J. Membrane Biol. 118, 93–105 (1990)

The Journal of
Membrane Biology

Topical Review

Oscillating Intracellular Ca^{2+} Signals Evoked by Activation of Receptors Linked to Inositol Lipid Hydrolysis: Mechanism of Generation

Ole H. Petersen and Makoto Wakui*
M.R.C. Secretory Control Research Group, Department of Physiology, University of Liverpool, Liverpool, L69 3BX, United Kingdom

Introduction

A Ca^{2+} signal is, for the purpose of this review, defined as an increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) and can be generated either by release of Ca^{2+} from intracellular stores or by opening of Ca^{2+} channels in the plasma membrane. Here we are concerned solely with Ca^{2+} signals evoked by hormone or neurotransmitter activation of receptors linked via GTP-binding proteins (G proteins) to the enzyme phosphoinositidase C (also often referred to as phospholipase C). Binding of neurotransmitters or hormones to such receptor types causes breakdown of the membrane-bound inositol lipid phosphatidylinositol (4, 5) bisphosphate (PIP_2) producing initially two separate messengers, namely the lipid soluble diacylglycerol (DAG) and the water soluble inositol (1,4,5) trisphosphate (Ins(1,4,5)P_3) (Berridge, 1987). In 1983 it was shown for the first time that Ins (1,4,5) P_3 releases Ca^{2+} from an intracellular nonmitochondrial store (Streb et al., 1983), and soon thereafter it became clear that this Ca^{2+} store was in the endoplasmic reticulum (Streb et al., 1984; Prentki et al., 1984).

The Ca^{2+} signals often oscillate (Fig. 1) and since receptor-mediated repetitive Ca^{2+} spikes can be observed in the absence of extracellular Ca^{2+} (Berridge & Galione 1988; Berridge & Irvine, 1989) it would appear that pulsatile release of Ca^{2+} from intracellular stores (Fig. 1) is primarily responsible for the oscillations.

Pulsatile Ins (1,4,5) P_3 Formation is not Required for Pulsatile Intracellular Ca^{2+} Release

From the simplest possible model concept shown in Fig. 1 it seems that pulsatile Ins (1,4,5) P_3 formation could provide a straightforward explanation for the oscillating Ca^{2+} signal, and indeed Rink and Jacob (1989) stated that "most of those who have discussed this topic have explicitly or implicitly invoked fluctuating levels of Ins (1,4,5) P_3 as a key component. . ." Three possible explanations for these postulated oscillations in Ins (1,4,5) P_3 levels have been suggested: (i) A negative feedback loop whereby DAG generated as a consequence of the phosphoinositidase C activation could inhibit the enzyme via protein kinase C-mediated phosphorylation (Woods, Cuthbertson & Cobbold, 1987). (ii) Ca^{2+} activation of phosphoinositidase C in conjunction with strong cooperative interaction between Ins (1,4,5) P_3 and its receptor could explain a rapid rise in the cytoplasmic concentrations of both Ins (1,4,5) P_3 and Ca^{2+} which would be terminated when the Ins (1,4,5) P_3-sensitive Ca^{2+} pool was emptied. The rapid fall in Ca^{2+} would terminate the phosphoinositidase C activation, and Ins (1,4,5) P_3 levels would also fall. The pool would then refill and the cycle could start again (Meyer, Holowka & Stryer, 1988; Meyer & Stryer, 1988). (iii) Ca^{2+}-activation of Ins (1,4,5) P_3-3-kinase, the enzyme that causes phosphorylation of Ins (1,4,5) P_3 to inositol (1,3,4,5) tetrakisphosphate (Ins(1,3,4,5) P_4) (Biden & Wollheim, 1986) would reduce stimulated levels of Ins (1,4,5) P_3 and since Ins (1,3,4,5) P_4 by itself cannot release Ca^{2+} from the endoplasmic reticulum (Berridge, 1987) the Ca^{2+} signal would also disappear. This in turn would remove the stimulation of the Ins (1,4,5) P_3-3 kinase and Ins (1,4,5) P_3 levels would rise again (Yule & Gallacher, 1988).

* *Present address:* Department of Physiology, Tohoku University School of Medicine, Sendai, 980, Japan.

Key Words inositol trisphosphate · cytoplasmic Ca^{2+} concentration · Ca^{2+} signals · Ca^{2+} oscillation

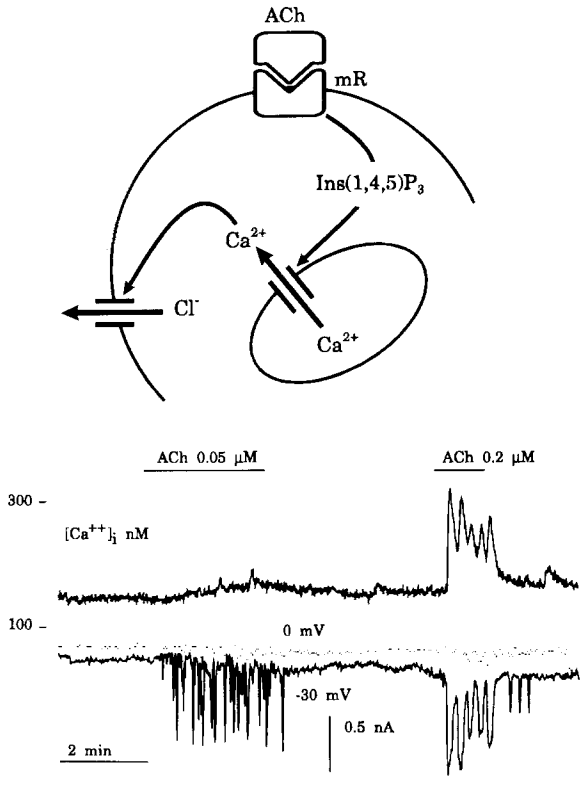

Fig. 1. Acetylcholine-evoked oscillations in $[Ca^{2+}]_i$ assessed simultaneously by two different methods. The lower part of the figure shows measurements from a single mouse pancreatic acinar cell studied in the patch-clamp whole-cell recording configuration. The bottom two traces are current recordings obtained at 0 and −30 mV membrane potentials, respectively. The trace at −30 mV shows the Ca^{2+}-dependent Cl^- current. The Cl^- concentrations inside and outside the cell were the same ($E_{Cl^-} \sim 0$), and there is therefore virtually no current changes at 0 mV membrane potential. The upper trace represents the result of the microfluorimetric measurement of $[Ca^{2+}]_i$ (nM) using the dye fura-2 which was present in the pipette solution. The upper part of the figure shows the simplest possible scheme explaining the events. It may appear that an increase in ACh concentration decreases the frequency of Ca^{2+}-dependent Cl^- current pulses, but what has actually happened is that the frequency has increased to the extent that individual short-lasting pulses have fused into much longer-lasting ones. The principal effect of increasing the ACh concentration is therefore prolongation of periods when $[Ca^{2+}]_i$ is elevated, in this case, to such an extent that longer pulses seem to take off from an elevated plateau. At a still higher ACh concentration of about 0.5–1 μM (*not shown*) a sustained elevation of both $[Ca^{2+}]_i$ and Cl^- current would have been observed. *ACh:* acetylcholine; *mR*; muscarinic receptor; *Ins (1,4,5) P_3*; inositol (1,4,5) trisphosphate. (Adapted from Osipchuk et al., 1990)

At this point in time it is not possible to measure the Ins (1,4,5) P_3 concentration in single cells with a high time resolution, and we therefore do not know whether Ins (1,4,5) P_3 levels oscillate during receptor activation. It is, however, possible to address the central question in a different manner by asking whether a constant concentration of Ins (1,4,5) P_3 can evoke pulsatile Ca^{2+} release.

Intracellular injections of Ins (1,4,5) P_3 into *Xenopus* oocytes have been shown to evoke repetitive membrane current changes or depolarizations due to Ca^{2+}-activation of Cl^- channels (Oron et al. 1985; Berridge, 1988). In such experiments a constant intracellular Ins (1,4,5) P_3 concentration cannot be achieved, but similar findings have been obtained when perfusing mouse pancreatic acinar cells or golden hamster egg cells internally with a constant Ins (1,4,5) P_3 concentration in patch-clamp or microelectrode experiments where the cytoplasmic Ca^{2+} fluctuations have been assessed by measuring the Ca^{2+}-dependent Cl^- current under voltage-clamp conditions (Fig. 2) (Wakui, Potter & Petersen, 1989) or the Ca^{2+}-dependent hyperpolarizing responses (Swann, Igusa & Miyazaki, 1989). Even such experiments can be criticized since it cannot be ruled out that metabolism and particularly Ca^{2+}-dependent phosphorylation of Ins (1,4,5) P_3 to Ins (1,3,4,5) P_4 (Biden & Wollheim, 1986) occurs. To solve this problem it was therefore necessary to use a nonmetabolizable Ins (1,4,5) P_3 analogue with Ca^{2+}-releasing activity. Inositol (1,4,5) trisphosphorothioate (Ins (1,4,5) PS_3) is an effective releaser of Ca^{2+} from intracellular stores, but is not metabolized by phosphatase or kinase pathways (Willcocks et al., 1988; Taylor et al., 1989). Ins (1,4,5) PS_3 perfused continuously via a patch pipette into single pancreatic acinar cells, evokes regular and repetitive spikes of Ca^{2+}-dependent Cl^- current (Fig. 2) showing that a constant messenger concentration can cause pulsatile intracellular Ca^{2+} release (Wakui et al., 1989). While these experiments cannot exclude that receptor activation results in pulsatile Ins (1,4,5) P_3 formation, it is an unnecessary complication to postulate that such a phenomenon occurs. The most economical hypothesis is that a constantly elevated Ins (1,4,5) P_3 concentration elicits pulsatile Ca^{2+} release in normal intact cells when stimulated by appropriate concentrations of hormones or neurotransmitters.

