Acta Physiol Scand 2000, **169**, 203–219

Ruled by waves? Intracellular and intercellular calcium signalling

J.-A. RØTTINGEN and J.-G. IVERSEN

Laboratory of Intracellular Signalling, Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

ABSTRACT

The field of calcium signalling has evolved rapidly the last 20 years. Physiologists had worked with cytosolic Ca^{2+} as the coupler of excitation and contraction of muscles and as a secretory signal in exocrine glands and in the synapses of the brain for several decades before the discovery of cellular calcium as a second messenger. Development of powerful techniques for measuring the concentration of cytosolic free calcium ions in cell suspensions and later in single cells and even in different cellular compartments, has resulted in an upsurge in the knowledge of the cellular machinery involved in intracellular calcium signalling. However, the focus on intracellular mechanisms might have led this field of study away from physiology. During the last few years there is an increasing evidence for an important role of calcium also as an intercellular signal. Via gap junctions calcium is able to co-ordinate cell populations and even organs like the liver. Here we will give an overview of the general mechanisms of intracellular calcium signalling, and then review the recent data on intercellular calcium signals. A functional coupling of cells in different tissues and organs by the way of calcium might be an important mechanism for controlling and synchronizing physiological responses.

Keywords calcium, cellular signalling, gap junctions, intercellular signals.

Received 19 January 2000, accepted 10 April 2000

In order to co-ordinate all the functions of the integrated human organism, the cells of the body have to communicate. The intercellular communication is mediated by soluble factors as, e.g. hormones, contact with extracellular matrix or by direct cell to cell interaction either by cell membrane proteins or through channels between the cells – gap junctions. All these interactions may induce some processes of intracellular signalling, but only the latter bypasses the transduction across the cell membrane. This transduction permits amplification and integration of signals, which may explain why cells may respond differently to apparently identical stimuli. On the other hand, intercellular signalling through gap junctions may contribute to co-ordinate and synchronise the function of the cells that are united in this way. Molecules smaller than approximately 1.5 kDa may pass through gap junctions, which implies that the most common second messengers as, e.g. cyclic AMP, inositol-1,4,5-trisphosphate (IP_3) and Ca^{2+} may be transferred. A Ca^{2+} signal, i.e. a temporary increase in cytosolic Ca^{2+}, is apparently propagated like a wave from one cell to the next, and is presumably responsible for a co-ordinated behaviour of these cells. It has been known for long that myocytes, e.g. in the heart, may behave as a functional unit, but the recent observation that glial cells communicate with each other, and probably also with nerve cells, in this way, has brought a new dimension to the analysis of brain function (Giaume & Venance 1998). Non-excitable cells, as, e.g. hepatocytes, seem also to co-ordinate their function by means of gap junctions and Ca^{2+} waves (Eugenin *et al.* 1998).

In this review, the properties of the calcium ion as both an intracellular and intercellular signalling substance will be outlined, and the mechanisms whereby a population of cells can synchronise their calcium signals will be focused on. Cytosolic Ca^{2+} is a versatile cellular signal. It is involved in the control of many different cellular functions ranging from fertilisation, proliferation and differentiation to secretion and contraction. The seminal role for intracellular Ca^{2+} has been recognised for several decades. Initially, the role of Ca^{2+} in excitation–contraction and excitation–secretion coupling and in synaptic transmission was demonstrated, and later Ca^{2+} as a second messenger was suggested (Rasmussen 1970). It was not until 1983

Correspondence: Prof. Jens-Gustav Iversen, Department of Physiology, PO Box 1103 Blindern, N-0317 Oslo, Norway.

that this signalling role for Ca^{2+} was demonstrated by showing IP_3-induced release of Ca^{2+} from intracellular stores (Streb *et al.* 1983). The history of this discovery has been described (Berridge 1992a), and the progress in the field over the last 15 years can be seen from a series of reviews of one the leading investigators in the field (Berridge & Irvine 1984, 1989, Berridge 1993a, Berridge *et al.* 1998).

Ca^{2+} AS A CELLULAR SIGNAL

Most of the cellular effects of a Ca^{2+} signal are mediated by Ca^{2+} binding proteins that alter their functional characteristics upon binding of Ca^{2+}. These are classified in three groups: Ca^{2+} storage proteins, EF hand motif containing proteins and the Ca^{2+}/phospholipid binding proteins (Niki *et al.* 1996).

The Ca^{2+} storage proteins, like calsequestrin and calreticulin, are important for the high capacity binding properties of the sarcoplasmic and endoplasmic reticulum (ER), respectively. However, calreticulin is also found outside the ER and has been implicated in modulation of nuclear receptor function and interaction with the cytoplasmic part of integrin α subunits. Recently, calreticulin was shown to mediate both cellular adhesion to fibronectin and the Ca^{2+} influx induced by fibronectin binding to integrins (Coppolino *et al.* 1997).

The EF-hand, which is able to bind one Ca^{2+} ion, normally exists in pairs and is usually a part of a larger α helical domain. Several proteins contain this motif. Troponin C and myosin light chains are important Ca^{2+} sensors in muscle contraction. The Ca^{2+} dependent protease calpain is a cysteine endopeptidase implicated in protein processing and necrosis. The best characterised EF-hand protein is calmodulin (Williams 1992). It is able to activate several enzymes like nitric oxide synthase, phosphodiesterases, adenylyl cyclase, Ca^{2+} pumps, phosphatases and kinases.

Ca^{2+}/phospholipid binding proteins bind phospholipid in a Ca^{2+} dependent manner, thereby allowing the proteins to translocate to the membrane upon a Ca^{2+} increase. The annexins have special Ca^{2+} binding domains and are involved in secretory processes by modulating membrane fusion (Rizo & Sudhof 1998). Synaptotagmins are proteins located in intracellular vesicles. They have two C_2 domains in their cytosolic part and are involved in vesicle transport and membrane fusion.

A typical Ca^{2+} signal contains a sudden increase in cytosolic Ca^{2+}, a spike, followed by a sustained increase often as oscillations or transients. Such regular fluctuations in cytosolic Ca^{2+} may have functional significance. Thus, gene regulation by Ca^{2+} signals is both amplitude and frequency modulated (Berridge 1997a). The transcription factor NF-AT (nuclear factor of activated T-cells) is activated by a sustained low amplitude Ca^{2+} increase, whereas others, as NF-κB (nuclear factor κB) and Jnk (c-Jun N-terminal kinase), are selectively turned on by a large high amplitude Ca^{2+} transient (Dolmetsch *et al.* 1997). High frequency Ca^{2+} spikes are more efficient positive regulators of NF-AT than low-frequency oscillations or a sustained high Ca^{2+} level (Li *et al.* 1998). Low-frequency transients can selectively turn on NF-κB, whereas both NF-κB and NF-AT are activated by high-frequency oscillations.

Cytosolic Ca^{2+} is involved in a vast area of cellular functions, and its physiological and pathophysiological roles have been reviewed extensively (Berridge 1993a, Berridge 1994, Berridge *et al.* 1998, Mooren & Kinne 1998). Here some aspects will be highlighted. At the beginning of life – fertilisation – the sperm evokes Ca^{2+} oscillations, which persist for some hours in the oocyte. Later in embryonal development a dorso-ventral IP_3 gradient is established and this may induce Ca^{2+} signals. Ca^{2+} is also involved in differentiation of muscle cells, the heart and the nervous system. Cell growth and proliferation are modulated by Ca^{2+} signals that affect cell cycle control and cell division (Berridge 1995).

At the other end of the life of the cell – cell death – Ca^{2+} is also important (Trump & Berezesky 1992, McConkey & Orrenius 1997). An increase in Ca^{2+} is always observed as the cells are dying, both in apoptosis/programmed cell death and accidental death, because of reduced levels of ATP, which leads to breakdown of ionic gradients across the cell membrane. The role of Ca^{2+} in cell death, i.e. whether an increase in Ca^{2+} is a primary or secondary event, is therefore somewhat difficult to resolve. In cerebral ischaemia neuronal damage is induced by an increase in Ca^{2+} caused by the release of glutamate. Several toxicological substances also induce cell death via Ca^{2+}. In the controlled type of cell death – apoptosis – Ca^{2+} is involved both in the initiation of the 'death signal' and as an effector activating endonucleases and proteases like calpain.

Ca^{2+} is involved in the process of cell spreading and adhesion to a surface. Integrin activation is important in these events, and integrins have been shown to induce Ca^{2+} signals (Sjaastad & Nelson 1997). Cytosolic Ca^{2+} is also important in cell motility and is able to control leukocyte chemotaxis (Pettit & Fay 1998). In the nervous system Ca^{2+} is an important signalling substance (Berridge 1998). The axonal action potential leads to a brief and localised Ca^{2+} signal that triggers the secretion of the transmitter to the synaptic cleft. In addition, Ca^{2+} is involved in forming synaptic plasticity, which is important for learning and memory. Both Ca^{2+} influx through the NMDA (*N*-methyl-D-aspartate) receptors and release of Ca^{2+} either by calcium-

induced calcium release (CICR) or via the inositol-1,4,5-trisphosphate receptor (IP_3R) is implicated in both early and late (via gene regulation) long-term potentiation.

