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Review

Regulation of the type III $InsP_3$ receptor and its role in β cell function

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Abstract. The type III inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is an important intracellular calcium (Ca^{2+}) release channel in the pancreatic β cell. Pancreatic β cells secrete insulin following a characteristic change in membrane potential that leads to an increase in cytoplasmic Ca^{2+}. Both extracellular Ca^{2+} and Ca^{2+} mobilized from $InsP_3$-sensitive stores contribute to this increase. RIN-m5F cells, an insulin-secreting β cell line, preferentially express the type III $InsP_3R$ [1]. These cells have been useful in determining the regulatory properties of the type III $InsP_3R$ and the role of this isoform in an intact cell. The type III $InsP_3R$ is ideal for signal initiation because high cytoplasmic Ca^{2+} does not inhibit its activity [2]. Altered insulin secretion, the result of changes in Ca^{2+} handling by the β cell, has significant clinical consequences.

Key words. Ion channel gating; calcium channels; inositol 1,4,5-trisphosphate; kinetics; lipid bilayers.

Introduction

$InsP_3R$

The $InsP_3R$ is an intracellular Ca^{2+} release channel that controls Ca^{2+} signaling in most cells [3, 4]. When $InsP_3$ binds to the $InsP_3R$, Ca^{2+} passes from the lumen of endoplasmic reticulum into the cytosol. $InsP_3$-mediated changes in intracellular Ca^{2+}, for example, control the expression of transcription factors [5], the formation of the fertilization envelope during egg activation [6], stimulus-contraction coupling in smooth muscle [7] and the development of long-term depression [8–10].

The $InsP_3R$ exists as a homotetramer in which each subunit is approximately 300 kDa. Three $InsP_3R$ isoforms have been cloned [11–16], and isoform expression varies with cell type and isoform-specific functions [17]. Cerebellar Purkinje cells, for example, express almost exclusively the type I $InsP_3R$. Pancreatic acinar cells express types II and III $InsP_3R$, whereas several epithelia express all three isoforms [1, 18, 19]. The formation of heterotetramers due to the association of different isoform subunits into a functional $InsP_3R$ enables an additional level of receptor diversity [20–22].

Structure of the $InsP_3R$

The types II and III $InsP_3R$ have an overall sequence homology with the type I $InsP_3R$ of 69 and 64%, respectively [13, 14]. Despite their similarity, the three isoforms possess different affinities for $InsP_3$ with a relative order of affinity of type II > type I > type III [13, 15, 23]. The three $InsP_3R$ isoforms, however, are remarkably similar in their domain structure. Each one contains a large regulatory domain between the $InsP_3$ binding site (at the N-terminal end) and the pore-forming region (the C-terminal end, which contains six

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membrane-spanning regions) where intracellular compounds are able to exert their modulatory effects (fig. 1) [24]. The regulatory domain contains several Ca^{2+} binding sites that control the activity of the $InsP_3R$ with respect to the free cytosolic Ca^{2+} concentration. The regulatory domain also contains specific binding sites for ATP which were identified through biochemical studies [25].

Additional regulatory control of the $InsP_3R$ is possible through associated proteins [26, 27] that bind to the regulatory domain and permit the wide range of responses that are seen for $InsP_3$-induced Ca^{2+} release. Finally, the phosphorylation state of the $InsP_3R$ affects the nature of Ca^{2+} signaling [28]. Pancreatic cells, for example, display different Ca^{2+} responses depending on the agonist (such as acetylcholine and cholecystokinin) that is used to stimulate the cells [29–33] because cholecystokinin, but not acetylcholine, causes rapid phosphorylation of the $InsP_3R$ [28].

Pancreatic *β* cells

Electrical activity and insulin secretion

Insulin release occurs in pancreatic *β* cells following stimulus-secretion coupling. This coupling allows precise control of the insulin level with respect to circulating glucose concentrations. The *β* cell functions as an efficient sensor of the extracellular glucose concentration for two reasons. First, glycogen stores are low within *β* cells under normal conditions so that ATP production is dependent upon extracellular glucose. Second, the rate-limiting step for glucose metabolism is the phosphorylation of the sugar by glucokinase [34, 35]. For this reason, any glucose that passively enters the *β* cell is quickly used in glycolysis and the Krebs cycle to generate ATP. ATP is an important part of stimulus-secretion coupling because it initiates electrical activity in *β* cells. Other levels of regulation occur through factors which have an immediate or delayed effect on the secretory process (discussed below).

The resting membrane potential of *β* cells is determined primarily by the high potassium (K^+) permeability of the plasma membrane. Stimulation with glucose depolarizes the *β* cell membrane and triggers a distinctive pattern of electrical activity (fig. 2) [36]. A rise in the glucose level causes an initial depolarization of 10–15 mV, which brings the membrane potential to a threshold at which electrical activity starts. A long phase of continuous spike activity is followed by a partial repolarization without spikes and finally by the development of slow waves. Each slow wave is characterized by a fast depolarization from a threshold potential (V_t) to a plateau potential (V_p) where spike activity occurs. Following the spike activity, the membrane repolarizes to a level which is slightly more negative than V_t (the repolarization potential, V_r). During

