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Inositol Trisphosphate Receptor and Ca<latex>$^{2+}$</latex> Signalling

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Phil. Trans. R. Soc. Lond. B 1993 **340**, 345-349
doi: 10.1098/rstb.1993.0077

Inositol trisphosphate receptor and Ca^{2+} signalling

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SUMMARY

Inositol 1,4,5-trisphosphate ($Ins P_3$) is a second messenger that releases Ca^{2+} from the intracellular stores. The $Ins P_3$ receptor ($Ins P_3$-R) was purified and its cDNA was cloned. We have found that $Ins P_3$-R is identical to the P_{400} protein identified as a protein enriched in the cerebellar Purkinje cells. We generated an L fibroblast cell transfectant that produced cDNA derived $Ins P_3$-R. The expressed protein displays high affinity and specificity for $Ins P_3$. $Ins P_3$ induces Ca^{2+} release from the membrane vesicles of the transfected cells. Incorporation of purified $Ins P_3$-R into a lipid bilayer showed $Ins P_3$ induced Ca^{2+} release. These result suggest that $Ins P_3$-R is a Ca^{2+} release channel. Immunogold method using monoclonal antibodies against the receptor showed that it is highly condensed on the smooth surfaced endoplasmic reticulum (ER) and slightly on the outer nuclear membrane and rough ER. Cross linking experiments show that the $Ins P_3$-R forms a homotetramer. The approximately 650 N-terminal amino acids are highly conserved between mouse and *Drosophila melanogaster*, and this region has the critical sequences for $Ins P_3$ binding. We found novel subtypes of the $Ins P_3$-R resulting from RNA-splicing that are expressed in a tissue-specific and developmentally specific manner and also resulting from different genes. It is believed that there are two Ca^{2+} release mechanisms, $Ins P_3$-induced Ca^{2+} release (IICR) and Ca^{2+}-induced Ca^{2+} release (CICR). Eggs are good materials to analyse the machanism of Ca^{2+} signalling: fertilized hamster eggs exhibit repetitive Ca^{2+} transients as well as the Ca^{2+} wave. A monoclonal antibody to the $Ins P_3$-R inhibited both IICR and CICR respectively upon injection of $Ins P_3$ and Ca^{2+} into hamster eggs. The antibody completely blocked sperm-induced Ca^{2+} waves and Ca^{2+} oscillations. The results indicate that Ca^{2+} release in fertilized hamster eggs is mediated solely by the $Ins P_3$-R, and that Ca^{2+}-sensitized IICR, but not CICR, generates Ca^{2+} waves and Ca^{2+} oscillations.

1. HISTORY OF $Ins P_3$ RECEPTOR

Inositol 1,4,5-trisphosphate ($Ins P_3$), generated from phosphatidyl inositol 4,5-bisphosphate in response to various extracellular stimuli, mediates the release of intracellular Ca^{2+} to exert physiological functions of cells (figure 1) (Berridge & Irvine 1989). It is essential to know how $Ins P_3$ releases Ca^{2+} and where the $Ins P_3$-sensitive Ca^{2+} pool is located in order to understand the mechanism of intracellular signal transduction. It is therefore necessary to know the molecular properties of the receptor that specifically recognizes $Ins P_3$. The $Ins P_3$ receptor ($Ins P_3$-R) was first characterized in 1979 as a protein called P_{400} that was abundant in the cerebellum but was virtually absent in the cerebella from Purkinje cell-deficient mutant mice (Mikoshiba *et al.* 1979, 1985) long before the importance of $Ins P_3$ was recognized as a second messenger to release Ca^{2+}. This protein was also characterized by other groups as PCPP-260 (Walaas *et al.* 1986) and GP-A (Groswald & Kelly 1984). Purification was performed in 1988 independently by two groups as an $Ins P_3$ binding protein (Supattapone *et al.* 1988) and also as a Purkinje cell-enriched protein (Maeda *et al.* 1988). Both proteins were shown to be identical immunologically (Maeda *et al.* 1990).

