypertrophic response (e.g. mechanical load, loss of myocytes and endocrine factors such as endothelin) seem to act through the Ca^{2+} signalling system, which appears to be particularly important for inducing some of the key transcriptional events responsible for activating the foetal genes (Fig. 5). However, the heart receives continuous pulses of Ca^{2+} to drive contraction under normal conditions without initiating a change in transcription. So what is it about the hypertrophic Ca^{2+} signals that initiate the remodelling of cardiac gene transcription? It has been proposed that hypertrophic stimuli induce subtle changes in the characteristics of the individual Ca^{2+} transients (e.g. increases in frequency, amplitude or width) that are sufficient to activate the foetal transcriptional events that induce the phenotypic remodelling that leads to hypertrophy [81–83]. An important feature of this proposal is that the changes in the temporal properties of each transient may reflect an IP_3-dependent alteration in the spatial organization of each transient [83] (Fig. 6). During the normal transients, each Ca^{2+} spark is located primarily in the cytoplasm where it functions to drive contraction. However, in the presence of agonists that increase the level of IP_3, this cytosolic signal may be amplified by the release of Ca^{2+} by IP_3 receptors located near the nucleus to give a nuclear-cytosolic signal capable of stimulating the transcriptional events responsible for hypertrophy. This explanation is based on the fact that the IP_3R is a coincident detector in that its activation requires the presence of both IP_3 and Ca^{2+} [44–46]. In the absence of IP_3, the IP_3Rs will fail to respond to the depolarization-induced Ca^{2+} sparks that occur mainly in the cytoplasm. Likewise, the endothelin-induced elevation in IP_3 will also fail to stimulate the release of Ca^{2+} during diastole when the Ca^{2+} concentration is low. However, during the course of the brief Ca^{2+} transient, the simultaneous presence of both Ca^{2+} and IP_3 will result in the activation of the perinuclear IP_3 receptors (Fig. 6). In effect, the IP_3Rs will create a nuclear Ca^{2+} microdomain responsible for driving the nuclear transcriptional processes such as HDAC5 phosphorylation [84]. The normal Ca^{2+} signals responsible for excitation–contraction coupling were not able to activate the CaMKII responsible for stimulating the phosphorylation of HDAC5. However, this phosphorylation, which was activated by the endothelin-dependent formation of $InsP_3$, seemed to depend on the local release of Ca^{2+} from perinulear $InsP_3$ receptors [84]. There is now experimental evidence to show that IP_3 receptors can function to enhance nuclear Ca^{2+} transients [77]. This

is an excellent example of how IP_3 can alter the properties of Ca^{2+} signalling within discrete cellular microdomains [83].

2. Conclusion

I have been privileged to live through a period when many of the major signalling pathways used for cell communication were being discovered. I was caught up in the excitement of the discovery of cyclic AMP, which introduced the concept of second messengers and this prepared me for my work on the role of IP_3 as a second messenger linking inositol lipid hydrolysis to Ca^{2+} signalling. There has been much excitement along the way. In addition to the real buzz of making new discoveries in the laboratory, I have also enjoyed immensely the intellectual challenge of trying to use all this new information to understand how specific cell functions as divergent as fertilization, cell proliferation, muscle contraction and synaptic plasticity are controlled.

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