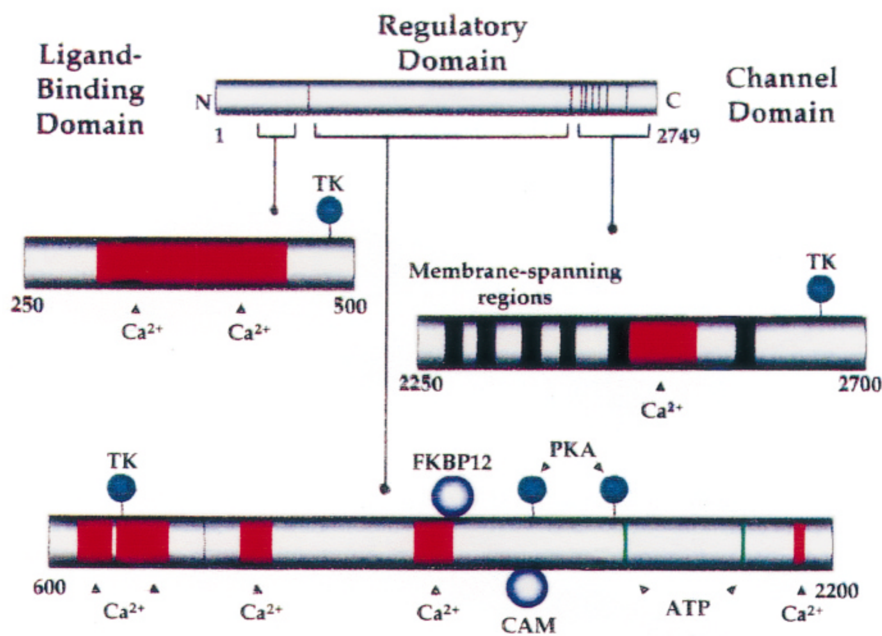

Figure 1. Domain structure of the $InsP_3R$. The $InsP_3R$ consists of an N-terminal ligand binding domain, a large regulatory domain and a C-terminal channel domain that contains six transmembrane regions. Ca^{2+} binding sites are located throughout the polypeptide sequence. ATP binding sites, phosphorylation sites by tyrosine kinase and PKA, and sites of interaction with accessory proteins (FKBP12 and calmodulin) are also shown. Adapted with permission from: Patel, S., Joseph, S. K. & Thomas, A. P. (1999) Molecular properties of inositol 1,4,5-trisphosphate receptors. Cell Calcium **25:** 247–264.

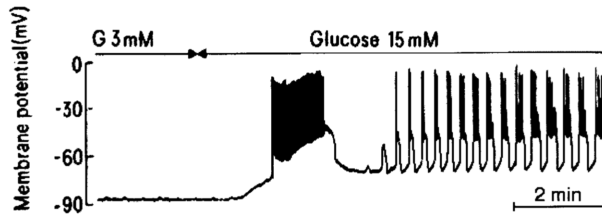

Figure 2. Raising the external glucose concentration from 3 to 15 mM causes a characteristic change in the membrane potential of a β cell. Initially, continuous spike activity occurs following an initial depolarization. After partial repolarization, slow waves appear. Reprinted with permission from: Henquin, J. C. & Meissner, H. P. (1984) Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells. Experientia **40:** 1043–1052.

the interval between slow waves, the membrane slowly depolarizes until the threshold is reached for the initiation of another slow wave.

The initial depolarization leading to β cell electrical activity involves a decrease in K^+ permeability due to the closure of ATP-sensitive K^+ channels following ATP production in the β cell [37]. This depolarization leads to the activation of voltage-dependent calcium (Ca^{2+}) channels and an increase in Ca^{2+} conductance. The increase in Ca^{2+} conductance, which underlies the slow waves, is supported by two observations: slow waves are abolished by Ca^{2+} omission in the extracellular fluid [38] and by Ca^{2+} channel blockers [39, 40]. Additional Ca^{2+} enters the β cell during the spikes generated on the plateau of the slow waves, which are thought to be Ca^{2+} action potentials [39]. The rise that occurs in intracellular Ca^{2+} during β cell electrical activity ultimately initiates exocytosis of insulin-containing secretory vesicles—an event which follows the activation of Ca^{2+}/calmodulin-dependent protein kinase [41]. The mobilization of extracellular Ca^{2+} is important for sustained insulin secretion [42]. Rapid insulin secretion, however, can also occur following the release of Ca^{2+} from intracellular $InsP_3$-sensitive stores [42].

Additional regulation of insulin release occurs through the effects of two types of factors. One group exerts an immediate and direct effect upon insulin release by the pancreatic β cell. They include hormones and neurotransmitters which may potentiate or inhibit insulin secretion by modulation of stimulus-secretion coupling via effects on ion channels, cytosolic second messengers or the secretory machinery itself. For example, cyclic AMP (cAMP) increases the frequency of the slow waves with little change in their duration [43]. The second group influences the secretory behavior of the β cell in a delayed fashion. Examples in this group include ontogenic, nutritional and endocrine factors.