2. STRUCTURE AND FUNCTION OF $Ins P_3$ RECEPTOR

By the cDNA cloning of mouse $Ins P_3$-R, it was shown that the transmembrane domain exists near the C-terminus, and both the large N-terminal and the short C-terminal portion are in the cytoplasmic compartment. The amino acid sequence deduced from the cDNA sequence of mouse (Furuichi *et al.* 1989; Ross *et al.* 1992) rat (Mignery *et al.* 1990; Sudhof *et al.* 1991), *Drosophila melanogaster* (Yoshikawa *et al.* 1992) and human $Ins P_3$-R (Ross *et al.* 1992) showed that the sequence of the receptor is highly conserved. Transfection of the cDNA to L-fibroblasts (Miyawaki *et al.* 1990), NG108–15 (Furuichi *et al.* 1989; Yoshikawa *et al.* 1992) or COS (Mignery *et al.* 1990) cells showed enhanced $Ins P_3$ binding activity. It was an interesting question to know if $Ins P_3$-R is itself a Ca^{2+} channel or if Ca^{2+} channel is a distinct molecule from $Ins P_3$-R. Two approaches were undertaken to answer the

Phil. Trans. R. Soc. Lond. B (1993) **340,** 345–349
Printed in Great Britain

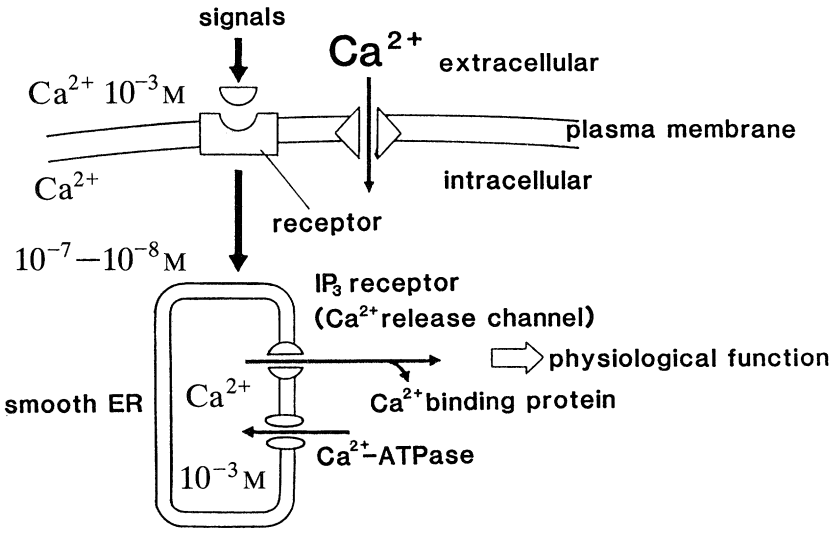

Figure 1. Regulation of intracellular Ca^{2+} concentration.

question. One approach was by reconstitution into a proteoliposome or lipid bilayer (Ferris *et al.* 1989; Maeda *et al.* 1991). InsP_3-R showed InsP_3 induced Ca^{2+} movement. The second approach was by cDNA transfection experiments. Transfection of the cDNA to L-fibroblast cells showed enhanced Ca^{2+}releasing activity (Miyawaki *et al.* 1990). From these data, it was concluded that InsP_3-R is a Ca^{2+} channel.

The primary sequence of the InsP_3-R shares no homology with the voltage-gated Ca^{2+} channels on the plasma membrane (Furuichi *et al.* 1989; Mori *et al.* 1991) but shares fragmentary significant homology with the ryanodine receptor (Furuichi *et al.*1989; Mignery *et al.* 1989) on the sarcoplasmic reticulum in skeletal and cardiac muscle (Takeshima *et al.* 1989). About 650 N-terminal amino acid residues within the large cytoplasmic portion of the InsP_3-R are highly conserved among different species (Furuichi *et al.* 1989; Mignery & Sudhof 1990; Miyawaki *et al.* 1991). Deletion of any small fragment within the region abolished InsP_3 binding activity, suggesting that this region is important for InsP_3 binding (Mignery & Sudhof 1990; Miyawaki *et al.* 1991). This region is enriched with positively charged Arg and Lys residues, and it bound heparin. The transmembrane domain is localized near the C-terminal portion with six (Yoshikawa *et al.* 1992), or eight (Mignery & Sudhof 1990) membrane-spanning regions predicted from the hydropathy profile of the amino acid sequence. The amino acid sequence is conserved, especially at the last two membrane-spanning regions, among different species (Furuichi *et al.* 1989; Sudhof *et al.*1991; Yoshikawa *et al.* 1992). Cross linking experiments (Ohtsu *et al.* 1990; Maeda *et al.* 1991) and electron microscopic observation (Maeda *et al.* 1990) demonstrated that the receptor forms a homotetrameric structure (figure 2). Each subunit of InsP_3-R binds one InsP_3 molecule as revealed by the InsP_3 binding kinetics (Maeda *et al.* 1991) and also by the deletion mutant study of the transmembrane domain (Mignery & Sudhof 1990; Miyawaki *et al.* 1991). The kinetics of the InsP_3 binding to the receptor showed that the Hill coefficient was almost one, indicating that there is no cooperativity in InsP_3 binding (Ehrlich & Watras 1988).