INTRACELLULAR CALCIUM SIGNALLING

Cellular Ca^{2+} homeostasis

The cytosolic free calcium ion concentration ($[Ca^{2+}]_c$) at around 100 nM is about 10 000 times lower than the extracellular concentration. In addition to this steep concentration gradient, there is an electrical gradient because of the negative resting membrane potential approximately increasing the electrochemical potential difference by a factor of 100. The reason for the evolution of this low intracellular Ca^{2+} level might be that Ca^{2+} precipitates the important metabolite phosphate (Clapham 1995). In the cell Ca^{2+} is either free in cytosol, bound to proteins or other molecules or trapped in organelles with high Ca^{2+} concentrations like the endoplasmic reticulum and the mitochondria. More than 90% of cytoplasmic Ca^{2+} is bound to Ca^{2+} buffers. Ca^{2+} binding proteins can either have high or low affinity. Ca^{2+} buffering proteins like calsequestrin and calreticulin in the Ca^{2+} stores have low affinity, but have a high capacity because of their relatively high concentration, whereas Ca^{2+} effector proteins typically have high affinity binding sites (Lytton & Nigam 1992).

The mitochondria were for a period thought to have a buffering role, but have now been shown to take a more active part in the Ca^{2+} signal (see below). The mitochondria takes up Ca^{2+} by a uniporter with low affinity and release Ca^{2+} through Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers and the permeability transition pore (see below). Because of the negative membrane potential (around –150 mV) across the mitochondrial inner membrane, there is an electrochemical driving force for Ca^{2+} into the mitochondria even if the concentration of Ca^{2+} is 2–3 times higher on the inside than in cytosol.

The basal Ca^{2+} concentration is kept low by pumps and exchangers that move Ca^{2+} from the cytosol to intracellular stores or across the cell membrane (Mooren & Kinne 1998). A membrane Na^+/Ca^{2+}-antiporter is able to exchange 3 Na^+ for 1 Ca^{2+}, and with its high capacity it is especially important in cells that undergo large changes in intracellular Ca^{2+}, e.g. excitable cells like heart muscle cells. The plasma membrane Ca^{2+}-ATPase is important in all cells and regulates, together with the pump of internal stores, the resting Ca^{2+} level. It is activated by binding of calmodulin (CaM), which increases the sensitivity for Ca^{2+}, and by phosphorylation by protein kinase A and C, whereas it is inhibited by La^{3+} (Carafoli 1991, 1992). The Ca^{2+} pump of the endoplasmic reticulum is activated by phosphorylation by a CaM-kinase (CaMK) either directly or indirectly via phospholamban (mainly in muscle cells), which in an unphosphorylated state inhibits the pump (MacLennan *et al.* 1997). This pump can be inhibited by exogenous substances like thapsigargin, cyclopiazonic acid or tBuBHQ.

Cytosolic Ca^{2+} can be increased by opening channels either in the plasma membrane or in the membrane of internal stores. There are several types of Ca^{2+} channels in the cell membrane (Capiod *et al.* 1989, Tsien & Tsien 1990). Voltage-operated channels (VOCs) open upon depolarisation and are mainly present in excitable cells (nerves and muscle). Receptor-operated channels (ROCs) open directly upon interaction with a receptor activated by binding of its ligand. Examples include the NMDA glutamate receptor and the P_{2X} ATP receptor. Second-messenger operated channels (SMOCs) that can be activated by IP_3 or IP_4 have been described, but the main focus are now the store-operated channels (SOCs), also called calcium release activated channels (CRACs) (Sage 1992). Stretch or mechanically activated channels are found in epithelial and endothelial cells. In addition, tonically activated or leak divalent cation channels have been found. The Ca^{2+} release channels of the internal stores include the IP_3 receptor (IP_3R) and the ryanodine receptor (RyR) (see below).

Ca^{2+} release from internal stores

The releasable internal Ca^{2+} stores are mainly the endoplasmic reticulum or sarcoplasmic reticulum in striated muscle. Earlier a specialised calciosome has been proposed, but this idea is now abandoned (Meldolesi & Pozzan 1998a). There has been some debate on whether the stores are continuous or discrete pools. Two of the reasons for the latter view are that pools seem to have different IP_3 sensitivity, some even being insensitive, and different sensitivity to pump inhibitors like thapsigargin. Ca^{2+} translocation or fusion of these pools could be brought about by GTP (Rink & Merritt 1990). Later some of this observed discontinuity has been ascribed to fractionation of the ER induced by cell permeabilization (Bootman 1994). The concentration of Ca^{2+} inside the ER is around 1 mM, i.e. similar to the extracellular concentration, and Ca^{2+} inside the ER has also been shown to exert functions like modulating protein folding and vesicle transport between the Golgi apparatus and the ER (Meldolesi & Pozzan 1998b).

Several different substances that are able to release Ca^{2+} from the ER have been described. The best characterised substance is IP_3, which is produced by phospholipase C (PLC). Phosphatidylinositol specific

PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to IP_3 and diacylglycerol (DAG). DAG activates protein kinase C (PKC) and has also been shown to activate guanine nucleotide exchange factors for the small G-proteins Ras and Rho (Topham & Prescott 1999). There have also been reports on phosphatidylcholine specific PLC activities, but no such mammalian enzyme has been found. Here only the well characterised phosphatidylinositol specific PLC family will be discussed.

Three types of PLC have been found and there exists four isoforms of PLCβ, two of PLCγ and four of PLCδ (Rhee & Bae 1997, Katan 1998). All have similar structure with an N-terminal pleckstrin homology (PH) domain and two domains (X and Y) that constitute the catalytic domain. PLCδ has a short C-terminal region, while PLCβ has a larger region responsible for interaction with G-proteins. PLCγ has a region between X and Y that contains PH, SH2 and SH3 domains (pleckstrin and *src* homology domains) responsible for interaction with its upstream regulators. PLCβ is activated by the α-subunits of the G_q-family. In addition the $\beta\gamma$-dimer also activates PLCβ. As the G_q-family is pertussis toxin resistant, activation by $\beta\gamma$ subunits probably accounts for activation of PLCβ by pertussis toxin sensitive G-protein coupled receptors (GPCRs) coupled to G_i. α and $\beta\gamma$ bind to different regions of PLCβ and can act additively. Different PLCβ isozymes show different sensitivity to α and $\beta\gamma$. The PLC pathway can also be negatively modulated by the action of protein kinase A (PKA) and PKC at the level of the GPCR, the G-protein or PLC itself.

PLCγ is activated by tyrosine kinases either of the receptor type like the receptors for PDGF and EGF or non-receptor type like Src, Btk and JAKs (Janus family kinases). Tyrosine phosphorylation of the kinase or an adaptor molecule leads to binding of PLCγ by its SH2 domain and tyrosine phosphorylation of PLCγ. Both the phosphorylation and the association to the other molecule seem to be important for the activation of PLCγ. Recently several GPCRs have been shown to activate PLCγ by tyrosine phosphorylation, possibly via Pyk and Src. Other activators of PLCγ include PIP_3, phosphatidic acid and arachidonic acid interacting with a microtuble-associated protein. PLCδ can be activated by a newly described heterodimeric G-protein (G_h) linked to an α-adrenergic receptor and an oxytocin receptor. PLCδ also has the highest sensitivity to Ca^{2+} and is therefore activated when cytosolic Ca^{2+} is increased, thereby reinforcing the Ca^{2+} signal initiated by the other PLCs.

IP_3 releases Ca^{2+} by binding to the IP_3 receptors in the membranes of the ER. Three receptor subtypes and a splice variant have been described, and more than one type is usually expressed in most cells (Ferris & Snyder 1992, Taylor 1998, Patels *et al.* 1999). The IP_3R is a tetramer with an IP_3 binding site on each subunit. It is homologous to the ryanodine receptor. Two characteristics of the IP_3-induced Ca^{2+} release have been described, i.e. its quantal properties and its biphasic sensitivity to cytosolic Ca^{2+} (Irvine 1990, Cheek 1991, Bootman 1994, Taylor 1998).

Quantal release denotes that a certain concentration of IP_3 seems to release only a fraction of the Ca^{2+} in the stores and not all at a slower rate. Two explanations for this have been suggested. A steady-state model proposed that luminal Ca^{2+} could regulate the IP_3 sensitivity of the IP_3R such that an initial release would lower luminal Ca^{2+} to a level where it reduced the sensitivity of the channel to IP_3. The all-or-none model suggested that the Ca^{2+} stores were discrete pools with different IP_3 sensitivity and that IP_3 released all Ca^{2+} in each pool. The evidence for luminal control of the IP_3 sensitivity is inconsistent. The sensitivity of IP_3Rs to IP_3 differs between the different isoforms and can be modulated by phosphorylation by several kinases (PKA, PKC, CaMK and PKG) and by interaction with many accessory proteins (MacKrill 1999). The latter hypothesis is therefore the most favoured today.

Intermediate concentrations of cytosolic Ca^{2+} stimulate the IP_3R while high and low concentrations do not, i.e. a bell-shaped dose–response curve or a bimodal effect (Taylor 1998). This is important as Ca^{2+} can thereby reinforce its own release by CICR. This was first described for the RyR, although at somewhat higher Ca^{2+} concentrations. A difference between the two channels is that CICR through IP_3R is dependent on binding of ligand, while this is not necessary for the RyR. The biphasic effect is now thought to be mediated by the following model: IP_3 binding to the channel unmasks a Ca^{2+} binding site and the channel opens upon Ca^{2+} binding. The channel is autoinactivated by a fast mechanism where the affinity to IP_3 is increased but the channel is closed. When cytosolic Ca^{2+} is increased it binds to a site of an accessory protein that inhibits the channel. In this way the IP_3R is under control of two ligands (Berridge 1997b). Luminal Ca^{2+} probably controls release by leakage, thereby activating the channel at high luminal Ca^{2+} levels, whereas there is some evidence that low levels of luminal Ca^{2+} can reduce the IP_3 sensitivity by interaction with the luminal loop of the IP_3R.