Ca^{2+} handling by the β cell

Calcium enters the β cell by diffusion along an inwardly directed electrochemical gradient. The maintenance of this ionic gradient (approximately 10,000 in the resting state) depends on Ca^{2+}-extruding mechanisms at the plasma membrane and Ca^{2+} sequestration by intracellular organelles (fig. 3). At the plasma membrane, two processes are involved in transporting Ca^{2+} out of the cell: a Ca^{2+} pump which derives its energy from ATP hydrolysis and a Na^+/Ca^{2+} exchanger driven by the inward Na^+ gradient. The endoplasmic reticulum (ER) and mitochondria both can accumulate Ca^{2+} due to the action of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and the mitochondrial Ca^{2+} pump, respectively. However, rat insulinoma mitochondria are only able to buffer ambient [Ca^{2+}] to 1.0 μM. Since this Ca^{2+} level is one order of magnitude higher than the resting cytosolic Ca^{2+}, the ER is required to lower ambient [Ca^{2+}] to the value found in the intact cell (100 nM) [44].

Mobilization of intracellular Ca^{2+}

Several neurotransmitters, growth factors and hormones have been shown to mobilize stored Ca^{2+} by enhancing the turnover of phosphoinositides [45] and leading to accumulation of $InsP_3$ [46]. $InsP_3$ then opens a Ca^{2+} channel in the ER, releases Ca^{2+} from this internal store and leads to an increase in the intracellular Ca^{2+} concentration. In support of this view, the purified $InsP_3R$ from cerebellum has been shown to support $InsP_3$-induced Ca^{2+} fluxes from vesicles [47, 48] and to form Ca^{2+}-permeable channels in planar lipid bilayers [25, 27, 49, 50]. In addition, $InsP_3$ has been shown to release Ca^{2+} from a nonmitochondrial pool in permeabilized pancreatic acinar cells [51].

Exposure of rat pancreatic islets to high concentrations of glucose has several important effects. First, glucose stimulation rapidly increases the transcription and expression of the type III $InsP_3R$ in isolated rat pancreatic islets [52]. Second, glucose stimulation enhances the incorporation of palmitate into phospholipids [53]. Secretagogues such as glucose not only stimulate phospholipid biosynthesis directly but, by generating glycerol-3-phosphate, they also supply one of the metabolic precursors for phospholipid biosynthesis. Enhanced phospholipid synthesis generates phosphatidyl inositol 4,5-bisphosphate (PIP_2), which can be cleaved by phospholipase C into diacylglycerol (DAG) and $InsP_3$. Finally, rapid increases in $InsP_3$ occur following PIP_2 hydrolysis in pancreatic islets in response to glucose [54]. Thus, glucose increases the expression of the type III $InsP_3R$, the $InsP_3$-generating capability of β cells and the production of $InsP_3$ in β cells. These effects ultimately promote insulin secretion by raising the intracellular Ca^{2+} concentration.

RIN-m5F cells

Origin of cell line

The RIN-m cell line was derived from a radiation-induced transplantable rat islet cell tumor [55]. The line was established from a nude mouse xenograft of the tumor [56]. The cells produce and secrete islet polypeptide hormones (insulin, somatostatin and glucagon) and contain L-dopa-decarboxylase (a marker for cells having amine precursor uptake and decarboxylase, or APUD, activity). The RIN-m5F cell line, a clone derived from the RIN-m cell line, also produces and secretes insulin. Unlike the parental line, however, RIN-m5F cells do not produce somatostatin.

General properties

The resting intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in RIN-m5F cells, a rat insulinoma *β* cell line, is approximately 100 nM [57]. The microsomal fraction is primarily responsible for buffering cytosolic Ca^{2+} within this range; neither mitochondria nor secretory