3. LOCALIZATION OF THE InsP_3 RECEPTOR

InsP_3-R was highly enriched in cerebellar Purkinje cells but it was also expressed in the hippocampus (especially in the CA1 region), striatum and cerebral cortex where the PI system appears to work. Electron microscopic observation demonstrated that the receptor was localized mostly on the smooth surfaced endoplasmic reticulum (SER) (Mignery *et al.* 1989; Ross *et al.* 1989; Ohtsu *et al.* 1990; Satoh *et al.* 1990) in the Purkinje cell. The receptor is more abundant on the stacked type SER than on the non-stacked type (Ohtsu *et al.* 1990; Satoh *et al.* 1990). The InsP_3-R is also present in the rough ER and outer nuclear

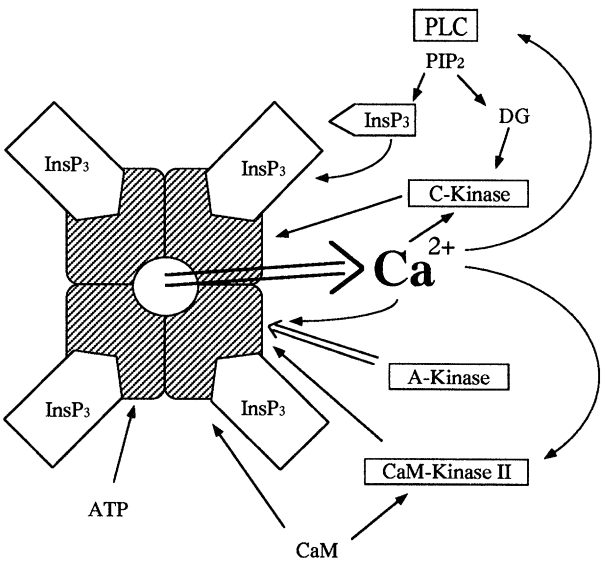

Figure 2. Interaction of the InsP_3 receptor with other cell signalling systems.

membrane. It is not expressed on mitochondria and Golgi apparatus. InsP_3-R has a fuzzy structure that corresponds to the large N-termini similar to the feet-like structure of the ryanodine receptor (Ohtsu *et al.* 1990; Satoh *et al.* 1990). InsP_3-R was not only highly expressed in the brain, but also expressed in the smooth muscle of arteries, oviduct and uterus, and also in the contractile smooth muscle of the aorta, intestine and esophagus (Furuichi *et al.* 1990). InsP_3-R is also enriched in mature oocytes (Furuichi *et al.* 1990; Volpe *et al.* 1991). Recently, we succeeded in defining the primary structure of InsP_3-R of *Drosophila melanogaster* (Yoshikawa *et al.* 1992) and found that InsP_3-R was more enriched in the antennae and legs than in the brain (Yoshikawa *et al.* 1992). In the head of the *eyes absent* (*eya*) mutant where the retina is deficient, there was a great decrease in its content (Yoshikawa *et al.* 1992). These data suggested that InsP_3-R plays an important role also in the sensory system (visual and olfactory tissue) and in muscle contraction in invertebrates.