The RyR was first described in skeletal muscle and upon depolarisation of the muscle it is opened both by direct protein interaction with the voltage sensitive Ca^{2+} channel dihydropyridine receptor and by Ca^{2+} influx. RyR is abundantly expressed in most tissues, and its function was thought to be mediating CICR. Caffeine activates this channel whereas ruthenium red and dantrolene inhibit it. Since CICR of the RyR was

discovered before the similar properties of the IP_3R, the RyR was important in a two-pool model for Ca^{2+} oscillations where CICR was an important mechanism. Now an endogenous ligand for RyR has also been found – cyclic ADP ribose (cADPR) (Lee 1996). It activates the RyR in a similar way that IP_3 modulates its receptor. cADPR has also been shown to be a true second messenger as cyclic GMP produced by NO activation of guanylyl cyclase activates a ADP-ribosyl cyclase that produces cADPR from NAD^+ (Berridge 1993b).

Two other Ca^{2+} releasing molecules have also been described. The same enzymes that generate cADPR can produce NAADP form $NADP^+$, and NAADP has been shown to release Ca^{2+} independent of IP_3R and RyR (Lee 1996, Cancela *et al.* 1999, Guse 1999). This might be modulated by cAMP instead of cGMP. Sphingolipids are also able to release Ca^{2+} (Meyer zu Heringdorf *et al.* 1998) and a channel called SCaMPER (sphingolipid Ca^{2+}-release-mediating protein from the endoplasmic reticulum) has been cloned but is not well described (Mao *et al.* 1996).

CICR from the mitochondria has also been described. Mitochondria has a large capacity to store Ca^{2+}, but the free Ca^{2+} concentration is only somewhat higher than in cytosol (Rizzuto *et al.* 1993). The Ca^{2+} levels in a normal global signal are not high enough to give a substantial influx to the mitochondria via the uniporter, but now it has been demonstrated that because of the proximity between the ER and the mitochondria, the high local concentration outside the Ca^{2+} releasing channels activates the uniporter (Robb-Gaspers *et al.* 1998, Csordas *et al.* 1999). The Ca^{2+} uptake by the mitochondria thereby modulates the Ca^{2+} signals in the cytosol by suppressing the local feedback effects of Ca^{2+} on the IP_3R and resulting in subcellular heterogeneous 'IP_3 sensitivity' (Hajnoczky *et al.* 1999). The resulting Ca^{2+} transients in the mitochondria are similar to the Ca^{2+} signal in the cytosol and are able to modulate the metabolic activity of the mitochondria. The Ca^{2+} transient in the mitochondria has also been shown to open the permeability transition pore, thereby releasing Ca^{2+} to the cytosol after the electrochemical gradient for Ca^{2+} is reversed as the opening of the pore abolishes the proton gradient (Ichas *et al.* 1997, Jouaville *et al.* 1998). This is called mitochondrial CICR.

Ca^{2+} can also be released from the nuclear envelope into the nucleus. There has been much debate on the nature of nuclear Ca^{2+} signalling (Santella & Carafoli 1997, Malviya & Rogue 1998). Even though the size of the nuclear pore should allow rapid diffusion of both Ca^{2+} and other small signalling molecules, cytosolic and nuclear Ca^{2+} signals have been shown to be uncoupled. This implicates that the transport of Ca^{2+} through the pore is probably regulated, and free diffusion at low cytosolic Ca^{2+} and partially restricted diffusion at high levels have been described (Genka *et al.* 1999). The nuclear envelope is in continuity with the endoplasmic reticulum and may act as a Ca^{2+} store for the nucleus, as Ca^{2+} is released from this store by similar mechanisms as the cytosolic stores, i.e. via IP_3R on the inner membrane of the nuclear envelope.

Capacitative Ca^{2+} entry

The most important Ca^{2+} influx pathway in non-excitable cells is the store-operated Ca^{2+} influx. The capacitative model originally suggested that depletion of the intracellular stores resulted in direct refilling of the stores from the outside of the cell, but in its revised form it was concluded that the emptying of the stores led to Ca^{2+} influx across the cell membrane (Putney 1990). Several mechanisms have been suggested for signalling store depletion to a store-operated Ca^{2+} influx channel (SOC) (Penner *et al.* 1993, Putney 1993, Parekh & Penner 1997). There has to be a Ca^{2+} sensor in the store that can transduce the level of Ca^{2+} to a signal regulating Ca^{2+} influx. The IP_3R could be such a sensor, but the effects of luminal Ca^{2+} on the receptor is contradictory. Another sensor could be calreticulin or other calcium binding proteins in the store.

The IP_3R has been proposed to interact directly with the SOC so that the channel is open both ways when IP_3 and IP_4 (or only IP_3) are present and when luminal Ca^{2+} is low (Irvine 1992). A diffusible messenger called calcium influx factor (CIF) has also been demonstrated (Randriamapita & Tsien 1993), but the direct contact hypothesis has been strongly supported by recently published findings (Clapham 1993, Parekh *et al.* 1993, Putney 1999). Cyclic GMP and NO stimulate a SOC, but this might be an indirect effect of depleting cADPR-sensitive stores. Other signals that have been demonstrated to be involved are small G-proteins, pertussis toxin-sensitive G-proteins, cytochrome P-450, arachidonic acid, kinases and phosphatases. The results are conflicting and some could be secondary to changes in the membrane potential. Several SOCs have been demonstrated using the patch clamp technique. The first channel that was characterised was the I_{CRAC}, which is highly specific for Ca^{2+} and has a low conductance (0.02 pS). Other SOCs with higher conductance and lower specificity for Ca^{2+}, including non-selective cation channels, have been described.

The capacitative route is important for Ca^{2+} influx at high concentrations of agonist, its role in modulating Ca^{2+} oscillations has been recently questioned (Shuttleworth 1999). Capacitative Ca^{2+} entry has been regarded important during the interspike period to refill the stores. However, the slow activation kinetics of the

SOCs compared with the short duration of the Ca^{2+} spikes, the relatively low Ca^{2+} extrusion compared with Ca^{2+} recycling back to the stores during a transient, and an obvious need for Ca^{2+} influx before any store depletion, have led to another conclusion, i.e. that influx is important for triggering the transient. This implies that other influx pathways than SOCs is probably the main route during Ca^{2+} oscillations, and that SOCs are activated only at high concentrations of agonists, which induce sustained responses and a marked depletion of the stores.

In addition to SOCs also the SMOCs may be important downstream Ca^{2+} influx pathways of the PLC-system (Barritt 1999). Channels that rely on either IP_3 or IP_4, or both, Ca^{2+} (i.e. Ca^{2+}-induced Ca^{2+} influx), Ca^{2+} and IP_4, or G-proteins have been described, but the results are difficult to interpret. In conclusion, the capacitative entry, although its mechanisms are uncertain, is the most abundant and well demonstrated Ca^{2+} influx pathway in non-excitable cells.

Fundamental, elemental and global Ca^{2+} signals

So far the different components taking part in a cellular Ca^{2+} signal have been described. Now the events and mechanisms that constitute a typical Ca^{2+} signal will be outlined (Bootman & Berridge 1995, Thorn 1996, Berridge 1997b,c, Berridge *et al.* 1999). A Ca^{2+} signal has been thought of as a uniform biphasic signal covering the whole cell with an initial spike produced by release from internal stores and a second shoulder phase brought about by influx through the capacitative route. With the improvements in imaging techniques this has proved to be wrong. Because of all the buffering capacity in the cytosol the diffusion of Ca^{2+} is very limited (15–65 $\mu m^2\ s^{-1}$), whereas IP_3 diffuses fast (230 $\mu m^2\ s^{-1}$). The calculated effective ranges of free Ca^{2+} before it is buffered were thus 0.1 μm, buffered Ca^{2+} 5 μm, and IP_3 24 μm determined from their diffusion coefficients and lifetimes (Allbritton *et al.* 1992). A local increase can therefore be kept more or less localised. In the resting state there are spontaneous openings of the release channels of the internal stores. These are the fundamental events called blips producing a localised increase in the Ca^{2+} concentration of about 30 nM and with a diameter of about 1 μm. Because of the negative feedback on the channel of both IP_3 and Ca^{2+} the blip is terminated.

When the cell is stimulated, the IP_3 level is increased and the frequency of blips increases. Because of the dual ligand properties of the IP_3R, Ca^{2+} can coactivate adjacent IP_3Rs. The concerted action of such a cluster of Ca^{2+} channels produces a larger but still localised elevation of Ca^{2+}. This elementary event in Ca^{2+} signalling is called a puff and is large enough to produce effects like exocytosis, Ca^{2+} response in the mitochondria (see above) and activation of ion channels. When the level of stimuli is increased, the concentration of IP_3 is further elevated. Now the puff in the region of the cell with the highest sensitivity to IP_3 is large enough to control the other events and a global Ca^{2+} wave spreads around the cell.

This Ca^{2+} wave is regenerative starting from the same region of the cell every time. It travels rather slowly with a velocity of 10–50 $\mu m\ s^{-1}$, indicating that the synchronisation of the global cellular Ca^{2+} signal takes about 1 s in most cells (Amundson & Clapham 1993, Thomas *et al.* 1996). The propagation speed is rather constant and not dependent on the level of agonist or IP_3, indicating that there is no IP_3 gradient in the cell. The Ca^{2+} wave is disseminated by CICR through both IP_3Rs and RyRs. In most cells the wave spreads like a tide until the Ca^{2+} level throughout the cell is increased. Then Ca^{2+} is restored to basal level simultaneously all over the cell. In larger cells like oocytes the wave has both a front and a trailing edge, and waves can start from several foci. Both planar and radial waves have been observed, and when two waves meet they annihilate. In addition complex spiral waves can form.