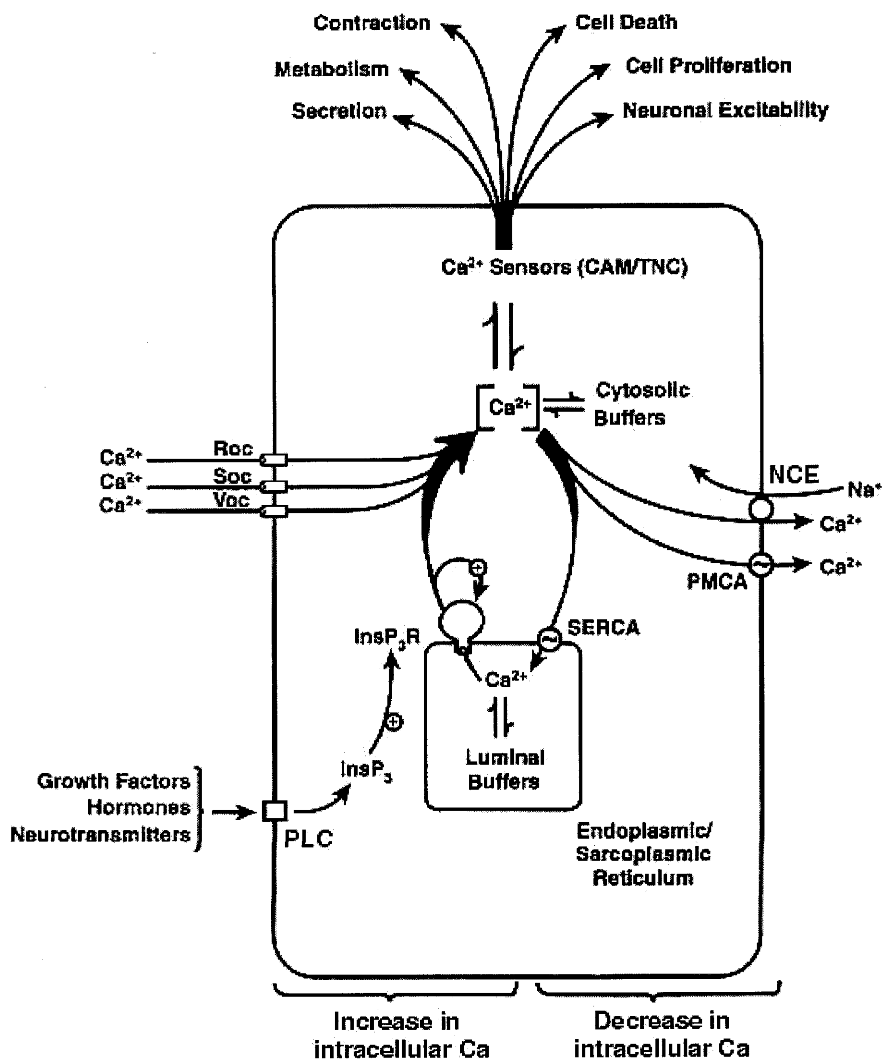

Figure 3. The cytosolic Ca^{2+} level in resting cells is normally kept low (10–100 nM), but stimulation raises it to 500–1000 nM, a level necessary to effect many cellular processes. Mechanisms for Ca^{2+} entry include voltage-operated channels (VOC), receptor-operated channels (ROC) and store-operated channels (SOC) as well as two families of intracellular Ca^{2+} release channels, the ryanodine receptor (RyR) and $InsP_3R$. Ca^{2+} is transported out of the cell by the Na^+/Ca^{2+} exchanger (NCE) and plasma membrane Ca^{2+} ATPase (PMCA), and pumped into internal stores by the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). Adapted with permission from: Berridge, M. J. (1999) Calcium Metabolism. Calbiochem: Signal Transduction Catalog and Technical Reference, 54–56.

vesicles are able to sequester Ca^{2+} to this extent [57]. Insulin release from RIN-m5F cells can be evoked by a variety of secretagogues with the notable exception of glucose; this lack of glucose responsiveness has been attributed to a deficiency in the high K_m phosphorylating enzyme, glucokinase [58]. Since this enzyme catalyzes the rate-limiting step in glucose metabolism in normal β cells, it effectively controls glucose-induced insulin release and appears to be the likely deficiency in RIN-m5F cells.

Mobilization of intracellular Ca^{2+}

The addition of glyceraldehyde (10 mM) to a suspension of RIN-m5F cells elevates $[Ca^{2+}]_i$ by threefold within 2 min and follows the change in membrane potential [57]. When cells are exposed to verapamil prior to the addition of glyceraldehyde, the change in $[Ca^{2+}]_i$ is only reduced by 50%. For this reason, glyceraldehyde appears to raise $[Ca^{2+}]_i$ in RIN-m5F cells not only by promoting Ca^{2+} influx but also by Ca^{2+} mobilization from internal stores. Additional evidence indicates that Ca^{2+} enters the cytoplasm from these two sources. First, activation of muscarinic receptors by carbamylcholine raises $[Ca^{2+}]_i$ in RIN-m5F cells even in the absence of extracellular Ca^{2+} [59]. Second, a residual elevation in $[Ca^{2+}]_i$ remains following the addition of other secretagogues (alanine or K^+) even when maximal concentrations of diltiazem, a different Ca^{2+} channel blocker, are used [60]. These results support the hypothesis that glucose uses both intracellular and extracellular Ca^{2+} to raise $[Ca^{2+}]_i$ [42, 61].

As found for permeabilized pancreatic acinar cells [51], $InsP_3$ can release Ca^{2+} from a nonmitochondrial pool in RIN-m5F cells [62]. The accumulation of $InsP_3$ precedes both the rise in intracellular Ca^{2+} and the peak in insulin secretion [63]. When $InsP_3$ was added to the microsomal fraction from RIN-m5F cells, a rapid Ca^{2+} release was observed [44]. The action was specific for the triphosphate derivative since myo-inositol, inositol monophosphate ($InsP_1$) and inositol 1,4-bisphosphate ($InsP_2$) were ineffective. $InsP_3$ is able to evoke this response by binding to a cytoplasmic receptor located in the ER, the $InsP_3R$. Once activated, the $InsP_3R$ releases stored Ca^{2+}. Interestingly, the response is transient and desensitizes the microsomes to subsequent $InsP_3$ additions (fig. 4).