Ca^{2+}-Induced Ca^{2+} Release is Important for the Generation of Ca^{2+} Oscillations Initiated by Ins (1,4,5) P_3

Since Ins (1,4,5) P_3 at a constant concentration can evoke repetitive spikes of intracellular Ca^{2+} release (Wakui et al. 1989) and since the simplest suggestion would be that Ins (1,4,5) P_3 primarily evokes a constant movement of Ca^{2+} from the Ins (1,4,5) P_3-sensitive Ca^{2+} pool, it is pertinent to ask whether a small constant flow of Ca^{2+} into the cytosol can

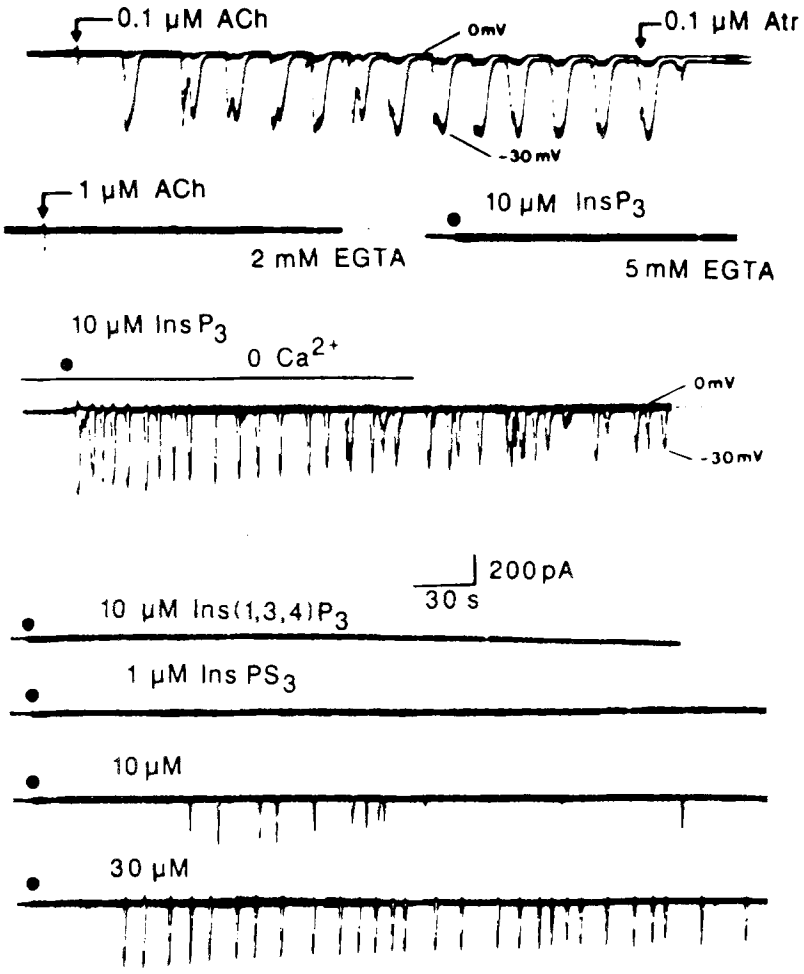

Fig. 2. Effects of acetylcholine (*ACh*), Ins (1,4,5) P_3 (*InsP_3*) and inositol (1,4,5) trisphosphorothioate (*Ins PS_3*) on Ca^{2+}-dependent Cl^- current in single internally perfused pancreatic acinar cells. ACh (0.1 μM) is seen to evoke repetitive pulses of Ca^{2+}-dependent Cl^- current, and this effect is blocked by atropine (*Atr*) and when the cell is filled with a high concentration of the Ca^{2+}-chelator EGTA. Internal perfusion with Ins P_3 also evokes regular pulses of Ca^{2+}-dependent Cl^- current and this effect is also blocked by a high internal EGTA concentration, but not by removal of external Ca^{2+} (0 Ca^{2+}). Inositol (1,3,4) trisphosphate (*Ins (1,3,4) P_3*) which is known not to cause intracellular Ca^{2+} release (Berridge, 1987) has no effect, but Ins PS_3 causes a dose-dependent response consisting of repetitive pulses of Ca^{2+}-activated Cl^- current. (Adapted from Wakui et al., 1989)

evoke repetitive transport of Ca^{2+} from another pool.

Ca^{2+}-induced Ca^{2+} release was originally discovered in skinned cardiac muscle cells where a small increase in the Ca^{2+} concentration of the fluid in contact with the sarcoplasmic reticulum causes a large Ca^{2+} release (Endo, Tanaka & Ogawa, 1970). In the presence of caffeine, skinned muscle fibers exhibit spontaneous contractions when bathed in a solution with a relatively low Ca^{2+} concentration (Endo et al., 1970). In intact cardiac cells Ca^{2+} oscillations observed during exposure to ouabain or solutions with low Na^+ concentration, giving rise to aftercontractions and transient inward Ca^{2+}-dependent currents, have been explained on the basis of Ca^{2+}-induced Ca^{2+} release from cells overloaded with Ca^{2+} (Kass et al., 1978; Allen, Eisner & Orchard, 1984). If Ca^{2+}-induced Ca^{2+} release is directly responsible for the cytoplasmic Ca^{2+} oscillations evoked by stimuli generating Ins (1,4,5) P_3 as suggested by Berridge (Berridge, 1988; Berridge & Irvine, 1989), then intracellular Ca^{2+} infusion should be able to mimick the effect of Ins (1,4,5) P_3. Figure 3 shows the result of an experiment in which Ca^{2+} was infused into a single cell while $[Ca^{2+}]_i$ was assessed both by microfluorimetry using fura-2 and by measurement of Ca^{2+}-dependent Cl^- current (Osipchuk et al., 1990). In this type of experiment the electrical current trace monitors $[Ca^{2+}]_i$ in the immediate vicinity of the inner surface of the plasma membrane, whereas the microfluorimetrical recording reports the average $[Ca^{2+}]_i$ in the cell. As seen in Fig. 3 the intracellular Ca^{2+} infusion results in a gradual rise in $[Ca^{2+}]_i$, and this increase is reversed when the Ca^{2+} infusion is stopped and a Ca^{2+} chelator applied. In the cytoplasmic space near the plasma membrane there are additional short-lasting repetitive Ca^{2+} spikes which are particularly pronounced just before the major sustained $[Ca^{2+}]_i$ rise and in the phase after discontinuation of the Ca^{2+} infusion. These results indicate that Ca^{2+} can induce pulses of Ca^{2+} release primarily from pools very close to

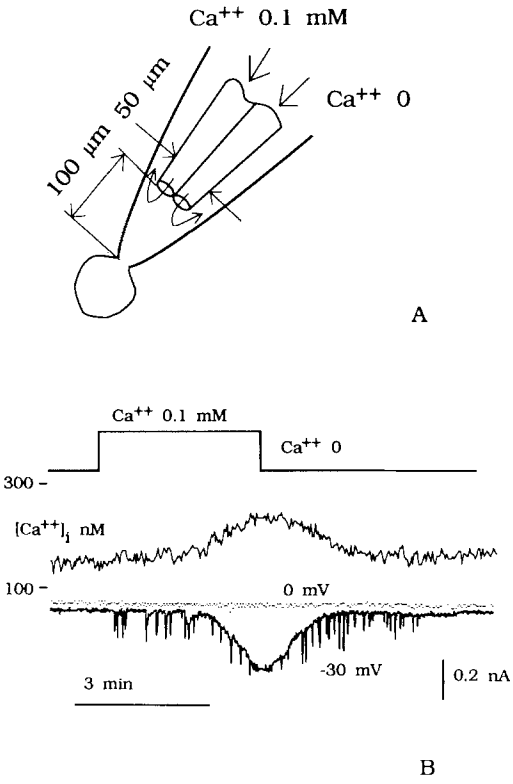

Fig. 3. Ca^{2+}-induced repetitive Ca^{2+} release from stores close to the cell membrane in single mouse pancreatic acinar cell. The cartoon in *A* shows the arrangement used to exchange solution at the tip of the patch-clamp pipette. Application of 200 mm Hg pressure to the tube containing 0.1 mM Ca^{2+} resulted in a slow rise of $[Ca^{2+}]_i$ and Ca^{2+}-dependent Cl^- current (bottom trace in *B*) and also in repetitive short-lasting spikes of Ca^{2+}-dependent Cl^- current (bottom trace in *B*). Application of pressure to the tube with Ca^{2+}-free solution (also containing the Ca^{2+} chelator EGTA) reversed the effects. (Adapted from Osipchuk et al., 1990)

the cell membrane. In the internally perfused mouse pancreatic acinar cells Ins (1,4,5) P_3 infusion also evokes Ca^{2+} spikes near the cell membrane which are mostly not reflected in the average $[Ca^{2+}]_i$ recording, although in small cells it is possible to detect Ins (1,4,5) P_3 evoked Ca^{2+} oscillations synchronously with both microfluorimetry and electrophysiology (Osipchuk et al., 1990). Low doses of ACh also predominantly evoke Ca^{2+} spikes seen only at the cell membrane, whereas larger doses cause slightly broader Ca^{2+} signals seen throughout the cell (Fig. 1) (Osipchuk et al., 1990). The conclusion from these experiments is therefore that Ca^{2+} infusion can mimick the action of Ins (1,4,5) P_3.