4. HETEROGENEITY OF THE InsP_3 RECEPTOR

The subtypes of InsP_3-R were shown to be present, arising from the alternative splicing of two segments SI and/or SII. SI lies in the InsP_3 binding domain corresponding to 45 nucleotides (present [SI+] or absent [SI−] form) (Sudhof *et al.* 1991; Nakagawa *et al.* 1991), and SII in the regulatory domain (Nakagawa *et al.* 1991; Danoff *et al.* 1991) just between the two phosphorylation sites (120 nucleotides that consist of three further splicing subsegments, A, B and C, producing SII+, SIIB−, SIIBC−, SIIABC−) (Nakagawa *et al.* 1991). The isoforms carrying any of the subsegments within the SII are expressed only in the nervous system, and the 120 b.p.-deleted form SIIABC− is expressed ubiquitously (Nakagawa *et al.* 1991) and peripheral tissues express only the SII− type (Nakagawa *et al.* 1991; Danoff *et al.* 1991).

New types of InsP_3-R from different genes have been reported (Sudhof *et al.* 1991; Ross *et al.* 1992). The original InsP_3-R is now called the cerebellar type or type-1 receptor. Type-2 InsP_3-R was shown to have a significant homology with the type-1 receptor especially in the InsP_3 binding and transmembrane domains (Sudhof *et al.* 1991). Type-2 receptor has a higher affinity with InsP_3 than the type-1. Therefore, it is plausible that InsP_3 mediated Ca^{2+} release is directed by various forms of InsP_3-R from different genes and also from spliced variants. These receptors may have different functional properties in Ca^{2+} release.

Recent reports on olfactory neurons (Restrepo *et al.* 1991), human T lymphocytes (Kuno & Gardner 1987), and Jurkat lymphocytes (Khan *et al.* 1992) have let us to consider the presence of InsP_3-R localized at the plasma membrane. We observed for the first time by electron microscopy that an InsP_3-R-like molecule was localized at the caveola structure of the plasma membrane of endothelium, smooth muscle and keratinocytes (Fujimoto *et al.* 1992). It is of further interest to know what type of InsP_3-R is involved in the plasma membrane type.

5. ROLE OF InsP_3 RECEPTOR IN Ca^{2+} SIGNALLING

The cerebellar InsP_3-R in the lipid bilayer system exhibits four subconductance levels, which suggested that InsP_3 molecules open the channel in an additive manner (Maeda *et al.* 1991; Ehrlich & Watras 1988). However, it is reported, by using the permeabilized basophilic leukemia cells, that opening of the Ca^{2+} channel required binding of four InsP_3 molecules (Meyer *et al.* 1988). The discrepancy of the results might be attributed to the difference in the tissues used or to methodological differences.

ATP enhanced InsP_3-dependent Ca^{2+} release in a reconstituted cerebellar membrane (Maeda *et al.* 1991) as well as in a microsomal fraction from aorta (Ehrlich & Watras 1988). ATP binding sites are located near the phosphorylation sites (Maeda *et al.* 1991; Ehrlich & Watras 1988). The receptor bound ATP with the stoichiometry of equivalent moles (Maeda *et al.* 1991). Protein kinase A (A-kinase) phosphorylated InsP_3-R, and protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaM kinase II) also phosphorylated it but less efficiently than A-kinase (figure 2) (Furuichi *et al.* 1989; Danoff *et al.* 1991; Ferris & Snyder 1992; Yamamoto *et al.* 1989). Two serine residues around both ends of the SII spliced segment were shown to be phosphorylated by A-kinase (figure 3) (Furuichi *et al.* 1989; Danoff *et al.* 1991).

The InsP_3-R of *Drosophila melanogaster* (Yoshikawa *et al.* 1992) is of considerable interest in the regulation of the receptor with regard to phosphorylation, since the consensus sites for A-kinase phosphorylation were absent in the receptor. The rat type-2 InsP_3-R (Sudhof *et al.* 1991) also lacked phosphorylation sites.

InsP_3-induced Ca^{2+} flux showed a bell-shaped curve depending on the Ca^{2+} concentration using the cerebellar microsomal fraction incorporated into the lipid bilayer. The data suggested that InsP_3-induced Ca^{2+} release is regulated by Ca^{2+} (Bezprozvanny *et al.* 1991). It was recently demonstrated that InsP_3-R binds Ca^{2+} (Mignery *et al.* 1992).