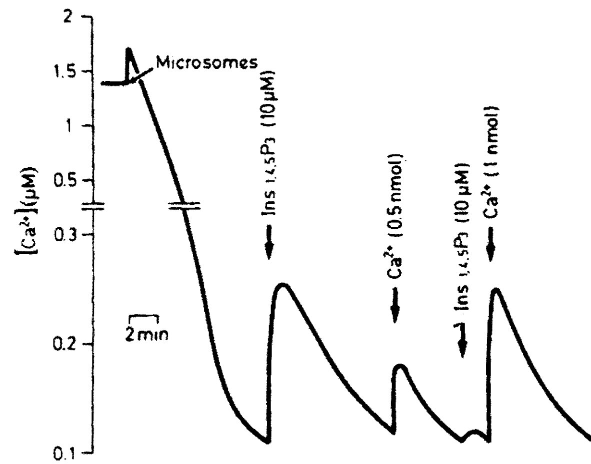

Figure 4. Extramicrosomal ambient free Ca^{2+} concentration maintained by insulinoma microsomes was monitored following stimulation by $InsP_3$. A pulse addition of $InsP_3$ resulted in an increase in medium free Ca^{2+} to approximately 0.25 µM. However, a second addition of $InsP_3$ produced only a very small increase in medium free Ca^{2+}. The free Ca^{2+} returned to baseline after each condition due to Ca^{2+} reuptake. Reprinted with permission from: Prentki, M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J. & Wollheim, C. B. (1984) Rapid mobilization of Ca2+ from rat insulinoma microsomes by inositol- 1,4,5-trisphosphate. Nature **309:** 562–564. Macmillan Magazines Ltd.

The type III $InsP_3R$

RIN-m5F cells as a source for the type III $InsP_3R$

The distribution of $InsP_3R$ isoforms is often cell specific even within the same tissue. For example, cerebellar Purkinje cells contain predominately the type I isoform, whereas cerebellar astrocytes contain predominately type III [21]. Cardiac myocytes contain only type II $InsP_3R$ [17]. Although three isoforms have been identified [11–16], early work on the $InsP_3R$ characterized the type I $InsP_3R$ at the single channel level [64]. The permeation properties of the type II $InsP_3R$ are similar to those of the type I $InsP_3R$ even though the type II $InsP_3R$ has greater affinity for $InsP_3$ [17]. Since RIN-m5F cells preferentially express the type III isoform with 96% relative abundance [1], they are an excellent source for the type III $InsP_3R$. When studied in planar lipid bilayers, the type III $InsP_3R$ forms channels that can release Ca^{2+} from intracellular stores [2].

Regulation by physiological modulators

Ca^{2+} release by the type I $InsP_3R$ is regulated by a variety of cofactors and cellular processes, including cytosolic and intraluminal free Ca^{2+}, phosphorylation of the $InsP_3R$ and intracellular pH [65–70]. Regulation by Ca^{2+} is bell shaped with maximal channel activity occurring at 300 nM free Ca^{2+}; Ca^{2+}-dependent activation and inhibition occurs over the normal physiological range of Ca^{2+} concentrations [71]. ATP has been shown to enhance the gating of the type I $InsP_3R$ in *Xenopus* oocyte nuclei [72] and canine cerebellum [73] by allosteric regulation of the $InsP_3R$. In this way, ATP

is able to shape the extent and duration of cytoplasmic Ca^{2+} signals depending upon stimulus intensity and a cell's metabolic state. The experiments involving ATP have particularly interesting implications for pancreatic β cells since stimulus-secretion coupling in these cells (and ultimately insulin release) involves an increase in the cytoplasmic ATP concentration.

Regulation of the type III $InsP_3R$ by $InsP_3$, ATP and Ca^{2+} has been studied. Like the type I and II $InsP_3R$, $InsP_3$ is necessary to activate the type III $InsP_3R$ [2]. Ion permeation and channel gating properties for the type III $InsP_3R$ are similar to the type I $InsP_3R$ [74]. The type III $InsP_3R$ reaches a higher level of maximal activity than the type I $InsP_3R$ but requires a 10-fold higher $InsP_3$ concentration to reach this level of activity (fig. 5) [75]. When $InsP_3$ is present, ATP and Ca^{2+} both function as allosteric regulators. Low ATP concentrations (< 6 mM) increase the open probability by increasing the mean open time and decreasing the mean closed time [75]. The type III $InsP_3R$ does not show the same bell-shaped Ca^{2+}-dependence curve as the type I $InsP_3R$; activity increases monotonically with increasing cytosolic Ca^{2+} (fig. 6) [2]. This property was predicted because $InsP_3$ binding to the type III $InsP_3R$ is not inhibited by elevated Ca^{2+} [76, 77]. Thus, the type III $InsP_3R$ forms a Ca^{2+}-permeable channel that has properties which are distinct from those of the type I $InsP_3R$.

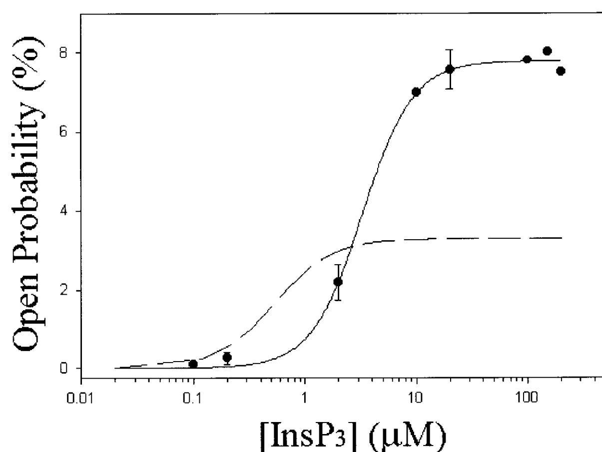

Figure 5. $InsP_3$ dependence of the types I and III $InsP_3R$. Channel activity, plotted as open probability, was observed at several $InsP_3$ concentrations with 0.5 mM ATP and 150 nM free Ca^{2+}. The type III $InsP_3R$ (circles and solid line) was activated maximally at higher $InsP_3$ concentrations than the type I $InsP_3R$ (dashed line). The EC_{50} for the type III $InsP_3R$ was 3.2 μM versus 0.5 μM for the type I $InsP_3R$. Reprinted with permission from: Hagar, R. E. & Ehrlich, B. E. (2000) Regulation of the Type III $InsP_3R$ by $InsP_3$ and ATP. Biophys J. **79:** 271–278.