In the course of normal signal transduction, Ins (1,4,5) P_3 most likely evokes a small steady flow of Ca^{2+} into the cytosol that subsequently causes repetitive pulses of Ca^{2+} release from separate stores and a minimal quantitative model based on this concept has recently been developed (Goldbeter, Dupont & Berridge, 1990). According to this model, two different types of Ca^{2+} channels must exist in intracellular Ca^{2+} storing organelles, namely Ins (1,4,5) P_3-activated and Ca^{2+}-activated Ca^{2+} release channels (Fig. 4). It is useful to review briefly what is known about these two channel types before discussing attempts to critically test the hypothesis outlined in Fig. 4.

Ins (1,4,5) P_3 and Ca^{2+} Activate Two Separate Types of Ca^{2+} Release Channels

The Table summarizes some of the properties of the two functionally very different Ca^{2+} release channels from intracellular Ca^{2+} storing organelles. The pharmacology is of particular significance. The Ins (1,4,5) P_3-activated Ca^{2+} channel is inhibited by heparin, whereas this substance has no effect on the Ca^{2+}-induced Ca^{2+} release channel (Ehrlich & Watras, 1988). The Ca^{2+}-induced Ca^{2+} release channel is inhibited by ruthenium red and activated by caffeine, substances that have no effect on the Ins (1,4,5) P_3-activated Ca^{2+} channel (Ehrlich & Watras, 1988). The Ca^{2+}-induced Ca^{2+} release channel, often referred to as the ryanodine receptor, since it has a very high affinity for the plant alkaloid ryanodine (Lai et al., 1988), has been characterized mainly in muscle tissues. In skeletal muscle this channel normally functions by releasing Ca^{2+} from the sarcoplasmic reticulum in response to depolarization of the T-tubular cell membrane. A junctional complex has been described consisting of the voltage-sensing dihydropyridine receptor in the T-tubule membrane, and the ryanodine receptor Ca^{2+} channel (foot) complex in the sarcoplasmic reticulum. In cardiac cells it would appear that the dihydropyridine-receptor functions as a Ca^{2+} channel and the Ca^{2+} entering from the outside during membrane depolarization then evokes Ca^{2+} release through the ryanodine receptor channel (Fleischer & Inui, 1989). In general, this channel can therefore be activated either by electromechanical transduction, in a way not completely understood, or by Ca^{2+} binding to a site on the cytoplasmic side of the sarcoplasmic reticulum membrane, thereby inducing the conformational change presumably required for the increase in open-state probability (Fleischer & Inui, 1989). It is clear that the concentrations of ATP and Mg^{2+} in particular are important as ATP promotes opening, whereas Mg^{2+} has the opposite effect (Table). It has recently been shown that the Ins (1,4,5) P_3-activated

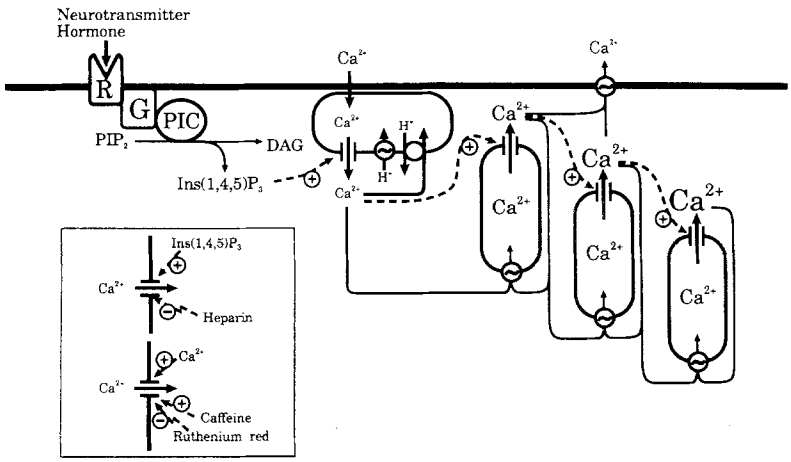

Fig. 4. Working hypothesis of mechanisms underlying cytoplasmic Ca^{2+} oscillation evoked by receptor activation. The basic concept is based on the models by Berridge (Berridge & Galione, 1988; Berridge & Irvine, 1989). This present version of the model emphasizes the different characteristics of the two types of Ca^{2+} channels in intracellular Ca^{2+} storing organelles which are now known and also highlights the progression of the Ca^{2+} signal from the surface cell membrane to the cell interior. This idea is based on the data from Osipchuk et al. (1990). The proposal that the Ca^{2+} uptake mechanisms in the Ins (1,4,5) P_3-sensitive and insensitive pools are different is based on the data from Thevenod et al. (1989)

Table. Some characteristics of the two Ca^{2+} release channels from nonmitochondrial intracellular Ca^{2+} stores

	Ins (1,4,5) P_3-activated Ca^{2+} channel	Ca^{2+}-activated Ca^{2+} channel
Structure	Probably homotetramer (each of the 4 polypeptides with $M_r \sim 250{,}000$) with square shape (Maeda et al., 1990). The amino acid sequence has been determined (Furuichi et al., 1989)	Homotetramer (each of the 4 polypeptides with $M_r \sim 400{,}000$) forming four-leaf clover 'feet' (Lai et al., 1988; Anderson et al., 1989). The amino acid sequence has been determined (Takeshima et al., 1989).
Activator	Ins (1,4,5) P_3 Ins (2,4,5) P_3 (Ferris et al., 1989)	Ca^{2+} (Smith et al., 1986; Lai et al., 1988).
Potentiator		Caffeine (Rousseau et al., 1988; Rousseau & Meissner, 1989) ATP and ATP analogues (Meissner, 1984; Smith et al., 1985).
Inhibitor	Heparin (Ferris et al., 1989)	Ruthenium red and Mg^{2+} (Meissner et al., 1986)
Single-channel conductance	10 pS (Ehrlich & Watras, 1988)	~100 pS (Smith et al., 1986; Lai et al., 1988; Ehrlich & Watras, 1988)

Ca^{2+} channel can also be influenced by ATP. In studies on purified Ins (1,4,5) P_3 receptors reconstituted in lipid vesicles it was shown that 1–10 μM ATP increased the maximal Ins (1,4,5) P_3-induced Ca^{2+} flux by about 50% whereas ATP in a higher concentration (10^{-4} to 10^{-3} M) inhibited this effect (Ferris, Huganir & Snyder, 1990).

When the skeletal muscle ryanodine receptor is expressed in Chinese hamster ovary cells there is no depolarization-evoked Ca^{2+} release, but caffeine effectively releases Ca^{2+} (Penner, et al., 1989). In pancreatic acinar cells surface membrane depolarization does not cause Ca^{2+} release (Matthews, Petersen & Williams, 1973), but Ca^{2+} -induced Ca^{2+} release occurs (Osipchuk et al., 1990) and caffeine can evoke cytoplasmic Ca^{2+} oscillations under certain conditions (Wakui & Petersen, 1990). It is therefore clear that the Ca^{2+}-induced Ca^{2+} release channel does not necessarily have to be functionally linked to a plasma membrane voltage sensor and in electrically nonexcitable cells, for example those found in epithelia, the absence of such a coupling is probably normal.

A Ca^{2+} channel has recently been studied in endoplasmic reticulum vesicles from rat pancreatic acinar cells (Schmid et al., 1990) fused by a dehydration/rehydration method (Schmid et al., 1988). In excised patches single Ba^{2+} and Ca^{2+} selective chan-

nels with a mean conductance of about 50 pS (50 mM Ba^{2+} in bath and 75 mM K^+ in pipette solution) were found. The channel activity was voltage dependent with a very low open state probability at −10 mV and virtually complete opening at +10 mV. Caffeine (10 mM) markedly increased the channel open state probability in about half the experiments and ruthenium red (10 μM) closed the channels completely. Neither Ins (1,4,5) P_3 or heparin had any effect on channel activity and variations in $[Ca^{2+}]_i$ in the bath solution had also no effect (Schmid et al., 1990). It is assumed that the cytoplasmic side of the endoplasmic reticulum membrane faces the pipette solution in these experiments and the failure of Ins (1,4,5) P_3 and Ca^{2+} to cause activation could therefore be due to the fact that these potential stimulants were added to the insensitive side. More work on the pancreatic endoplasmic reticulum Ca^{2+} channel is clearly needed to clarify its characteristics and in particular to establish whether it can be activated by low (micromolar) Ca^{2+} concentrations acting on the cytoplasmic side. It seems possible that the findings of Schmid et al. (1990) indicate the presence of a caffeine-sensitive channel in the pancreas that could explain Ca^{2+}-induced Ca^{2+} release and caffeine potentiation of receptor-activated responses observed in this tissue (Osipchuk et al., 1990).