In a temporal domain these regenerative waves appear like repetitive transients or baseline oscillations. There is a slow elevation, i.e. a pacemaker phase, preceding the transient and this is caused by an increasing frequency and amplitude of blips and puffs, which produce an elevation in the global Ca^{2+} level (Bootman *et al.* 1997). The cytosol can be viewed as an excitable medium with a threshold for excitation. The gradual increase in excitability in the pacemaker phase of a temporal signal is probably dependent on store-operated Ca^{2+} influx, which increases both store loading and the global basal Ca^{2+} level.

These intracellular Ca^{2+} waves and oscillations are induced when cells are stimulated with low or intermediate concentrations of agonists. Because of the fast diffusion of IP_3, this leads to uniform and intermediate levels of IP_3 that are sufficient to set up a wave from the most sensitive region of the cell. When high concentrations of agonists are used, the IP_3 level is high enough to induce fast release from most of the stores simultaneously. There is no wave but only a fast spike with a continuously elevated Ca^{2+} level, i.e. a traditional biphasic signal. For many agonists this is probably an unphysiological response and may explain why the biological responses induced by Ca^{2+} signals often have left-shifted dose–response curves compared with the dose–response curve for Ca^{2+}. *In vivo* the concentrations of agonists increase gradually and not abruptly like those used in most experimental conditions and this

will also start the Ca^{2+} signal in a more physiological way, i.e. from fundamental via elemental to a global response.

Ca^{2+} oscillations

Temporal oscillations in cytosolic free Ca^{2+} were described when it became possible to study Ca^{2+} at the single cell level. A train of transients was first observed in the non-excitable hepatocytes (Woods *et al.* 1986) and later in excitable cells (Schlegel *et al.* 1987). Two types of Ca^{2+} oscillations are generally described – baseline oscillations and sinusoidal oscillations. The latter are the only oscillations *strictu sensu* and are symmetrical fluctuations superimposed on an elevated Ca^{2+} level (Bird *et al.* 1993). These are ascribed to negative feedback from PKC, either on PLC, the G-protein or the receptor. Upon PLC activation DAG is generated and PKC is activated. PKC then feedback inhibits PLC. This leads to lower levels of DAG, which again reduces the activation of PKC, thereby halting the inhibition on PLC. These oscillations in DAG/PKC will parallel oscillations in IP_3 and this produces the regular sinusoidal Ca^{2+} oscillations. The sinusoidal oscillations usually have a constant frequency and are independent of the concentration of agonist. They are generally brought about at rather high concentrations of agonist.

Baseline oscillations are repetitive spikes with an interspike interval at baseline Ca^{2+} levels. The rising phase of the oscillations is rapid and usually highly similar, whereas the declining phase is slower and often differs among oscillations induced by different agonists. Usually the amplitude of the spike is constant, whereas the latency before the first spike and the frequency of the oscillations are dependent on the concentration of agonist. Single cells stimulated by the same level of agonist usually oscillate with distinct frequencies, and this has been called the cell's Ca^{2+} fingerprint (Thomas *et al.* 1991). This demonstrates the heterogeneous sensitivity of the cells. Several mechanisms and models explaining these baseline oscillations have been suggested (Rink & Merritt 1990, Tsien & Tsien 1990, Berridge & Dupont 1994, Thomas *et al.* 1996). There are both membrane and cytosolic oscillators. The former are Ca^{2+} oscillations caused by periodic Ca^{2+} influx across the cell membrane. These are usually dependent upon alterations in the membrane potential, which induces opening of voltage-operated Ca^{2+} channels. The cytosolic oscillators are more complex.

The models explaining baseline Ca^{2+} oscillations can be divided into two classes dependent upon whether the level of IP_3 oscillates in parallel with Ca^{2+} or not. Cobbold *et al.* (1991), who were the first to describe Ca^{2+} oscillations, suggested that PKC acted by negative feedback on PLC, and expanded this model later by including positive feedback from Ca^{2+} on PLC. Another model relied on this latter positive feedback, co-operativity of IP_3 and Ca^{2+} and desensitisation or emptying of the IP_3 sensitive stores (Meyer & Stryer 1988). One model based on a constant level of IP_3 suggests that negative feedback by Ca^{2+} or IP_3 on the IP_3R is enough by itself (Champeil *et al.* 1989). Berridge (1991) originally proposed a two-pool model where CICR from an IP_3 insensitive pool was the main positive feedback cycle. When the IP_3R was shown to be sensitive to Ca^{2+} and the RyR was proven not to be important in several cell types, this model was modified to a one-pool model that relied on CICR from the IP_3 sensitive store (Berridge 1992b).

Today most experimental data support a model where the IP_3 level is rather constant and Ca^{2+} feedback activates its own release and later blocks it via interaction with IP_3 on the IP_3R. Nevertheless, more complex modulating mechanisms are probably also involved, including positive feedback on PLCδ by Ca^{2+}. Recently, IP_3 oscillations in parallel with Ca^{2+} oscillations have been demonstrated in single cells for the first time (Hirose *et al.* 1999). This study suggested a bimodal effect of Ca^{2+} on the generation of IP_3 with an enhancing effect at intermediate concentrations and an inhibitory effect at high concentrations, thus resembling the effects of Ca^{2+} on the IP_3R. If this will be confirmed by other investigators, it seems likely that the bimodal effect of Ca^{2+} on the IP_3R and PLC, are both responsible for controlling the Ca^{2+} and IP_3 oscillations.

Several quantitative mathematical models have also been developed to elucidate the mechanisms for both Ca^{2+} oscillations and waves (Dupont & Goldbeter 1992, Sneyd *et al.* 1995a). Models that simulate both membrane oscillators (Colding-Jorgensen *et al.* 1992) and one-pool (Somogyi & Stucki 1991, Li & Rinzel 1994, Li *et al.* 1995) and two-pool oscillators (Goldbeter *et al.* 1990, Dupont *et al.* 1991) have been used to mimic experimental observations. Two-dimensional models describing intracellular Ca^{2+} waves have also been constructed (Girard *et al.* 1992, Dupont & Goldbeter 1994).

INTERCELLULAR CA^{2+} SIGNALLING

Intercellular Ca^{2+} waves

In addition to being an intracellular signal, Ca^{2+} is also able to act as an intercellular messenger. Ca^{2+} can spread out in a population of cells as an intercellular Ca^{2+} wave, and a group of cells can co-ordinate their oscillations and thereby generate a synchronous oscillatory Ca^{2+} signal (Sanderson *et al.* 1994). In ciliated airway epithelial cells, the beat frequency is increased

upon mechanical stimulation and this cilial response spreads like a wave to adjacent cells (Sanderson *et al.* 1988). By Ca^{2+} imaging this was shown to be controlled by an intercellular Ca^{2+} wave (Sanderson *et al.* 1990). The Ca^{2+} wave spreads with the same velocity as intracellular waves, i.e. around 25 μm s^{-1}, and is delayed 0.5–1 s when crossing over to the next cell. When extracellular Ca^{2+} is removed, the wave still propagates, but no Ca^{2+} increase is seen in the stimulated cell. After this observation a mechanical stimulus has been shown to induce Ca^{2+} waves in several cell types like endothelial cells (Domenighetti *et al.* 1998), different glial cells (Giaume & Venance 1998), mammary (Enomoto *et al.* 1992) and liver epithelial cells (Frame & de Feijter 1997), osteoblasts (Jorgensen *et al.* 1997), mast cells (Osipchuk & Cahalan 1992) and pancreatic β-cells (Cao *et al.* 1997).

In addition to mechanical stress, other stimuli have also been shown to induce intercellular Ca^{2+} signals. Glutamate induces Ca^{2+} waves in astrocytes (Cornell-Bell *et al.* 1990) and focal electrical stimulation can evoke waves in astrocytes (Hassinger *et al.* 1996) and from astrocytes to neurons (Nedergaard 1994). A special example is how angiotensin II binds to receptors on follicular cells and induces a Ca^{2+} wave through gap junction to the oocyte (Sandberg *et al.* 1990).

These intercellular Ca^{2+} signals could be brought about either by paracrine effects of a secreted diffusible messenger or by diffusion of an intracellular messenger through gap junctions. In general most of the mechanically-induced waves are dependent on gap junctions, but in mast cells that lack coupling, ATP is released and induces the Ca^{2+} responses in adjacent cells (Osipchuk & Cahalan 1992).

Sanderson (1995), who was the first to describe the mechanically-induced Ca^{2+} waves, has developed the following model. The mechanical stimulus activates PLC and induces Ca^{2+} influx. IP_3 diffuses to the adjacent cell through gap junctions and releases Ca^{2+} from internal stores. This release is reinforced by CICR and an intracellular Ca^{2+} wave spreads through the cell. IP_3 then diffuses to cells further away and sets up new intracellular waves. The resulting intercellular Ca^{2+} wave is self-limited and the model proposes that IP_3 is only produced in the first cells and that the wave is damped because of limited diffusion. Mathematical models have been developed to explain both waves induced by passive diffusion of IP_3 (Sneyd *et al.* 1995b, 1998) and waves generated by a regenerative process (Wilkins & Sneyd 1998).

Synchronised Ca^{2+} oscillations

Ca^{2+} oscillations observed in single cells are usually asynchronous with variable frequency, and this will appear as a constantly elevated Ca^{2+} level when responses from many cells are summarised. Therefore, when Ca^{2+} signals are recorded from a population of cells either in suspension or grown on coverslips generally only a biphasic response has been observed. However, in 1988 synchronous sinusoidal Ca^{2+} oscillations in endothelial monolayers (Sage *et al.* 1989) and pancreatic acini (Pralong *et al.* 1988) were reported. As then, synchronisation has been reported in several cell types (Berridge & Dupont 1994). In excitable cells like neuronal cells (Gray & McCormick 1996), cardiac muscle (Kimura *et al.* 1995) and smooth muscle cells (Weissberg *et al.* 1989) and spontaneously synchronised Ca^{2+} oscillations have been observed. These are mediated by electrical coupling through gap junctions. Ca^{2+} oscillations in excitable endocrine cells like pancreatic β-cells (Gylfe *et al.* 1991, Hellman *et al.* 1997) and anterior pituitary cells (Guerineau *et al.* 1998) are membrane oscillators, synchronised by gap junctions. In non-excitable cells synchrony is generally brought about by other mechanisms (see below). However, recently spontaneous synchronised Ca^{2+} spiking caused by Ca^{2+} action potentials in fibroblasts – hitherto classified as non-excitable – has been reported (DeRoos *et al.* 1997a,b).