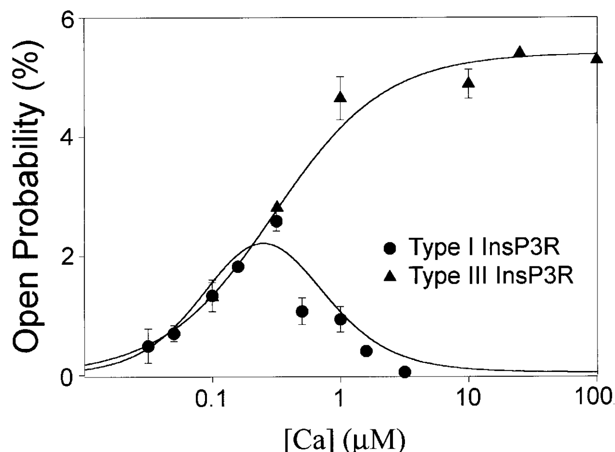

Figure 6. Single-channel open probability for the types I and III $InsP_3R$ as a function of free Ca^{2+} concentration. Channel activity was monitored in the presence of 2 μM $InsP_3$ and 0.5 mM ATP. Activity for the type I $InsP_3R$ (circles) shows both Ca^{2+}-dependent activation and inhibition. Activity for the type III $InsP_3R$ (triangles), however, increases monotonically as a function of free cytosolic Ca^{2+} concentration. Reprinted with permission from: Hagar, R. E., Burgstahler, A. D., Nathanson, M. H. & Ehrlich, B. E. (1998) Type III $InsP_3$ receptor channel stays open in the presence of increased calcium. Nature **396:** 81–84. Macmillan Magazines Ltd.

Since high cytosolic Ca^{2+} does not inhibit the type III $InsP_3R$, inactivation of this isoform must occur at another level. Several possibilities have been suggested as additional regulatory mechanisms of the $InsP_3R$ such as receptor phosphorylation [65, 66, 78, 79], accelerated degradation of the $InsP_3R$ [1, 80] and the activity of phospholipase C (PLC). Phosphorylation of PLC provides a mechanism for very rapid desensitization whereas PLC internalization provides delayed desensitization [81]. Both of these processes, however, lead to decreased levels of $InsP_3$ and DAG. In RIN-m5F cells, $InsP_3$ can regulate its own levels through the activity of inositol 1,4,5-trisphosphate 3-kinase [82]. This Ca^{2+}-calmodulin-sensitive kinase is responsible for the 'early disposal' of $InsP_3$ during cellular stimulation [83, 84]. Finally, accessory proteins may also play a role in the regulation of the different $InsP_3R$ isoforms [27, 85]. These proteins can modulate the intrinsic activity of the $InsP_3R$ in ways that may be direct and indirect, isoform-specific or dependent upon changes in the Ca^{2+} concentration [26].

Intracellular Ca^{2+} signaling

Ca^{2+} waves and oscillations have been observed in cells from a large number of animals [86, 87]. These waves

and oscillations play an important role in regulating cell function [4] through effects on secretion [88–90], cell migration [91], gene expression [92, 93] and intercellular communication in hepatocytes [94–96]. The unique biophysical properties of the type I $InsP_3R$, particularly the presence of both Ca^{2+}-dependent activation and inhibition, is essential for Ca^{2+} oscillations and for the propagation of regenerative Ca^{2+} waves [97–99]. Localized, nonpropagating increases in cytosolic Ca^{2+} have also been observed when small amounts of $InsP_3$ were photoreleased inside SKHep1 cells [2]. These liver-derived cells, which express the type I but not the type III $InsP_3R$, display localized Ca^{2+} signals that are similar in duration to other mammalian cells [33, 100, 101].

Agonist-induced Ca^{2+} waves that travel from the apical to the basal pole have been observed in polarized epithelia, including pancreatic [33, 89, 100, 102], lacrimal [103], and salivary acinar cells [104] and hepatocytes [105]. The direction of Ca^{2+} waves in these cells probably results from the polarized distribution of the $InsP_3R$ (see below). These waves are initiated by $InsP_3$-mediated Ca^{2+} release in pancreatic acinar cells [33, 100]. The increases in Ca^{2+} originate at the site of highest density for the type III $InsP_3R$ [19, 104, 106], and $InsP_3R$ antagonists can block Ca^{2+} signaling in these cells [33, 100]. Stimulation of muscarinic or cholecystokinin receptors also induces rapid apical-to-basal Ca^{2+} waves in pancreatic acinar cells [102]. The type III $InsP_3R$ is localized to the apical pole of salivary gland cells [104], nonpigmented ciliary epithelia [107] and pancreatic acinar cells [19, 106]. This region has been called the 'trigger zone' from which Ca^{2+} signals originate in pancreatic acinar cells (fig. 7) [100]. Since the type III $InsP_3R$ lacks Ca^{2+}-dependent inhibition by high cytoplasmic Ca^{2+} [2], this $InsP_3R$ isoform is ideal for signal initiation. When stimulated continuously with external ATP to trigger Ca^{2+} release by the $InsP_3$ cascade [108, 109], RIN-m5F cells responded with a single, large Ca^{2+} transient [2]. Oscillations did not occur, and internal Ca^{2+} stores were depleted. In addition, a global response was elicited when threshold amounts of caged $InsP_3$ were photoreleased in RIN-m5F cells; Ca^{2+} signals localized to small regions of a RIN-m5F cell were never observed [2].