Caffeine and Heparin Can be Used to Define Involvement of Two Separate Ca^{2+} Channels in Intracellular Organelles

The model shown in Fig. 4 can be tested using the knowledge gained about the pharmacology of the two Ca^{2+} release channels summarized in the Table. It could be argued that results of the type shown in Fig. 3 demonstrating Ca^{2+}-induced Ca^{2+} oscillations could also be explained by Ca^{2+}-induced Ins (1,4,5) P_3 formation due to the reported Ca^{2+}-sensitivity of phosphoinositidase C (Taylor et al., 1986; Meyer & Stryer, 1988). It is therefore possible that intracellular Ca^{2+} infusion mimicks the action of intracellular Ins (1,4,5) P_3, not because of direct Ca^{2+}-activation of Ins (1,4,5) P_3-insensitive Ca^{2+} release channels, but simply because it generates Ins (1,4,5) P_3. There are, however, data in the literature that seem to indicate that in intact cells Ca^{2+}-activation of phosphoinositidase C is not of great importance. Takemura et al. (1989) have used the tumor promoter thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+} pump, to cause marked elevation of $[Ca^{2+}]_i$ in parotid acinar cells and in such experiments they did not find evidence of PIP_2 breakdown or Ins (1,4,5) P_3 formation. This was not due to thapsigargin inhibition of phosphoinositidase C since the tumor promotor did not inhibit PIP_2 breakdown or Ins (1,4,5) P_3 formation induced by muscarinic receptor activation (Takemura et al., 1989). Another approach is to test whether a Ca^{2+} ionophore, such as A23187, evokes an increase in Ins (1,4,5) P_3 levels. In pancreatic acinar cells Matozaki and Williams (1989) have shown that this is not the case. Although it would therefore appear that an elevation of $[Ca^{2+}]_i$ achieved in the absence of receptor stimulation does not generate measurable quantities of Ins (1,4,5) P_3, it is still desirable to test as directly as possible the proposition that Ca^{2+} infusion causes Ca^{2+} oscillations via pulsatile Ca^{2+} release from an Ins (1,4,5) P_3-insensitive store.

Heparin is a useful tool for testing the model shown in Fig. 4 since the prediction is that this substance should inhibit receptor-activated and Ins (1,4,5) P_3-induced, but not Ca^{2+}-induced, Ca^{2+} oscillations. If, on the other hand, Ca^{2+} infusion worked via Ins (1,4,5) P_3 generation then heparin should also block the Ca^{2+}-induced Ca^{2+} spikes. Using internally perfused single pancreatic acinar cells, it has recently been shown that pulsatile Ca^{2+}-dependent Cl^- current responses to external acetylcholine or internal Ins (1,4,5) P_3 application are blocked by intracellular infusion of heparin, whereas in the same experiments the Ca^{2+} oscillations evoked by Ca^{2+} infusion are unaffected (Wakui, Osipchuk & Petersen[1]). This is direct evidence showing that Ca^{2+} infusion evokes Ca^{2+} release that is not mediated by the Ins (1,4,5) P_3-activated Ca^{2+} channels.

In order to test the hypothesis that the Ca^{2+}-induced Ca^{2+} release in electrically nonexcitable cells is mediated by channels of the type found to be responsible for this effect in muscle cells, it is useful to employ caffeine, a well-established potentiator of Ca^{2+}-induced Ca^{2+} release in muscle (Endo, 1977), and a drug that has been shown to activate the ryanodine receptor Ca^{2+} release channel from skeletal and cardiac muscle sarcoplasmic reticulum (Rousseau et al., 1988; Rousseau & Meissner, 1989). The prediction from the model shown in Fig. 4 is that caffeine should enhance responses not only to submaximal receptor activation and Ins (1,4,5) P_3 application, but also to submaximal Ca^{2+} infusion.

We have recently shown that 1 mM caffeine can evoke cytoplasmic Ca^{2+} oscillations when applied in the presence of a subthreshold dose of acetylcholine. This effect of caffeine was rapid and rapidly reversible. Caffeine also markedly enhanced the Ca^{2+} signals evoked by direct G-protein activation (using internal application of GTP-γ-S) or by infusion of

[1] Wakui, M., Osipchuk, Y.V., Petersen, O.H. 1990. (*Submitted*)

Ins (1,4,5) P_3 (Osipchuk et al., 1990). Caffeine (1 mM) evoked regular Ca^{2+} spikes when applied in the presence of a subthreshold intracellular Ca^{2+} infusion (Wakui et al.[1]) but in the absence of stimulation caffeine did not evoke any effect (Osipchuk et al., 1990). This does not necessarily mean that a normal intact cell is unresponsive to caffeine. In internal cell perfusion studies it is normal to use a Ca^{2+}-free pipette solution with a low concentration (about 0.2 to 0.5 mM) of the Ca^{2+} chelator EGTA. In addition, the fluorescent dye fura-2, which also has the properties of a Ca^{2+} chelator, is often present. Thus the Ca^{2+} buffering capacity inside the cell is enhanced under such experimental conditions. If an internal perfusion solution not containing EGTA is used and electrophysiological recordings alone are employed, obviating the need for fura-2, then caffeine (1 mM) does evoke Ca^{2+} spikes when added alone without any other stimulants (Wakui & Petersen, 1990; Wakui et al.[1]). It is therefore now apparent that caffeine, a drug that lowers the threshold for Ca^{2+}-induced Ca^{2+} release in muscle (Endo, 1977; Fleischer & Inui, 1989) and modulates as well as induces oscillatory activity in neurons (Kuba & Nishi, 1976; Lipscombe et al., 1988) can under the right circumstances evoke regular Ca^{2+} oscillations also in electrically nonexcitable cells. This indicates a functional role for Ca^{2+}-induced Ca^{2+} release channels in the generation of pulsatile Ca^{2+} outflow from intracellular Ca^{2+} storage organelles.

In rat chromaffin cells there are often spontaneous cytoplasmic Ca^{2+} oscillations. Caffeine (2–10 mM) induces an increase in the fluctuation frequency or evokes oscillations in cells that are initially silent. Similar effects are evoked by the agonists bradykinin and histamine. The caffeine effects are not inhibited by neomycin, a drug that blocks Ins (1,4,5) P_3 formation, whereas the agonist-induced effects are abolished (Malgaroli, Fesce & Meldolesi, 1990). These findings again show that Ca^{2+}-induced Ca^{2+} release can generate cytoplasmic Ca^{2+} fluctuations independent of Ins (1,4,5) P_3.

Marty and Tan (1989) have shown in patch-clamp experiments on rat lacrimal acinar cells that immediately after the transition from the cell-attached to the whole-cell configuration there is a transient increase in Ca^{2+}-dependent Cl^- current if the pipette solution contains 0.2 or 1 mM Ca^{2+}. Unlike the situation in pancreatic acinar cells (Osipchuk et al., 1990), regular Ca^{2+} oscillations were not seen with this Ca^{2+} infusion protocol, but the initial Ca^{2+} transient represents Ca^{2+}-induced Ca^{2+} release and this phenomenon was abolished if 50 μM ruthenium red had been introduced into the cell prior to the Ca^{2+} challenge. These experiments (Marty & Tan, 1989) therefore represent further support for the model shown in Fig. 4 since ruthenium red is an inhibitor of the Ca^{2+}-induced Ca^{2+} release channel (Table).

Two Intracellular Nonmitochondrial Ca^{2+} pools

The model shown in Fig. 4 requires two separate Ca^{2+} pools with Ca^{2+} channels having different characteristics. The existence of at least two nonmitochondrial intracellular Ca^{2+} pools has been proposed on the basis of studies where it can be shown that Ins (1,4,5) P_3 is only able to release Ca^{2+} from a minor compartment of the endoplasmic reticulum Ca^{2+} pool (Biden, Wollheim & Schlegel, 1986; Thevenod et al., 1989). In the pancreatic acinar cells the two Ca^{2+} pools seem to have different types of Ca^{2+} uptake mechanisms. The Ins (1,4,5) P_3-insensitive pool has a normal Ca^{2+}-ATPase pump (Martonosi, 1984), whereas the Ins (1,4,5) P_3-sensitive pool accumulates Ca^{2+} via a machinery consisting of a H^+-ATPase pump and a Ca^{2+}-H^+ exchanger (Schulz, Thevenod & Dehlinger-Kremer, 1989; Thevenod et al., 1989). In parotid acinar cells Henne, Piiper and Söling (1987) have shown that Ins (1,4,5) P_3 and GTP induce Ca^{2+} release from different intracellular pools representing different functional domains of the endoplasmic reticulum.