Synchronisation in endothelial cells and pancreatic acinar cells

Synchronized Ca^{2+} oscillations were demonstrated in monolayers of endothelial cells where the Ca^{2+} response induced by bradykinin was recorded from around 10 000 cells (Sage *et al.* 1989). Synchrony was only observed in cells seeded at low density and grown to confluence and not in cells seeded at near confluence. Seeding at low density favours the development of gap junctions. Preconfluent cells did not oscillate, further establishing the need for intercellular communication. These sinusoidal oscillations were dependent on Ca^{2+} influx. Depolarisation by high K^+ abolished the oscillations. By measuring Ca^{2+} either directly or indirectly by the short-circuit current muscarinic agonists have been shown to induce similar sinusoidal Ca^{2+} oscillations in monolayers of sweat gland/duct cells (Pedersen 1991, Pickles *et al.* 1991).

van Breemen *et al.* have studied the mechanisms of synchronised endothelial monolayers further and demonstrated that hyperpolarization by removing extracellular K^+ favoured oscillations (Laskey *et al.* 1990). In a more detailed study they have shown that thapsigargin did not inhibit the oscillations, whereas the Ca^{2+} influx inhibitor SK & F 96365 abolished them (Laskey *et al.* 1992). Simultaneous measurement of the membrane potential suggested that oscillatory changes in membrane conductance are responsible for the Ca^{2+} oscillations. Altogether, this points towards a membrane oscillator and synchronisation by electrical

coupling. It can also be calculated that an intercellular Ca^{2+} wave can not be responsible for the process of synchronisation as its velocity is too slow to cover all the cells in the monolayer in due time. In another endothelial cell type histamine evoked synchronised baseline transients dependent on extracellular Ca^{2+}, but similar signals were also induced by caffeine (Neylon & Irvine 1990). This suggest that release from the stores with RyR and capacitative entry is responsible for these oscillations. The mechanism of synchronisation of these oscillations is not understood.

Synchronised Ca^{2+} oscillations have also been seen when recording from a whole pancreatic acinus (around 10 cells) (Pralong *et al.* 1988). These oscillations are inhibited by chelating extracellular Ca^{2+} by EGTA, but are not fully abolished. The relatively slow rising phase of these transients and the lower number of cells suggests that, contrary to the synchronised responses in the endothelial and epithelial cells above, the oscillations in pancreatic acini may be co-ordinated by chemical intercellular communication. By electrophysiological recordings it has been shown that a Ca^{2+} dependent current is increased when cells are coupled in pairs or triplets (Petersen & Petersen 1991). Pairs of pancreatic acinar cells stimulated by cholecystokinin oscillate in synchrony and electrical coupling has been ruled out as a synchroniser by clamping the membrane potential of the two cells to a common value (Ngezahayo & Kolb 1993).

In all these experiments, a global application of agonist has been used indicating that some cells have higher sensitivity for the agonist than the other cells and that these cells are leading the others, i.e. pacemakers. However, a global application of bombesin did not induce synchronous oscillations in all the cells of an acinus, whereas a focal stimulation of one cell did (Stauffer *et al.* 1993). Global stimulation with cholecystokinin also gave rise to asynchronous oscillations, but when gap junctional permeability was increased by NO_2^- the Ca^{2+} oscillations became synchronised. This indicates that global application of an agonist that allows for intercellular communication may set up a synchronous Ca^{2+} response, as we have observed (Røttingen *et al.* 1995, Camerer *et al.* 1996, Røttingen *et al.* 1997). We demonstrated that the coagulation factors VIIa (FVIIa) and Xa (FXa), thrombin and bradykinin were able to induce synchronized Ca^{2+} oscillations in MDCK cells. In Fig. 1, we show how bradykinin sets up synchronous oscillations probably by an intercellular Ca^{2+} wave and that this synchronization can be inhibited by the gap junctional blocker octanol. Different agonists may differ in their modulating roles on gap junctions. Cholecystokinin seems to inhibit gap junctional transport at least after a while (Ngezahayo & Kolb 1993, Stauffer *et al.* 1993, Yule *et al.* 1996). This is also documented for ATP (Ritter & Lang 1991, Røttingen *et al.* 1997). High intracellular concentrations of Ca^{2+} have been shown to block gap junctions, and Ca^{2+} can therefore mediate the inhibiting effects of these agonists. Prolonged Ca^{2+} influx through the capacitative route has been implicated (Chanson *et al.* 1999). However, brief Ca^{2+} transients like waves can pass through gap junctions demonstrating that Ca^{2+} per se is probably not enough to evoke this closure of gap junctions.

Synchronisation in hepatocytes and the liver

The best studied cells or organ regarding synchronous Ca^{2+} oscillations are the hepatocytes and the liver. Hepatocytes are coupled by gap junctions that allow the transport of both Ca^{2+} and IP_3 (Saez *et al.* 1989), and couplets of hepatocytes stimulated by Ca^{2+} mobilising agonists are synchronised through these gap junctions (Nathanson & Burgstahler 1992). Up to four coupled cells respond by oscillations that are synchronised by sequential activation of the cells. The sequence of activation is the same for each transient, indicating that the first responding cell has the highest sensitivity for agonist and control the others in the multiplet. When stimulating the cells with another agonist, the controlling cell was different, indicating heterogeneous agonist sensitivity.

Ca^{2+} signals have also been studied in the whole perfused liver (Robb-Gaspers & Thomas 1995). By infusion of agonists all hepatocytes in a lobule were shown to oscillate in synchrony. This synchronisation was produced by a Ca^{2+} wave that propagated from periportal cells to central cells during the start of each transient. The signal started in the same cell each time, suggesting a pacemaker role for this leading cell. The wave travelled as intracellular Ca^{2+} waves with constant velocity not dependent on the concentration of agonist, but delayed some seconds when propagating between cells. This delay was shortened when the agonist concentration was increased. When the concentration of agonist was elevated, more than one originating site was observed. The flow of infusion did not alter the direction of the wave, excluding a role for a paracrine messenger. Other investigators have observed a Ca^{2+} wave spreading from the pericentral to the periportal zone (Nathanson *et al.* 1995). This was in the opposite direction of the waves observed by the first group. The discrepancy could be explained by different potencies of the agonists utilised as an inverse direction of the wave was observed at high concentrations by the first group. However, receptor density measurements favour a gradient of sensitivity where the pericentral zone has most receptors (Tordjmann *et al.* 1998). The wave of the latter group did not halt at cell boundaries.

A more detailed study on the mechanisms of synchrony in hepatocytes utilised multiplets (Tordj-