After the release of Ca^{2+} in the trigger zone of pancreatic acinar cells by the type III $InsP_3R$, the mobilization of Ca^{2+} from stores that are sensitive to both caffeine and ryanodine is necessary for the rapid propagation of Ca^{2+} waves in these cells [102]. The ryanodine receptor (RyR) is present in pancreatic acinar cells [110], and increases in Ca^{2+} can be generated in these cells by the

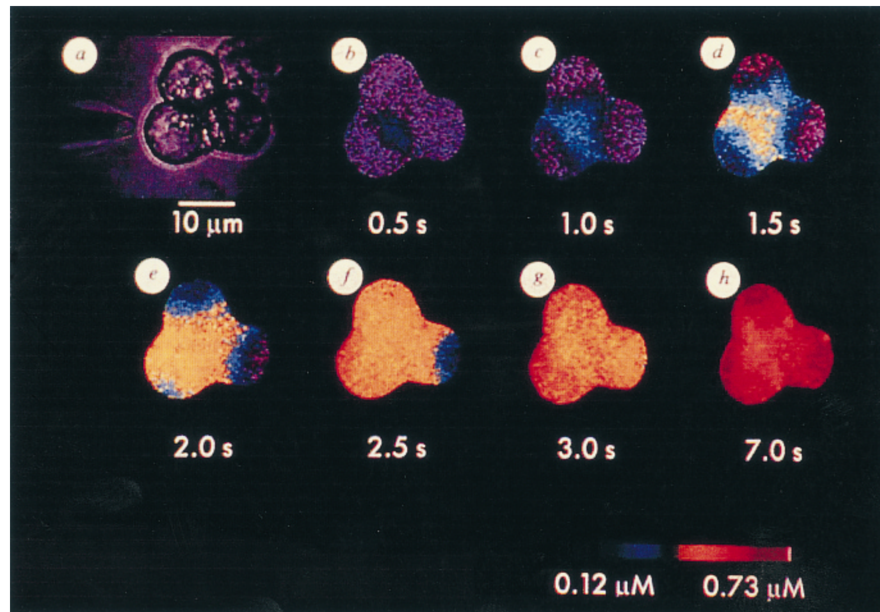

Figure 7. Acetylcholine induces apical-to-basal Ca^{2+} waves in pancreatic acinar cells. The waves begin at the apical end of the cell where the type III $InsP_3R$ is localized [19]. This region of the cell has been described as the trigger zone. Reprinted with permission from: Kasai, H. & Augustine, G. J. (1990) Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. Nature **348:** 735–738. Macmillan Magazines Ltd.

RyR agonist cyclic ADP-ribose (cADPR) [111]. Together, these findings suggest that apical release of Ca^{2+} (by $InsP_3$) leads to the sequential release of additional Ca^{2+} from ryanodine-sensitive stores that are distributed in an apical to basal pattern.

The subcellular distribution of the three $InsP_3R$ isoforms in a cell, along with differences in Ca^{2+}-dependent regulation [2, 17, 71], may allow cells to organize and coordinate subcellular Ca^{2+} signals. In nonpigmented ciliary epithelia (NPE), for example, the type I and III $InsP_3R$ are concentrated in different regions of the cell [107]. Acetylcholine-induced Ca^{2+} signals in these cells began in the apical region (where the type III $InsP_3R$ is localized) and then traveled from the apical to the basal pole. Localized increases in Ca^{2+} and repetitive Ca^{2+} spikes were observed at the basal pole (a region containing the type I $InsP_3R$) in half of the NPE cells [107]. In cells which lack the type III $InsP_3R$ (such as skate hepatocytes), Ca^{2+} signals begin at loci throughout the cell rather than as an organized apical-to-basal Ca^{2+} wave [112]. Thus, localized Ca^{2+} increases appear to be driven preferentially by Ca^{2+} released from the type I rather than the type III $InsP_3R$. In addition, the role of the type III $InsP_3R$ is to initiate cellular Ca^{2+} signals which travel in an apical-to-basal direction, whereas the type I $InsP_3R$ supports Ca^{2+} oscillations and regenerative responses.

Clinical implications

Diabetes mellitus

Diabetes mellitus (DM), the most common endocrine disease, is not a single hereditary disease but a heterogeneous group of diseases, all of which ultimately lead to an elevation of glucose in the blood (hyperglycemia) and the wasting of glucose into the urine. The disease is characterized by increased urine production (polyuria), excessive thirst (polydipsia) and excessive eating (polyphagia). Long-term complications involve the eyes, kidneys, nerves, and blood vessels. Diabetes is classified into two main categories: autoimmune (type 1) DM and nonautoimmune (type 2) DM.