It has been proposed that the Ins (1,4,5) P_3-sensitive Ca^{2+} pool in nonmuscle cells consists of calciosomes that are small vesicles found throughout the cell, separate from the endoplasmic reticulum and containing the Ca^{2+}-binding protein calsequestrin. The Ca^{2+} accumulation system in these organelles consists of the Ca^{2+}-ATPase pump with immunological properties similar to the muscle Ca^{2+} pump (Volpe et al., 1988). However, a recent Ca^{2+} imaging study on chromaffin cells does not readily fit in with this picture since the Ins (1,4,5) P_3-sensitive pool had a location corresponding to the endoplasmic reticulum whereas the caffeine-sensitive pool was present throughout the cell (Burgoyne et al., 1989). The calciosomes, according to this study, might therefore rather correspond to the caffeine-sensitive pool. Furthermore, a recent study of rat liver microsomes (Van, Peter & Söling, 1989) has not confirmed the presence of calsequestrin in the Ins (1,4,5) P_3-sensitive Ca^{2+} pool, but instead detected four other intracisternal Ca^{2+}-binding glycoproteins. A growing number of studies indicate that agonists acting via Ins (1,4,5) P_3 formation initially and primarily evoke internal Ca^{2+} release from a restricted region of a cell (Berridge & Irvine, 1989; O'Sullivan et al., 1989; Dissing, Nauntofte & Sten-Knudsen, 1990; Osipchuk et al., 1990). These findings also do not support the idea that the diffusely distributed calci-

osomes constitute the Ins (1,4,5) P_3-sensitive Ca^{2+} pool.

The Ins (1,4,5) P_3-binding protein (Supattapone et al., 1988; Maeda, Niinobe & Mikoshiba, 1990) has now been identified as the Ins (1,4,5) P_3-activated Ca^{2+} channel (Ferris et al., 1989) and has been localized by immunohistochemical techniques to intracellular particles associated with the endoplasmic reticulum in cerebellar purkinje cells (Ross et al., 1989).

In exocrine acinar cells there is evidence indicating that the major part of the internal Ca^{2+} release evoked as a consequence of the processes initiated by receptor activation primarily comes from stores very close to the plasma membrane. In single parotid acinar cells a high carbachol concentration evokes a rapid hyperpolarization, due to activation of Ca^{2+}-dependent K^+ channels (Petersen & Maruyama, 1984; 1989), that significantly precedes the rise in $[Ca^{2+}]_i$ measured by fura-2 microfluorimetry (Foskett et al., 1989). In single pancreatic acinar cells cytoplasmic Ca^{2+} oscillations evoked by low doses of acetylcholine, as well as by only just suprathreshold Ins (1,4,5) P_3 or Ca^{2+} stimuli applied internally, are detected near the cell membrane but not in the cell at large (Figs. 1 and 3). Caffeine potentiates these responses and makes them easily detectable in the whole cytoplasm (Osipchuk et al., 1990). This suggests that the Ca^{2+}-induced Ca^{2+} release which appears to be the major fraction of the Ca^{2+} signal, at least at relatively low intensities of stimulation, primarily occurs close to the cell membrane, although Ca^{2+}-induced Ca^{2+} release can be evoked throughout the cell interior with stronger stimulation or when Ca^{2+}-induced Ca^{2+} release is potentiated by caffeine.

GTP seems able to release Ca^{2+} from both Ins (1,4,5) P_3-sensitive and Ins (1,4,5) P_3-insensitive Ca^{2+} pools and this effect is independent of Ins (1,4,5) P_3. The effect of GTP cannot be mimicked by nonhydrolyzable GTP-analogues and seems to require GTP hydrolysis (Chueh & Gill, 1986). It has been proposed that GTP enlarges the Ins (1,4,5) P_3-sensitive Ca^{2+} pool by establishing links to the Ins (1,4,5) P_3-insensitive pool (Ghosh et al., 1989). A similar role has been suggested for Ins (1,3,4,5) P_4 since in the absence of extracellular Ca^{2+} this substance can enhance Ins (1,4,5) P_3-evoked internal Ca^{2+} release (Changya et al., 1989).

While the existence of separate Ins (1,4,5) P_3-sensitive and Ins (1,4,5) P_3-insensitive Ca^{2+} pools is well established at least in some cell types (Berridge & Irvine, 1989; Thevenod et al., 1989), there is still some uncertainty about the precise localizations (Milani et al., 1990) and the question about functional control of operational links between these pools cannot be regarded as settled.

Negative Feedback Exists

Although the minimal quantitative two-pool model to account for cytoplasmic Ca^{2+} oscillations (Goldbeter et al., 1990) proposes that individual Ca^{2+} spikes are terminated simply due to emptying of the relevant Ca^{2+} pool and that a new spike can only occur when the pool is full again, it is nevertheless useful to examine an alternative possibility involving Ca^{2+} inhibition of Ca^{2+} release.

Parker and Ivorra (1990) have recently reported the results of some very elegant experiments providing evidence for Ca^{2+} inhibition of Ins (1,4,5) P_3-evoked Ca^{2+} release. In *Xenopus* oocytes internal Ca^{2+} liberation was evoked by light flash photolysis of caged Ins (1,4,5) P_3 (Walker et al., 1987) to obtain a precisely controlled stimulus. Cytoplasmic Ca^{2+} levels were monitored by recording Ca^{2+}-dependent Cl^- current and by use of the fluorescent Ca^{2+} indicator fluo-3. In such experiments Ins (1,4,5) P_3-mediated Ca^{2+} release is inhibited by Ca^{2+} microinjection or by applying a conditioning Ins (1,4,5) P_3 pulse at varying times before the test pulse. The inhibition is not immediate, but takes 1–2 sec to develop and thereafter gradually declines. Parker and Ivorra (1990) propose that cytoplasmic Ca^{2+} oscillations are due to this delayed Ca^{2+} inhibition of Ins (1,4,5) P_3-evoked Ca^{2+} liberation that disappears after several seconds (recovery from inhibition is half-complete after about 6 sec) and suggest that these findings could explain pulsatile Ca^{2+} release at a constant Ins (1,4,5) P_3 concentration. In the *Xenopus* oocytes Parker and Ivorra (1990) do not find evidence for Ca^{2+}-induced Ca^{2+} release, although Berridge (1988) proposed his model for Ca^{2+} oscillations via Ca^{2+}-induced Ca^{2+} release on the basis of studies on oocytes from the same species. Parker and Ivorra (1990) suggest that Ca^{2+} inhibits release of Ca^{2+} from an Ins (1,4,5) P_3-sensitive pool.

Evidence for an inhibitory effect by Ca^{2+} on Ca^{2+} release has also been obtained in pancreatic acinar cells. In patch-clamp whole-cell recording studies, Ca^{2+} oscillations initiated by caffeine can be inhibited transiently by intracellular Ca^{2+} infusion and Ca^{2+} oscillations initiated by intracellular Ca^{2+} infusion can be inhibited by the Ca^{2+} ionophore ionomycin. Ionomycin also inhibits Ca^{2+} oscillations evoked by acetylcholine and Ins (1,4,5) P_3 (Wakui & Petersen, 1990). The important point is that Ca^{2+}-induced oscillations are inhibited by further Ca^{2+} mobilization, indicating that in this system the

negative feedback occurs at the level of the Ca^{2+} release from the Ins (1,4,5) P_3-insensitive pool. Although it cannot be excluded that in the experiments employing the Ca^{2+} ionophore ionomycin the inhibitory effects were due either to some direct interference by this lipophilic compound with the Ca^{2+} release mechanism or to ionophore-mediated emptying of the relevant Ca^{2+} pools, these explanations cannot apply to the inhibitory effects of Ca^{2+} infusion on, for example, caffeine-evoked oscillations (Wakui & Petersen, 1990).

In studies on isolated ryanodine-receptor channels from muscle, inhibitory effects of Ca^{2+} are observed at millimolar Ca^{2+} concentrations. These effects are similar to those exerted by millimolar Mg^{2+} concentrations (Meissner, Darling & Eveleth, 1986). It has not yet been investigated whether the inhibitory Ca^{2+} effects in oocytes (Parker & Ivorra, 1990) or pancreatic acinar cells (Wakui & Petersen, 1990) are specific for Ca^{2+} or could also be exerted by other divalent cations, but in these systems it would appear that the inhibitory effects are present at lower Ca^{2+} concentrations although a precise quantification has not yet been achieved.

An example of Ca^{2+} inhibition of Ca^{2+} release initiated by a Ca^{2+} stimulus is seen in Fig. 3. A tiny elevation of $[Ca^{2+}]_i$ above the resting level following start of intracellular Ca^{2+} infusion initiates repetitive pulses of Ca^{2+} release near the cell membrane, but as the average $[Ca^{2+}]_i$ rises the Ca^{2+} spike frequency decreases and at the peak level of $[Ca^{2+}]_i$ the spikes disappear altogether to reappear after $[Ca^{2+}]_i$ has fallen substantially. The mechanism underlying this negative feedback is not clear, but could involve Ca^{2+}-stimulated synthesis of a labile inhibitory agent.