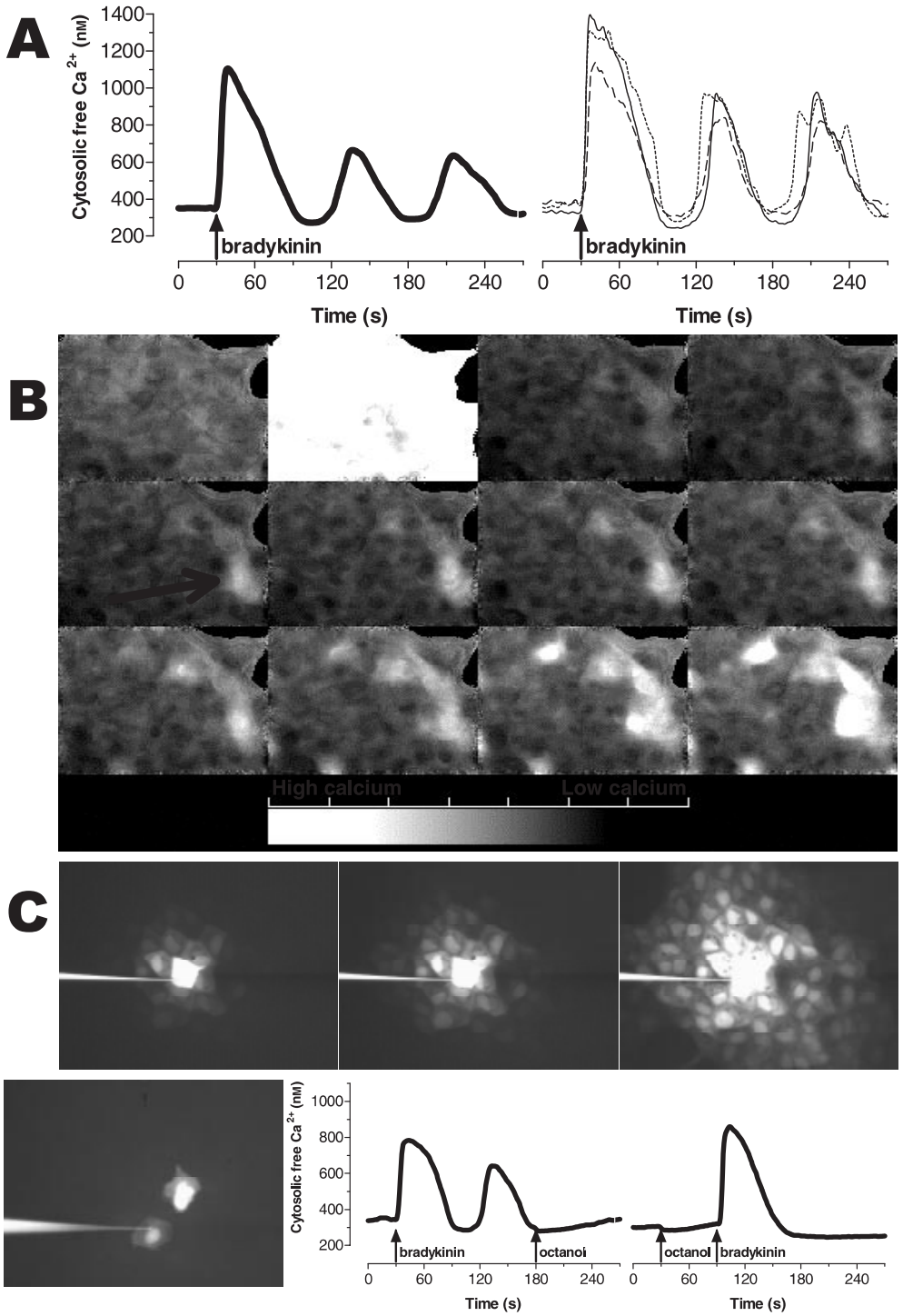

Figure 1 (a) Confluent MDCK cells treated with 10 nM bradykinin show synchronous Ca^{2+} oscillations. The left graph represents an average of 36 cells in the field of view, whereas in the right one traces from three different single cells are displayed. It is evident that all the cells are synchronised, but that some different temporal responses are seen when the single cells are studied. (b) Greyscale Ca^{2+} images of the cells in panel A. High Ca^{2+} levels are indicated by bright colours as shown by the bar. The first (upper left) image was taken 2 s before addition of bradykinin, the next at the first peak 8 s after adding bradykinin (high levels as all are white), the other images show the rising phase of the second transient (from 85 s and onwards with 1.2 s interval). The arrow is pointed towards the cell where Ca^{2+} increases first, and it can bee seen that a Ca^{2+} wave spreads to neighbouring cells from this 'pacemaker' cell. (c) *Top*: An MDCK cell is injected with Lucifer yellow and the dye is diffusing to neighbouring cells through gap junctions. The three images are taken 2, 3 and 5 min after injection. *Bottom*: Two different cells are injected with the dye when the cell layer is treated with the gap junctional blocker octanol at 1 mM. No spreading of dye can be seen. Octanol inhibits the synchronous oscillations both when given before and after bradykinin indicating that the synchrony is dependent on gap junctional traffic.

mann *et al.* 1997). When the intermediate cell of a triplet was excised or gap junctions were blocked, cell 1 and 3 oscillated with different frequencies with cell 3 as the slowest, whereas in normal conditions both cells oscillated with the same frequency as cell 1. This heterogeneous sensitivity has been shown to lie at the receptor level (Tordjmann *et al.* 1998). All the cells had to be stimulated by agonist to evoke synchronised oscillations, as focal stimulation of one cell did not induce a Ca^{2+} response in its neighbour. This is somewhat contradictory to earlier findings, where both injected IP_3 and agonists that elevate IP_3 are able to induce a Ca^{2+} signal in adjacent cells. It is probably a question of the concentration of IP_3. When injecting the middle cell in a triplet with heparin (a blocker of IP_3R), the Ca^{2+} signal was abolished in this cell and the oscillations in the two other cells became asynchronous. Mechanical stimulation of non-connected hepatocytes induces an intercellular Ca^{2+} wave by the release of ATP (Schlosser *et al.* 1996), but the superfusion used in the experiments by Tordjmann *et al.* abolished such a paracrine effect, ruling out ATP as a messenger for synchronised oscillations. The intercellular communication and synchrony in the liver is important, for instance the glycogenolytic response induced by hormones (Eugenin *et al.* 1998).

Synchronisation in situ

The advantage of doing experiments *in situ* is that the structure and architecture of cells is preserved, so that a physiological important variance in agonist sensitivity organised as a gradient is maintained. Others have also observed synchronised Ca^{2+} oscillations in 'organ' models. Synchronised spontaneous Ca^{2+} oscillations have been demonstrated *in situ* in the endothelium of venular lung capillaries (Ying *et al.* 1996). These oscillations were not dependent on extracellular Ca^{2+}. They were abolished by thapsigargin and were controlled by an intercellular Ca^{2+} wave from pacemaker endothelial cells. In salivary glands of blowfly, agonist-induced Ca^{2+} oscillations, synchronised by regenerative intercellular Ca^{2+} waves have been observed (Zimmermann & Walz 1997). These oscillations were not dependent on Ca^{2+} influx and relied on release and refilling of internal stores. No delays between cells were observed. Blocking gap junctions by octanol resulted in asynchronous oscillations. At intermediate agonist concentrations the waves of the first temporal Ca^{2+} transient were asynchronously initiated at several foci, but after some minutes the waves of later transients were initiated at one and the same site, i.e. the gland became more synchronised after a while. This is in agreement with our observation of the FVIIa and FXa-induced oscillations in MDCK cells that in general first synchronise after some period of time (Camerer *et al.* 1996). At low agonist concentrations, several asynchronous waves were either not able to propagate or just radiated at a short distance. Local application of the agonist did not induce a Ca^{2+} wave, but when pretreating the gland with a subthreshold agonist concentration, the local stimuli induced a wave and a synchronous signal. This parallels the results on the dependence of agonist in hepatocytes (see above).

Our observations on synchronised oscillations induced by a global stimulus in MDCK cells (Røttingen *et al.* 1995, 1997, Camerer *et al.* 1996) are carried out in a culture of cells supposedly with a uniform agonist sensitivity. However, stochastic differences in sensitivity will also necessarily exist in these cultures. The variance in sensitivity could be lower than in an intact organ, thereby making the observation of synchronous responses more difficult in cell cultures. In cultured chondrocytes similar synchronous oscillations have been observed (D'Andrea & Vittur 1996). ATP was able to set up synchronous Ca^{2+} spiking controlled by an intercellular Ca^{2+} wave. No pacemaker cell was observed as the wave initiated from different cells in each transient. 18α-glycyrrhetinic acid blocked gap junctions and the oscillations became asynchronous. A mechanically-induced Ca^{2+} wave had the same velocity and was completely inhibited by the gap junctional blocker.

Propagation of intercellular Ca^{2+} waves

The mechanism whereby intercellular Ca^{2+} waves are propagated is probably relevant also for the synchronisation of agonist-induced Ca^{2+} oscillations. In general cell coupling by gap junctions is the main route whereby mechanical or other focal stimuli induce intercellular Ca^{2+} signals (Sanderson *et al.* 1994, Giaume & Venance 1998). The necessity for gap junctions has been demonstrated by the following observations. The delay of the wave at cell boundaries would not be anticipated if an extracellular route was relevant. Blockers of gap junctions inhibit the wave. Cells without gap junctions have been transfected with connexins and are then able to propagate a wave. Directed fluid flow above the cells in one direction does not alter the direction of the wave.

Extracellular soluble ligand responsible for propagation

However, several observations have now demonstrated that an extracellular route is also relevant. This was first described in mast cells where ATP is released from the mechanically stimulated cell (Osipchuk & Cahalan 1992). In mammary epithelial cells both an extracellular and an intracellular route are utilised for propagating

Ca^{2+} waves, as cells without contact also took part in the wave, and fluid flow partly altered the radial wave (Enomoto *et al.* 1992). Suramine, a purinergic receptor blocker, inhibited an intercellular Ca^{2+} wave in gap junctional deficient epithelial cells and partly inhibited the wave in cells with gap junction demonstrating the role for both pathways (Frame & de Feijter 1997). In astrocytes there may be a species difference as rat astrocytes use gap junctions while mouse astrocytes seem to rely on released ATP (Hassinger *et al.* 1996, Giaume & Venance 1998, Guthrie *et al.* 1999). ATP is probably released from each cell along the route of the wave as the probability of crossing a cell free lane was not correlated to the distance from the stimulated cell (Hassinger *et al.* 1996).

In osteoblastic cells two types of intercellular Ca^{2+} waves have been described (Jorgensen *et al.* 1997). A slow type is dependent on gap junctions and Ca^{2+} influx, and a fast type is dependent on release from internal stores and activation of P_{2U} receptors. The wave in tracheal epithelia was in their hands and not dependent on gap junctions. Insulinoma cells (derived from pancreatic β-cells) without gap junctions were able to transduce a mechanically-induced Ca^{2+} wave by released ATP acting on P_{2U} receptors, whereas the same cells transfected with connexin 43 and in addition were able to propagate a wave dependent on gap junctions and VOCs (Cao *et al.* 1997). Recently, synchronous glucose-induced Ca^{2+} oscillations in contactless pancreatic β-cells were also proposed to be caused by an extracellular diffusible factor (Grapengiesser *et al.* 1999). Nitric oxide was suggested as the extracellular messenger, but ATP may be involved.

Gap junctional diffusible ligand responsible for propagation

The main mechanism proposed for intercellular Ca^{2+} waves dependent on gap junctions is diffusion of IP_3 through these intercellular connections (Sanderson 1995). The first observation that led to this conclusion was that heparin was able to block intercellular Ca^{2+} waves from follicular cells to oocytes (Sandberg *et al.* 1992) and Ca^{2+} waves in airway epithelia (Boitano *et al.* 1992). Later the lack of the effect of dantrolene, a proposed inhibitor of CICR, and the blocking effect of the PLC inhibitor U73122 led to the conclusion that IP_3 was the main propagating messenger (Giaume & Venance 1998). By transfection a nice model system has been developed where cells expressing the RyR and connexin 43 are surrounded by cells only expressing the connexin (Toyofuku *et al.* 1998). Caffeine is then able to set up an intercellular Ca^{2+} wave. As the adjacent cells do not have RyRs, the wave has to be mediated by release from IP_3 sensitive stores. The initial Ca^{2+} increase mediated by caffeine probably activates PLC thereby producing IP_3. This is substantiated by the fact that U73122 abolished the caffeine-induced Ca^{2+} wave.