By the time type 1 DM (also called insulin-dependent DM, IDDM) appears clinically, most of the β cells in the pancreas have already been destroyed. The destructive process is believed to be autoimmune in nature, although details surrounding this process remain unknown. A genetic susceptibility contributes to the disease even though an environmental event appears to initiate the process in susceptible individuals. The best evidence supporting an environmental insult as the precipitating factor involves studies in monozygotic twins. The concordance rate for diabetes in monozygotic twins is less than 50%, a finding which indicates that diabetes is not purely a genetic disorder.

There are two widely advocated hypotheses about the primary etiologic factor in type 2 diabetes (also called noninsulin-dependent DM, NIDDM). The first one holds that a primary β cell defect causes insufficient insulin secretion, resulting in hyperglycemia. Peripheral tissues (primarily muscle and liver) are initially insulin responsive, but may become insulin resistant in response to ongoing hyperglycemia. The alternate hypothesis proposes that the basic underlying abnormality is insulin resistance in the peripheral tissues. The β cells initially compensate to maintain normal glucose metabolism by increasing the amount of insulin that is secreted. However, demand eventually exceeds the ability to compensate, and pancreatic exhaustion results. Considerable evidence exists for both hypotheses. The first hypothesis is supported by several studies showing abnormalities in insulin secretion and normal insulin action in patients with type 2 diabetes [113]. The second hypothesis is supported by other studies which find insulin resistance (but normal glucose metabolism) in first-degree relatives of patients with type 2 diabetes [114].

In either case, altered insulin secretion underlies the pathogenesis of type 2 diabetes. Modulators of insulin secretion, especially second messengers such as $InsP_3$, affect the intracellular Ca^{2+} concentration directly. Other cytosolic compounds (such as calbindin and ATP) affect intracellular Ca^{2+} levels in a more subtle manner. Calcium buffering by calbindin depends upon the amount of protein expression, and an increase in calbindin expression has been associated with an increase in insulin release [115]. High ATP concentrations (> 4 mM) can inhibit $InsP_3$-induced Ca^{2+} release by the type I $InsP_3R$ [73]. Besides its effects on the $InsP_3R$, ATP is part of the stimulus-secretion pathway in β cells. Calcium is also interesting because it regulates its own release by the type I $InsP_3R$ [71]. Consequently, both Ca^{2+} and ATP play important roles within the β cell. Not only do Ca^{2+} and ATP affect Ca^{2+} handling by the β cell, but these two species also influence insulin secretion.

Hypoglycemic syndromes

Since the brain cannot use circulating free fatty acids as an energy source, hypoglycemia is much more dangerous than hyperglycemia in the short run. A lack of glucose to the brain produces deranged function, tissue damage and death if the deficit is prolonged. During the catabolic phase of metabolism, the liver produces glucose in order to maintain the plasma glucose concentration within a range which is safe for nervous system function. Short-chain fatty acid metabolites (acetoacetate and β-hydroxybutyrate) can be oxidized by the brain, but the development of ketosis requires several

hours, so that it is ineffective in protecting against acute hypoglycemia.

Hypoglycemia is traditionally classified as either postprandial (reactive) or fasting. The most common cause of postprandial hypoglycemia (PH) is alimentary hyperinsulinism. Rapid gastric emptying, brisk absorption of glucose and excessive insulin release cause glucose levels to fall more rapidly than insulin levels. The resulting insulin-glucose imbalance leads to hypoglycemia. In contrast, the causes of fasting hypoglycemia (FH) are numerous, but in all cases there is an imbalance between the production of glucose by the liver and its utilization in peripheral tissues. In some patients, FH is due primarily to a defect in glucose production, whereas in other patients the problem is excess glucose utilization. Glucose-6-phosphatase deficiency is an example of a defect in glycogen breakdown (impaired production). Excessive utilization of glucose can occur when insulin levels are high (insulinoma) or low (systemic carnitine deficiency, a condition in which the transport of fatty acids into mitochondria for oxidation is hindered).

Conclusions

This review compared regulation of the types I and III $InsP_3R$ and their effect on intracellular Ca^{2+} signaling. Most of the biophysical properties of the type III $InsP_3R$ are similar to the type I isoform with two important exceptions: regulation by $InsP_3$ and cytosolic Ca^{2+}. The type I $InsP_3R$ exhibits both Ca^{2+}-dependent activation and inhibition—two properties which are necessary for Ca^{2+} oscillations [97–99]. In contrast, the type III $InsP_3R$ requires a higher $InsP_3$ concentration than the type I $InsP_3R$ to reach maximal channel activity [75] and is not inhibited by high cytosolic Ca^{2+} [2]. Imaging experiments confirmed that these single-channel properties of the type III $InsP_3R$ are ideal for the initiation of intracellular Ca^{2+} waves [2].

Since the type III $InsP_3R$ is not inhibited by high cytosolic Ca^{2+}, it will remain open as long as $InsP_3$ is present. Inactivation of the type III $InsP_3R$ may require associated proteins [26, 27, 85] or occur at the level of $InsP_3$ production/degradation. In pancreatic β cells, Ca^{2+} signaling is especially important because altered insulin secretion has profound clinical implications. Since half of the $InsP_3R$ pool in these cells is the type III isoform [1], the type III $InsP_3R$ may play a role in the pathogenesis of diabetes mellitus or hypoglycemia due to its effects on Ca^{2+} handling.

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