Extracellular Ca^{2+} Plays a Role, but is not the Primary Source for the Cytoplasmic Ca^{2+} Oscillations

Up to now the receptor-activated cytoplasmic Ca^{2+} oscillations have been dealt with as if they were completely independent of extracellular Ca^{2+}, and the model shown in Fig. 4 does not have an element of regulated Ca^{2+} inflow. This is reasonable in the sense that oscillations in $[Ca^{2+}]_i$ have been observed in many systems in the absence of external Ca^{2+} (Kruskal & Maxfield, 1987; Prentki et al., 1988; Wakui et al., 1989), but in other studies receptor-activated Ca^{2+} spikes disappear when external Ca^{2+} is removed and rapidly reappear after Ca^{2+} readmission (Jacob et al., 1988; Yule & Gallacher, 1988). In internally perfused renal juxtaglomerular cells it has been shown that the frequency of Ca^{2+} spikes induced by GTP-γ-S can be increased by elevating the external Ca^{2+} concentration from the normal 2 to 10 mM and decreased by exposing cells to a nominally Ca^{2+}-free solution (Kurtz & Penner, 1989).

The mouse pancreatic acinar cells are particularly interesting since extracellular Ca^{2+} dependency is present or absent depending on the precise experimental situation. In intact single cells where the Ca^{2+} oscillations are studied by microfluorimetry after loading with fura-2 acetoxymethyl ester (Yule & Gallacher, 1988) acetylcholine evokes oscillations in $[Ca^{2+}]_i$ which in the initial phase (1–2 min) are independent of external Ca^{2+}. In the sustained phase of stimulation, however, the removal of Ca^{2+} immediately causes cessation of the oscillations and these reappear after external Ca^{2+} readmission. Surprisingly, the same cells isolated in exactly the same way in the same laboratory behave in a somewhat different manner when investigated in the patch-clamp whole-cell recording configuration. In such experiments both acetylcholine- and Ins (1,4,5) P_3-evoked Ca^{2+} oscillations are completely unaffected by removal and readmission of external Ca^{2+} for about 7–8 min after start of stimulation (Wakui et al., 1989), but external Ca^{2+} dependency thereafter gradually develops and finally becomes absolute (Fig. 5). These experiments show that the oscillation mechanism is not primarily linked to control of Ca^{2+} influx through the surface cell membrane, but indicate that during prolonged periods of stimulation intracellular Ca^{2+} deprivation occurs and reloading from the extracellular Ca^{2+} compartment becomes necessary. It therefore seems justified to focus on the intracellular transport events with regard to understanding the basic mechanisms underlying the cytoplasmic Ca^{2+} oscillations (Fig. 4). The regulation of Ca^{2+} influx and the possible role of inositol (1,3,4,5) tetrakisphosphate in this process is the subject of much discussion, but has been reviewed in detail elsewhere (Berridge & Irvine, 1989; Petersen, 1989, 1990).

Ca^{2+} Oscillations Can Occur Synchronously in Adjacent Cells Linked by Communicating Junctions

Cytoplasmic Ca^{2+} oscillations are normally studied in single cells, but most tissues consist of cells linked by junctional channels allowing both chemical and electrical communication (Loewenstein, 1981). Pralong, Wollheim and Bruzzone (1988) assessed $[Ca^{2+}]_i$ in small clusters of rat pancreatic acinar cells (acini) which are extremely well coupled (Petersen

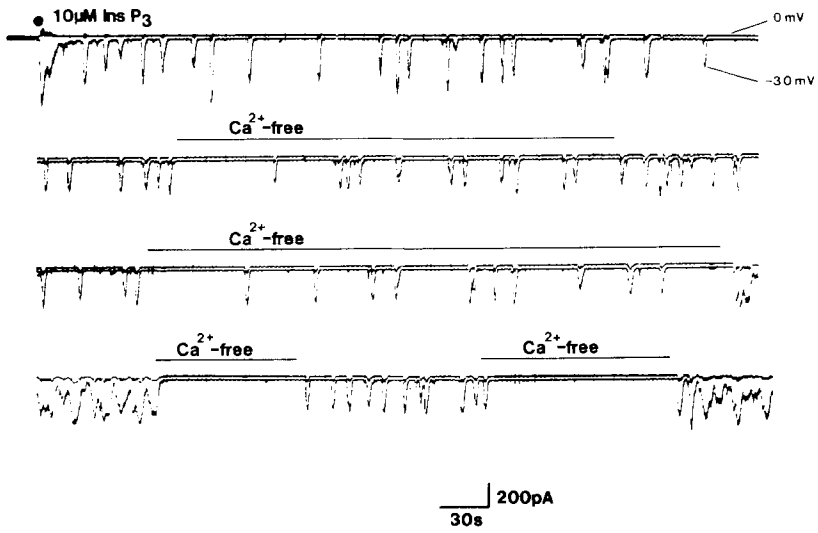

Fig. 5. The importance of extracellular Ca^{2+} for Ins (1,4,5) P_3-evoked $[Ca^{2+}]_i$ oscillations. The figure shows a continuous recording of Ca^{2+}-dependent Cl^- current measured by the patch-clamp method in the whole-cell recording configuration (Wakui et al., 1989). Ins (1,4,5) P_3 (*Ins P_3*) was present in a concentration of 10 μM in the pipette solution. Repetitive pulses of Ca^{2+}-dependent Cl^- current were generated. Early in the experiment external Ca^{2+} removal has little effect, but late in the same experiment the same procedure evokes complete and reversible cessation of the Ca^{2+} spiking process

& Findlay, 1987; Petersen & Maruyama, 1989) and found that the cholecystokinin analog caerulein evoked regular Ca^{2+} spikes in these units. Pralong et al.'s (1988) finding demonstrates that Ca^{2+} spikes occur synchronously in coupled cells. Synchronized oscillations in cytoplasmic Ca^{2+} concentration have also been demonstrated in confluent bradykinin-stimulated pulmonary artery endothelial cell monolayers (Sage, Adams & Van Bremen, 1989). A recent study of isolated parotid acini using imaging of fura-2 fluorescence has shown that the early rise in $[Ca^{2+}]_i$ following maximal carbachol stimulation occurs simultaneously in neighboring coupled cells (Dissing et al., 1990). It would be interesting to study systematically $[Ca^{2+}]_i$ oscillations with imaging techniques in cell clusters during normal conditions and during experimental situations where the cells had been uncoupled by, for example, heptanol or octanol (Meda et al., 1986).

On the basis of the limited evidence so far available it seems reasonable to expect major cytoplasmic Ca^{2+} oscillations to be occurring synchronously in adjacent well-coupled cells, but it is possible that short-lasting Ca^{2+} spikes restricted to the cytosolic space very near to the plasma membrane (Figs. 1 and 3) may not be seen simultaneously in all cells in a small communicating cell cluster. This point deserves further study as it could have considerable physiological implications.

There are Specific Receptor-Mediated Ca^{2+} Signatures

There is some controversy concerning the relationship between intracellular Ins (1,4,5) P_3 concentration and oscillation frequency. In studies on liver and endothelial cells the frequency of Ca^{2+} spikes increases with increasing agonist concentration (Woods et al., 1986; Jacob et al., 1988; Rooney, Sass & Thomas, 1989), whereas in parotid acinar cells no such relationship has been found (Gray, 1988). Although Yule and Gallacher (1988) did not find any clear relationship between acetylcholine concentration and frequency of $[Ca^{2+}]_i$ oscillation in intact mouse pancreatic acinar cells, the situation was different when exactly the same cells were investigated in the patch-clamp whole-cell recording configuration. In these internally perfused cells Wakui et al. (1989) found that the frequency of the Ca^{2+}-dependent Cl^- current pulses increased with increasing acetylcholine concentration. At high concentrations the Ca^{2+} spikes fused to give a sustained response. An increase in the concentration of the nonmetabolizable Ins (1,4,5) P_3-analogue inositol (1,4,5) trisphosphorothioate in the internal perfusion solution also appeared to cause an increase in the frequency of the evoked Ca^{2+} spikes.

According to the model shown in Fig. 4 one would expect different agonists to evoke the same pattern of cytoplasmic Ca^{2+} oscillations, even if they interacted with separate receptor sites (but, of course, both linked to inositol lipid breakdown), if the agonist doses were matched in such a way that the rate of Ins (1,4,5) P_3 formation was the same. The prediction seems incorrect, since in hepatocytes different agonists evoke specific patterns of changes in $[Ca^{2+}]_i$ (Woods et al., 1987; Rooney et al., 1989).