Recently, intercellular Ca^{2+} waves induced by focal agonist application or mechanical stimulation have been studied in endothelial cells (Domenighetti *et al.* 1998). These waves were dependent on both PLC-activity and CICR as evidenced by inhibiting effects of U73122 and dantrolene. Focal stimulation with ionomycin or caffeine did not induce a Ca^{2+} wave, indicating that an increase in cytosolic Ca^{2+} is not enough. By the use of caged compounds released by flash photolysis it has also been demonstrated that IP_3 and not Ca^{2+} is able to set up an intercellular Ca^{2+} wave in cocultures of astrocytes and endothelial cells (Leybaert *et al.* 1998). This has also been shown by injection of IP_3 and Ca^{2+} into pancreatic acinar cells where IP_3 induced a Ca^{2+} signal in adjacent cells, whereas Ca^{2+} was able to do this only when the cells had been stimulated by a subthreshold agonist concentration (Yule *et al.* 1996). The finding that Ca^{2+} is not able to propagate a wave on its own is contradictory to earlier reports demonstrating that injection of Ca^{2+} can induce a response in a neighbouring cell (Saez *et al.* 1989, Christ *et al.* 1992). The cause for this discrepancy is probably the use of different concentrations.

Models for gap junctional co-ordinated Ca^{2+} signalling

Based on the discussion above, we propose the following models where both Ca^{2+} and IP_3 act as intercellular messengers and coactivators of the IP_3R. When cells are mechanically stimulated or activated by a focal application of an agonist, PLC is activated and IP_3 diffuses to neighbouring cells. This sensitises the cells for the Ca^{2+} wave that propagates more slowly by CICR. In the adjacent cells the increase in Ca^{2+} activates PLC, thereby producing more IP_3, which can diffuse to cells further away. In this way, there exists two regenerative waves. The IP_3 wave is the faster one and the Ca^{2+} wave the slower but they are interdependent for their regenerative properties. Recently, a wave of IP_3 spreading from the stimulated cell in parallel with the Ca^{2+} wave has been described (Hirose *et al.* 1999).

When cells are stimulated globally by an agonist, PLC is activated in all cells and IP_3 is produced. However, the single cell level of IP_3 is dependent on the cell's sensitivity for the agonist. A Ca^{2+} wave is generated in a pacemaker cell with the highest sensitivity. This wave then spreads to adjacent cells where IP_3Rs are already sensitised by IP_3. The Ca^{2+} wave can in addition activate PLC and thereby increase the IP_3 level. In this way the intercellular Ca^{2+} wave originating from a pacemaker cell can co-ordinate a synchronised oscillatory Ca^{2+} signal. At low concentrations of

agonists the wave in the pacemaker cell will not be strong enough to set up a global wave and several local waves initiated in other cells will be observed, i.e. asynchronous temporal oscillations. At high agonist concentrations, IP_3 will be increased above threshold level simultaneously in most cells and a pacemaker will not be able to control the phase of the others, i.e. asynchronous oscillations or a sustained Ca^{2+} response. This model may be viewed as analogous to the model of fundamental and global Ca^{2+} signals in single cells, although at a higher level of organisation. Recently, a mathematical model has been developed describing synchronization of heterogenous hepatocytes by gap junctional Ca^{2+} fluxes (Hofer 1999). This quantitative model is similar to our simple conceptual model.

CONCLUDING REMARKS

The two general mechanisms for co-ordination of the behaviour of cells are hormonal and neuronal signals mediated by extracellular signalling substances and electrical coupling of the cells by gap junctions. The first mechanism produces intracellular signals in the target cells, but owing to cellular heteorgeneity this usually results in asynchronous responses. We have shown how an intracellular Ca^{2+} signal can spread to adjacent cells thereby constituting an intercellular signal that can synchronise an otherwise unco-ordinated response. In effect, this is a combination of the two hitherto known mechanisms, i.e. multiple extracellular signals is converted to a single co-ordinated intracellular signal via gap junctions. So far calcium is the best studied junctional signalling molecule, but a recent paper suggests that also IP_3 is a functional intercellular signal (Hirose *et al.* 1999) and colleagues will probably be described. The field of cellular signal transduction has thus moved an interesting step forward. Some would, however, say backward as we are going up to a higher organizational level, i.e. from cells to tissues and organs, making it a step back on the down-bound reductional ladder.

The authors are supported by grants from the Research Council of Norway, the Norwegian Cancer Society, the Jahre Foundation, and Rakel and Otto Bruun's Legacy.

REFERENCES

Allbritton, N.L., Meyer, T. & Stryer, L. 1992. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* **258,** 1812–1815.

Amundson, J. & Clapham, D. 1993. Calcium waves. *Curr Opin Neurobiol* **3,** 375–382.

Barritt, G.J. 1999. Receptor-activated Ca^{2+} inflow in animal cells: A variety of pathways tailored to meet different intracellular Ca^{2+} signalling requirements. *Biochem J* **337,** 153–169.

Berridge, M.J. 1991. Cytoplasmic calcium oscillations: A two pool model. *Cell Calcium* **12,** 63–72.

Berridge, M.J. 1992a. Discovery of the $InsP_3$-Ca^{2+} pathway. A personal reflection. *Adv Sec Mes Phosphoprot Res* **26,** 1–7.

Berridge, M.J. 1992b. Inositol trisphosphate and calcium oscillations. *Adv Sec Mes Phosphoprot Res* **26,** 211–223.

Berridge, M.J. 1993a. Inositol trisphosphate and calcium signalling. *Nature* **361,** 315–325.

Berridge, M.J. 1993b. A tale of two messengers. *Nature* **365,** 388–389.

Berridge, M.J. 1994. The biology and medicine of calcium signalling. *Mol Cell Endocrinol* **98,** 119–124.

Berridge, M.J. 1995. Calcium signalling and cell proliferation. *Bioessays* **17,** 491–500.

Berridge, M.J. 1997a. The AM and FM of calcium signalling. *Nature* **386,** 759–760.

Berridge, M.J. 1997b. Elementary and global aspects of calcium signalling. *J Exp Biol* **200,** 315–319.

Berridge, M.J. 1997c. Elementary and global aspects of calcium signalling. *J Physiol (Lond)* **499,** 291–306.

Berridge, M.J. 1998. Neuronal calcium signaling. *Neuron* **21,** 13–26.

Berridge, M.J., Bootman, M.D. & Lipp, P. 1998. Calcium – a life and death signal. *Nature* **395,** 645–648.

Berridge, M.J. & Dupont, G. 1994. Spatial and temporal signalling by calcium. *Curr Opin Cell Biol* **6,** 267–274.

Berridge, M.J. & Irvine, R.F. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312,** 315–321.

Berridge, M.J. & Irvine, R.F. 1989. Inositol phosphates and cell signalling. *Nature* **341,** 197–205.

Berridge, M.J., Lipp, P. & Bootman, M. 1999. Calcium signalling. *Curr Biol* **9,** R157–R159.

Bird, G.S., Rossier, M.F., Obie, J.F. & Putney, J.W. Jr 1993. Sinusoidal oscillations in intracellular calcium requiring negative feedback by protein kinase C. *J Biol Chem* **268,** 8425–8428.

Boitano, S., Dirksen, E.R. & Sanderson, M.J. 1992. Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science* **258,** 292–295.

Bootman, M. 1994. Questions about quantal Ca^{2+} release. *Curr Biol* **4,** 169–172.

Bootman, M.D. & Berridge, M.J. 1995. The elemental principles of calcium signaling. *Cell* **83,** 675–678.

Bootman, M.D., Berridge, M.J. & Lipp, P. 1997. Cooking with calcium: The recipes for composing global signals from elementary events. *Cell* **91,** 367–373.

Camerer, E., Røttingen, J.-A., Iversen, J.-G. & Prydz, H. 1996. Coagulation factors VII and X induce Ca^{2+} oscillations in Madin-Darby canine kidney cells only when proteolytically active. *J Biol Chem* **271,** 29034–29042.

Cancela, J.M., Churchill, G.C. & Galione, A. 1999. Coordination of agonist-induced Ca^{2+}-signalling patterns by NAADP in pancreatic acinar cells. *Nature* **398,** 74–76.

Cao, D., Lin, G., Westphale, E.M., Beyer, E.C. & Steinberg, T.H. 1997. Mechanisms for the coordination of intercellular calcium signaling in insulin-secreting cells. *J Cell Sci* **110,** 497–504.

Capiod, T., Mauger, J.-P., Binet, A. & Claret, M. 1989. Regulation of calcium in non-exitable cells. *Curr Opin Cell Biol* **1,** 211–214.

Carafoli, E. 1991. The calcium pumping ATPase of the plasma membrane. *Annu Rev Physiol* **53,** 531–547.

Carafoli, E. 1992. The Ca^{2+} pump of the plasma membrane. *J Biol Chem* **267,** 2115–2118.

Champeil, P., Combettes, L., Berthon, B., Doucet, E., Orlowski, S. & Claret, M. 1989. Fast kinetics of calcium release induced by *myo*-inositol trisphosphate in permeabilized rat hepatocytes. *J Biol Chem* **264,** 17665–17673.

Chanson, M., Mollard, P., Meda, P., Suter, S. & Jongsma, H.J. 1999. Modulation of pancreatic acinar cell to cell coupling during ACh-evoked changes in cytosolic Ca^{2+}. *J Biol Chem* **274,** 282–287.