InsP_3 did not desensitize Ca^{2+} release from isolated membrane vesicles (Nakade *et al.* 1991). But InsP_3-induced Ca^{2+} release activity was found to be changed following long-term stimulation of polyphosphoinositide hydrolysis at the cellular level. Chronic muscarinic stimulation reduced the InsP_3-R concentration in human neuroblastoma cells (Wojcikiewicz *et al.* 1992).

6. BLOCKING EFFECT OF InsP_3-R ANTIBODY ON Ca^{2+} SIGNALLING

Ca^{2+} oscillation upon various stimuli is a universal phenomenon in all living cells. Pharmacological experiments showed two Ca^{2+} release mechanisms, IICR (InsP_3-induced Ca^{2+} release) and CICR (Ca^{2+}-induced Ca^{2+} release) (Berridge 1990). Although

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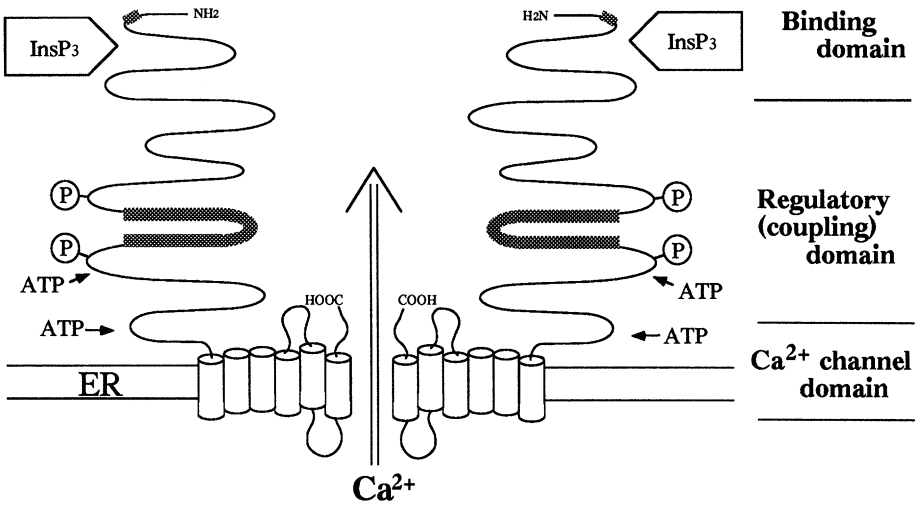

Figure 3. Schematic model of InsP_3 receptor representing the transmembrane topology. ER, endoplasmic reticulum; P, phosphorylation sites; ATP, ATP binding sites. A sequence of 650 amino acids at the N-terminal is essential for InsP_3 binding (Mignery & Sudhof 1990; Miyawaki *et al.* 1991). Splicing sites are shaded. Transmembrane domain is located near the C-terminal.

there is a report to show the biochemical heterogeneity of Ca^{2+} stores (Volpe *et al.* 1991), the actual molecular mechanism of interaction of IICR and CICR is still not known. A monoclonal antibody (mAb) 18A10 that recognizes the C-terminus of the type-1 InsP_3-R was found to suppress the Ca^{2+} channel activity of the receptor in an isolated cerebellar membrane fraction (Nakade *et al.* 1991). The C-terminal region of the InsP_3-R is considered to play a role in Ca^{2+} release. Golden hamster eggs were used to analyse the dynamic mechanism of Ca^{2+} signalling in the cell. The mAb 18A10 inhibited the Ca^{2+} waves upon injection of InsP_3 and Ca^{2+} into hamster eggs as well as sperm-induced Ca^{2+} waves (Miyazaki *et al.* 1992). Therefore, it is clear that Ca^{2+} release in fertilized hamster eggs is mediated solely by InsP_3-R, and that the Ca^{2+}-sensitized IICR, but not CICR, generates Ca^{2+} waves and Ca^{2+} oscillations (Miyazaki *et al.* 1992). Application of the mAb to the cells in other tissues will make clear the Ca^{2+} signalling mechanism in each cell in various tissues.

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