In the pancreatic acinar cells, cholecystokinin and acetylcholine, agents that are known to bind to separate receptor sites but both evoke inositol lipid breakdown and Ins (1,4,5) P_3 generation (Schnefel et al., 1988), induce somewhat different patterns of Ca^{2+} signals (Osipchuk et al., 1990). These findings show that the model in Fig. 4 is an oversimplification and that Ins (1,4,5) P_3 cannot be the only receptor-

generated internal message controlling Ca^{2+} transport. Diacylglycerol (DAG) is, of course, generated together with Ins (1,4,5) P_3, and it cannot be excluded that different proportions of the two messengers are produced by different agonists due to differences in the relative rates of breakdown of phosphatidylinositolphosphate and phosphatidylinositol bisphosphate (Berridge, 1987). It is also now known that many stimuli can activate a phospholipase D causing hydrolysis of phosphatidylcholine which would generate DAG (Billah et al., 1989). Varying the balance between phospholipase C and D stimulation would therefore produce different Ins (1,4,5) P_3/DAG ratios. Diacylglycerol is generally acting via activation of protein kinase C (Nishizuka, 1988) but other pathways cannot be totally excluded. There is evidence suggesting that phorbol esters, presumably acting via protein kinase C activation, inhibit receptor-activated Ins (1,4,5) P_3 production (Llano & Marty, 1987; Maruyama, 1989; Swann et al., 1989). Other messengers such as cyclic AMP and cyclic GMP may also play a role, and the possibility that an agent known to activate phosphoinositidase C (phospholipase C) also causes, for example, adenyl cyclase activation should not be ignored. In the case of cholecystokinin action on pancreatic acinar cell membranes it is known that there is, at least at high concentrations, activation of adenyl cyclase, whereas acetylcholine does not activate this enzyme (Schulz, 1989). Acetylcholine and cholecystokinin receptors interact with different G-proteins and while both these G-proteins can link up with phosphoinositidase C (Schnefel et al., 1988), they may also be able to connect other effectors and these could be different for the two types of G-proteins. It is interesting in this context that internal stimulation of pancreatic acinar cells with GTP-γ-S, that would undoubtedly interact with several different kinds of G-proteins, produces $[Ca^{2+}]_i$ changes that seem to combine the patterns characteristic for acetylcholine and cholecystokinin although being perhaps dominated by the latter (Osipchuk et al., 1990). In some situations it is likely that the agonist-specific patterns of Ca^{2+} spikes are due to specific modifications of Ca^{2+} extrusion from the cells and Ca^{2+} uptake into the various internal pools (Osipchuk et al., 1990), but at this stage there is no specific information on this point.

Conclusion

The two-pool model proposed by Berridge and Galione (1988) and Berridge and Irvine (1989) to account for receptor-activated cytoplasmic Ca^{2+} oscillations has been very fruitful as it has focussed the attention on a number of critical experiments that have now been carried out. Figure 4 is a slightly modified version of the Berridge model and several key points are now based on direct experimental evidence. The Ins (1,4,5) P_3-activated Ca^{2+} channel has been isolated and sequenced and most importantly its physiological and pharmacological properties investigated (Ehrlich & Watras, 1988; Ferris et al., 1989; Furuichi et al., 1989; Maeda et al., 1990). Direct evidence for Ca^{2+}-induced and caffeine-sensitive repetitive intracellular Ca^{2+} release in pancreatic acinar cells as well as evidence that the resulting Ca^{2+} spikes are similar to those produced by surface membrane receptor activation have been obtained (Osipchuk et al., 1990), showing that the Ca^{2+}-activated Ca^{2+} release channel is important for receptor-activated Ca^{2+} release. This channel has been isolated from skeletal and cardiac muscle and its basic chemical, structural, physiological and pharmacological properties are now well established (Meissner, 1984; Meissner et al., 1986; Lai et al., 1988; Rousseau et al., 1988; Takeshima et al., 1989). Evidence for the presence of a somewhat similar channel in nonmuscle tissue is now beginning to emerge since Schmid et al. (1990) have shown caffeine-activation of single Ca^{2+} channels isolated from the endoplasmic reticulum of pancreatic acinar cells.

Berridge and Galione (1988) proposed that Ca^{2+}-induced Ca^{2+} release was due to uptake of Ca^{2+} in an Ins (1,4,5) P_3-insensitive pool, eventual overfilling of the pool and therefore release due to the "Ca^{2+} pressure." The Ca^{2+} spike would be terminated when the pool was empty, and the interval between spikes would therefore depend on the time taken for renewed overfilling. The rate-limiting factor under most circumstances would be the Ins (1,4,5) P_3-evoked Ca^{2+} flow that provided the Ca^{2+} to be taken up into the Ca^{2+}-sensitive pool. According to this model concept Ca^{2+} release would be activated by the "Ca^{2+} pressure" inside the store. The Ca^{2+}-induced Ca^{2+} release (ryanodine) channel is, however, activated by Ca^{2+} acting on the outside (cytoplasmic side) of the channel (Lai et al., 1988), and it is therefore simpler to suggest that the Ca^{2+} released by Ins (1,4,5) P_3 into the cytoplasm acts directly on the ryanodine channel to cause opening as envisaged in more recent work (Goldbeter et al., 1990; Osipchuk et al., 1990). Whether the Ca^{2+}-sensitive Ca^{2+} pools are actually emptied during individual spikes is unknown. An alternative explanation for the oscillatory behavior of the system may be negative feedback by Ca^{2+} so that a rise in $[Ca^{2+}]_i$ at a critical site above the level that opens the ryanodine channels would switch them off. The concept of repetitive emptying and filling of Ca^{2+}-sensitive pools is appealing from the point of view of explaining the rela-

tionship between frequency of spikes and agonist dose seen in some cell types, but seems unattractive energetically. The amounts of Ca^{2+} released at low intensities of stimulation, apparently only generating Ca^{2+} spikes close to the cell membrane (Osipchuk et al., 1990) are probably quite small and therefore provide an energy-saving signalling mechanism. The sequential operation of two types of Ca^{2+} pools as shown in Fig. 4 is an attractive feature because it allows signal amplification in a simple manner. The possibility of the Ca^{2+} waves spreading by Ca^{2+}-induced Ca^{2+} release throughout the cell at higher intensities of stimulation (Osipchuk et al., 1990) is also useful since Ca^{2+} may serve as a messenger for many different types of events, not all necessarily controlled at the cell membrane. This view of Ca^{2+}-induced Ca^{2+} release is supported by data from chromaffin cells showing that caffeine induces a general cytoplasmic rise in $[Ca^{2+}]_i$ in contrast to the restricted elevation due to Ins (1,4,5) P_3 mobilizing agents (Burgoyne et al., 1989). By distributing the sensitivity of Ca^{2+} release to Ca^{2+} in particular ways it may be possible to control the spreading of Ca^{2+} signals at different intensities of stimulation. Precise characterization of the spatio-temporal organization of Ca^{2+} signals is now, in principle, possible with the help of high-resolution imaging techniques (Williams et al., 1985; O'Sullivan et al., 1989; Dissing et al., 1990), and this technique is now beginning to be applied systematically to study the mechanisms underlying receptor-activated cytoplasmic Ca^{2+} oscillations (Jacob, 1990).

We thank Professor R.D. Burgoyne (Liverpool, U.K.) and Professor G. Meissner (Chapel Hill, North Carolina) for valuable discussions and Beverly Fairfoull for great efficiency in preparing the manuscript in its final form. Our own work cited in this review was supported by a Programme Grant from The Medical Research Council (OHP).

References

Allen, D.G., Eisner, D.A., Orchard, C.H. 1984. *J. Physiol. (London)* **352:**113–128

Anderson, K., Lai, F.A., Liu, Q.-Y., Rousseau, E., Erickson, H.P., Meissner, G. 1989. *J. Biol. Chem.* **264;**1329–1335

Berridge, M.J. 1987. *Annu. Rev. Biochem.* **56:**159–193

Berridge, M.J. 1988. *J. Physiol. (London)* **403:**589–599

Berridge, M.J., Galione, A. 1988. *FASEB J.* **2:**3074–3082

Berridge, M.J., Irvine, R.F. 1989. *Nature (London)* **341:**197–205

Biden, T.J., Wollheim, C.B. 1986. *J. Biol. Chem.* **261:**11931–11934

Biden, T.J., Wollheim, C.B., Schlegel, W. 1986. *J. Biol. Chem.* **261:**7223–7229

Billah, M.M., Eckel, S., Mullmann, T.J., Egan, R.W., Siegel, M.I. 1989. *J. Biol. Chem.* **264;**17069–17077

Burgoyne, R.D., Cheek, T.R., Morgan, A., O'Sullivan, A.J., Moreton, R.B., Berridge, M.J., Mata, A.M., Colyer, J., Lee, A.G., East, J.M. 1989. *Nature (London)* **342:**72–74

Changya, L., Gallacher, D.V., Irvine, R.F., Potter, B.V.L., Petersen, O.H. 1989. *J. Membrane Biol.* **109:**85–93

Chueh, S.H., Gill, D.L. 1986. *J. Biol. Chem.* **261:**13883–13886

Dissing, S., Nauntofte, B., Sten-Knudsen, O. 1990. *Pfluegers Arch. (in press)*

Ehrlich, B.E., Watras, J. 1988. *Nature (London)* **336;**583–586

Endo, M. 1977. *Physiol. Rev.* **57:**71–108

Endo, M., Tanaka, M., Ogawa, Y. 1970. *Nature (London)* **228:**34–36

Ferris, C.D., Huganir, R.L., Snyder, S.H. 1990. *Proc. Natl. Acad. Sci. USA* **87:**2147–2151

Ferris, C.D., Huganir, R.L., Supattapone, S., Snyder, S.H. 1989. *Nature (London)* **342:**87–89

Fleischer, S., Inui, M. 1989. *Annu. Rev. Biophys. Biophys. Chem.* **18:**333–364

Foskett, J.K., Gunter-Smith, P.J., Melvin, J.E., Turner, R.J. 1989. *Proc. Natl. Acad. Sci. USA* **86:**167–171

Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., Mikoshiba, K. 1989. *Nature (London)* **342:**32–38

Ghosh, T.K., Mullaney, J.M., Tarazi, F.I., Gill, D.L. 1989. *Nature (London)* **340:**236–239

Goldbeter, A., Dupont, G., Berridge, M.J. 1990. *Proc. Natl. Acad. Sci. USA* **87:**1461–1465

Gray, P.T.A. 1988. *J. Physiol. (London)* **406:**35–53

Henne, V., Piiper, A., Söling, H.-D. 1987. *FEBS Lett.* **218:**153–158

Jacob, R. 1990. *Cell Calcium* **11:**241–249

Jacob, R., Merritt, J.E., Hallam, T.J., Rink, T.J. 1988. *Nature (London)* **335:**40–45

Kass, R.S., Lederer, W.J., Tsien, R.W., Weingart, E. 1978. *J. Physiol. (London)* **281:**187–208

Kruskal, B.A., Maxfield, F.R. 1987. *J. Cell. Biol.* **105:**2685–2693

Kuba, K., Nishi, S. 1976. *J. Neurophysiol.* **39:**547–563

Kurtz, A., Penner, R. 1989. *Proc. Natl. Acad. Sci. USA* **86:**3423–3427

Lai, F.A., Erickson, H.P., Rousseau, E., Liu, Q.-Y., Meissner, G. 1988. *Nature (London)* **331:**315–319

Lipscombe, D., Madison, D.V., Poenie, M., Reuter, H., Tsien, R.Y., Tsien, R.W. 1988. *Proc. Natl. Acad. Sci. USA* **85:**2398–2402

Llano, I., Marty, A. 1987. *J. Physiol. (London)* **394:**239–248

Loewenstein, W.R. 1981. *Physiol. Rev.* **61:**829–913

Maeda, N., Niinobe, M., Mikoshiba, K. 1990. *EMBO J.* **9:**61–67

Malgaroli, A., Fesce, R., Meldolesi, J. 1990. *J. Biol. Chem.* **265:**3005–3008

Martonosi, A.N. 1984. *Physiol. Rev.* **64:**1240–1320

Marty, A., Tan, Y.P. 1989. *J. Physiol. (London)* **419:**665–687

Maruyama, Y. 1989. *J. Physiol (London)* **417:**343–359

Matozaki, T., Williams, J.A. 1989. *J. Biol. Chem.* **264:**14729–14734

Matthews, E.K., Petersen, O.H., Williams, J.A. 1973. *J. Physiol. (London)* **234:**689–701

Meda, P., Bruzzone, R., Knodel, S., Orci, L. 1986. *J. Cell Biol.* **103:**475–483

Meissner, G. 1984. *J. Biol. Chem.* **259:**2365–2374

Meissner, G., Darling, E., Eveleth, J. 1986. *Biochem.* **25:**236–244

Meyer, T., Holowka, D., Stryer, L. 1988. *Science* **240:**653–656

Meyer, T., Stryer, L. 1988. *Proc. Natl. Acad. Sci. USA* **85:**5051–5055

Milani, D., Malgaroli, A., Guidolin, D., Fasolato, C., Skaper, S.D., Meldolesi, J., Pozzan, T. 1990. *Cell Calcium* **11:**191–199

Nishizuka, Y. 1988. *Nature (London)* **334:**661–665
Oron, Y., Dascal, N., Nadler, E., Lupu, M. 1985. *Nature (London)* **313:**141–143
Osipchuk, Y.V., Wakui, M., Yule, D.I., Gallacher, D.V., Petersen, O.H. 1990. *EMBO J.* **9:**697–704
O'Sullivan, A.J., Cheek, T.R., Moreton, R.B., Berridge, M.J., Burgoyne, R.D. 1989. *EMBO J.* **8:**401–411
Parker, I., Ivorra, I. 1990. *Proc. Natl. Acad. Sci. USA* **87:**260–264
Penner, R., Neher, E., Takeshima, H., Nishimura, S., Numa, S. 1989. *FEBS Letts.* **259:**217–221
Petersen, O.H. 1989. *Cell Calcium* **10:**375–383
Petersen, O.H. 1990. *In:* Intracellular Calcium Regulation. F. Bronner, editor. pp. 77–96. Liss-Wiley, New York
Petersen, O.H., Findlay, I. 1987. *Physiol. Rev.* **67:**1054–1116
Petersen, O.H., Maruyama, Y. 1984. *Nature (London)* **307:**693–696
Petersen, O.H., Maruyama, Y. 1989. *In:* Handbook of Physiology—The Gastrointestinal System. Vol. 3, pp. 25–50. J.G. Forte, editor. American Physiological Society Washington, D.C.
Pralong, W.F., Wollheim, C.B., Bruzzone, R. 1988. *FEBS Lett.* **242:**79–84
Prentki, M., Biden, T.J., Janjic, D., Irvine, R.F., Berridge, M.J., Wollheim, C.B. 1984. *Nature (London)* **309:**562–564
Prentki, M., Glennon, M.C., Thomas, A.P., Morris, R.L., Matschinsky, F.M., Corkey, B.E. 1988. *J. Biol. Chem.* **263:**11044–11047
Rink, T.J., Jacob, R. 1989. *Trends Neurosci.* **12:**43–46
Rooney, T.A., Sass, E.J., Thomas, A.P. 1989. *J. Biol. Chem.* **264:**17131–17141
Ross, C.A., Meldolesi, J., Milner, T.A., Satoh, T., Supattapone, S., Snyder, S.H. 1989. *Nature (London)* **339:**468–470
Rousseau, E., Ladine, J., Liu, Q.-Y., Meissner, G. 1988. *Arch. Biochem. Biophys.* **267:**75–86
Rousseau, E., Meissner, G. 1989. *Am. J. Physiol.* **256:**H328–H333
Sage, S.O., Adams, D.J., Van Bremen, C. 1989. *J. Biol. Chem.* **264:**6–9
Schmid, A., Gögelein, H., Kemmer, T.P., Schulz, I. 1988. *J. Membrane Biol.* **104:**275–282
Schmid, A., Kremer-Dehlinger, M., Schulz, I., Gögelein, H. 1990. *Nature (London)* **346:**374–376
Schnefel, S., Banfic, H., Eckhardt, L., Schultz, G., Schulz, I. 1988. *FEBS Lett.* **230:**125–130
Schulz, I., 1989. *In:* Handbook of Physiology. The Gastrointestinal System. Vol. 3, pp. 443–463. J.G. Forte, editor. American Physiological Society, Washington, D.C.
Schulz, I., Thevenod, F., Dehlinger-Kremer, M. 1989. *Cell Calcium* **10:**325–336
Smith, J.S., Coronado, R., Meissner, G. 1985. *Nature (London)* **316:**446–449
Smith, J.S., Coronado, R., Meissner, G. 1986. *J. Gen. Physiol.* **88:**573–588
Streb, H., Bayerdorffer, E., Haase, W., Irvine, R.F., Schulz, I. 1984. *J. Membrane Biol.* **81:**241–253
Streb, H., Irvine, R.F., Berridge, M.J., Schulz, I. 1983. *Nature (London)* **306:**67–69
Supattapone, S., Worley, P.F., Baraban, J.M., Snyder, S.H. 1988. *J. Biol. Chem.* **263:**1530–1534
Swann, K., Igusa, Y., Miyazaki, S. 1989. *EMBO J.* **8:**3711–3718
Takemura, H., Hughes, A.R., Thastrup, O., Putney, J.W. 1989. *J. Biol. Chem.* **264;**12266–12271
Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T., Numa, S. 1989. *Nature (London)* **339:**439–445
Taylor, C.W., Berridge, M.J., Cooke, A.M., Potter, B.V.L. 1989. *Biochem. J.* **259:**645–650
Taylor, C.W., Merritt, J.E., Putney, J.W., Rubin, R.P. 1986. *Biochem. J.* **238:**765–772
Thevenod, F., Dehlinger-Kremer, M., Kemmer, T.P., Christian, A.-L., Potter, B.V.L., Schulz, I. 1989. *J. Membrane Biol.* **109:**173–186
Van, P.N., Peter, F., Söling, H.-D. 1989. *J. Biol. Chem.* **264:**17494–17501
Volpe, P., Krause, K.H., Hashimoto, S., Zorzato, F., Pozzan, T., Meldolesi, J., Lew, D.P. 1988. *Proc. Natl. Acad. Sci. USA* **85:**1091–1095
Wakui, M., Petersen, O.H. 1990. *FEBS Lett.* **263:**206–208
Wakui, M., Potter, B.V.L., Petersen, O.H. 1989. *Nature (London)* **339:**317–320
Walker, J.N., Somlyo, A.V., Goldman, Y.E., Somlyo, A.P., Trentham, D.R. 1987. *Nature (London)* **327:**249–251
Willcocks, A.L., Potter, B.V.L., Cooke, A.M., Nahorski, S.R. 1988. *Eur. J. Pharmacol.* **165:**181–183
Williams, D.A., Fogarty, K.E., Tsien, R.Y., Fay, F.S. 1985. *Nature (London)* **318:**558–561
Woods, N.M., Cuthbertson, K.S.R., Cobbold, P.H. 1986. *Nature (London)* **319:**600–602
Woods, N.M., Cuthbertson, K.S.R., Cobbold, P.H. 1987. *Cell Calcium* **8:**79–100
Yule, D.I., Gallacher, D.V. 1988. *FEBS Lett.* **239:**358–362

Received 26 March 1990; revised 14 May 1990