Cheek, T.R. 1991. Calcium regulation and homeostasis. *Curr Opin Cell Biol* **3,** 199–205.

Christ, G.J., Moreno, A.P., Melman, A. & Spray, D.C. 1992. Gap junction-mediated intercellular diffusion of Ca^{2+} in cultured human corporal smooth muscle cells. *Am J Physiol* **263,** C373–C383.

Clapham, D.E. 1993. A mysterious new influx factor? *Nature* **364,** 763–764.

Clapham, D.E. 1995. Calcium signaling. *Cell* **80,** 259–268.

Cobbold, P.H., Sanchez-Bueno, A. & Dixon, C.J. 1991. The hepatocyte calcium oscillator. *Cell Calcium* **12,** 87–95.

Colding-Jorgensen, M., Ostergaard Madsen, H., Bodholdt, B. & Mosekilde, E. 1992. Minimal model for Ca^{2+}-dependent oscillations in excitable cells. *J Theor Biol* **156,** 309–326.

Coppolino, M.G., Woodside, M.J., Demaurex, N., Grinstein, S., St-Arnaud, R. & Dedhar, S. 1997. Calreticulin is essential for integrin-mediated calcium signalling and cell adhesion. *Nature* **386,** 843–847.

Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S. & Smith, S.J. 1990. Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* **247,** 470–473.

Csordas, G., Thomas, A.P. & Hajnoczky, G. 1999. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J* **18,** 96–108.

D'Andrea, P. & Vittur, F. 1996. Gap junctions mediate intercellular calcium signalling in cultured articular chondrocytes. *Cell Calcium* **20,** 389–397.

DeRoos, A.D.G., Willems, P.H., Peters, P.H., van, Z.E. & Theuvenet, A.P. 1997a. Synchronized calcium spiking resulting from spontaneous calcium action potentials in monolayers of NRK fibroblasts. *Cell Calcium* **22,** 195–207.

DeRoos, A.D.G., Willems, P.H., van Zoelen, E.J. & Theuvenet, A.P. 1997b. Synchronized Ca^{2+} signaling by intercellular propagation of Ca^{2+} action potentials in NRK fibroblasts. *Am J Physiol* **273,** C1900–C1907.

Dolmetsch, R.E., Lewis, R.S., Goodnow, C.C. & Healy, J.I. 1997. Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* **386,** 855–858.

Domenighetti, A.A., Beny, J.L., Chabaud, F. & Frieden, M. 1998. An intercellular regenerative calcium wave in porcine coronary artery endothelial cells in primary culture. *J Physiol (Lond)* **513,** 103–116.

Dupont, G., Berridge, M.J. & Goldbeter, A. 1991. Signal-induced Ca^{2+} oscillations: Properties of a model based on Ca^{2+}-induced Ca^{2+} release. *Cell Calcium* **12,** 73–85.

Dupont, G. & Goldbeter, A. 1992. Oscillations and waves of cytosolic calcium: Insights from theoretical models. *Bio Essays* **14,** 485–493.

Dupont, G. & Goldbeter, A. 1994. Properties of intracellular Ca^{2+} waves generated by a model based on Ca^{2+} induced Ca^{2+} release. *Biophys J* **67,** 2191–2204.

Enomoto, K., Furuya, K., Yamagishi, S. & Maeno, T. 1992. Mechanically induced electrical and intracellular calcium responses in normal and cancerous mammary cells. *Cell Calcium* **13,** 501–511.

Eugenin, E.A., Gonzalez, H., Saez, C.G. & Saez, J.C. 1998. Gap junctional communication coordinates vasopressin-induced glycogenolysis in rat hepatocytes. *Am J Physiol* **274,** G1109–G1116.

Ferris, C.D. & Snyder, S.H. 1992. Inositol 1,4,5-trisphosphate-activated calcium channels. *Annu Rev Physiol* **54,** 469–488.

Frame, M.K. & de Feijter, A.W. 1997. Propagation of mechanically induced intercellular calcium waves via gap junctions and ATP receptors in rat liver epithelial cells. *Exp Cell Res* **230,** 197–207.

Genka, C., Ishida, H., Ichimori, K., Hirota, Y., Tanaami, T. & Nakazawa, H. 1999. Visualization of biphasic Ca^{2+} diffusion from cytosol to nucleus in contracting adult rat cardiac myocytes with an ultra-fast confocal imaging system. *Cell Calcium* **25,** 199–208.

Giaume, C. & Venance, L. 1998. Intercellular calcium signaling and gap junctional communication in astrocytes. *Glia* **24,** 50–64.

Girard, S., Lückhoff, A., Lechleiter, J., Sneyd, J. & Clapham, D. 1992. Two-dimensional model of calcium waves reproduces the patterns observed in *Xenopus* oocytes. *Biophys J* **61,** 509–517.

Goldbeter, A., Dupont, G. & Berridge, M.J. 1990. Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation. *Proc Natl Acad Sci* **87,** 1461–1465.

Grapengiesser, E., Gylfe, E. & Hellman, B. 1999. Synchronization of glucose-induced Ca^{2+} transients in pancreatic beta-cells by a diffusible factor. *Biochem Biophys Res Commun* **254,** 436–439.

Gray, C.M. & McCormick, D.A. 1996. Chattering cells: Superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex [see comments]. *Science* **274,** 109–113.

Guerineau, N.C., Bonnefont, X., Stoeckel, L. & Mollard, P. 1998. Synchronized spontaneous Ca^{2+} transients in acute anterior pituitary slices. *J Biol Chem* **273,** 10389–10395.

Guse, A.H. 1999. Cyclic ADP-ribose: A novel Ca^{2+}-mobilising second messenger. *Cell Signal* **11,** 309–316.

Guthrie, P.B., Knappenberger, J., Segal, M., Bennett, M.L., Charles, A.C. & Kater, S.B. 1999. ATP released from astrocytes mediates glial calcium waves. *J Neurosci* **19,** 520–528.

Gylfe, E., Grapengiesser, E. & Hellman, B. 1991. Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic beta-cells exposed to glucose. *Cell Calcium* **12,** 229–240.

Hajnoczky, G., Hager, R. & Thomas, A.P. 1999. Mitochondria suppress local feedback activation of inositol 1,4,5-trisphosphate receptors by Ca^{2+}. *J Biol Chem* **274,** 14157–14162.

Hassinger, T.D., Guthrie, P.B., Atkinson, P.B., Bennett, M.V. & Kater, S.B. 1996. An extracellular signaling component in propagation of astrocytic calcium waves. *Proc Natl Acad Sci USA* **93,** 13268–13273.

Hellman, B., Gylfe, E., Bergsten, P. *et al.* 1997. Oscillatory signaling and insulin release in human pancreatic beta-cells exposed to strontium. *Endocrinology* **138,** 3161–3165.

Hirose, K., Kadowaki, S., Tanabe, M., Takeshima, H. & Iino, M. 1999. Spatiotemporal dynamics of inositol 1,4,5-trisphosphate that underlies complex Ca^{2+} mobilization patterns. *Science* **284,** 1527–1531.

Hofer, T. 1999. Model of intercellular calcium oscillations in hepatocytes: Synchronization of heterogeneous cells. *Biophys J* **77,** 1244–1256.

Ichas, F., Jouaville, L.S. & Mazat, J.P. 1997. Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell* **89,** 1145–1153.

Irvine, R.F. 1990. 'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates – a possible mechanism. *FEBS* **263,** 5–9.

Irvine, R.F. 1992. Inositol phosphates and Ca^{2+} entry: Toward a proliferation or a simplification? *FASEB J* **6,** 3085–3091.

Jorgensen, N.R., Geist, S.T., Civitelli, R. & Steinberg, T.H. 1997. ATP- and gap junction-dependent intercellular calcium signaling in osteoblastic cells. *J Cell Biol* **139,** 497–506.

Jouaville, L.S., Ichas, F. & Mazat, J.P. 1998. Modulation of cell calcium signals by mitochondria. *Mol Cell Biochem* **184,** 371–376.

Katan, M. 1998. Families of phosphoinositide-specific phospholipase C: Structure and function. *Biochim Biophys Acta* **1436,** 5–17.

Kimura, H., Oyamada, Y., Ohshika, H., Mori, M. & Oyamada, M. 1995. Reversible inhibition of gap junctional intercellular communication, synchronous contraction, and synchronism of intracellular Ca^{2+} fluctuation in cultured neonatal rat cardiac myocytes by heptanol. *Exp Cell Res* **220,** 348–356.

Laskey, R.E., Adams, D.J., Cannell, M. & Breemen, C. 1992. Calcium entry-dependent oscillations of cytoplasmic calcium concentration in cultured endothelial cell monolayers. *Procedings Natl Acad Sci* **89,** 1690–1694.

Laskey, R.E., Adams, D.J., Johns, A. & Rubanyi, G.M. & van Breemen, C. 1990. Membrane potential and $Na(+)$-K^+ pump activity modulate resting and bradykinin-stimulated changes in cytosolic free calcium in cultured endothelial cells from bovine atria. *J Biol Chem* **265,** 2613–2619.

Lee, H.C. 1996. Modulator and messenger functions of cyclic ADP-ribose in calcium signaling. *Recent Prog Horm Res* **51,** 355–88.

Leybaert, L., Paemeleire, K., Strahonja, A. & Sanderson, M.J. 1998. Inositol-trisphosphate-dependent intercellular calcium signaling in and between astrocytes and endothelial cells. *Glia* **24,** 398–407.

Li, W., Llopis, J., Whitney, M., Zlokarnik, G. & Tsien, R.Y. 1998. Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* **392,** 936–941.

Li, Y.X., Keizer, J., Stojilkovic, S.S. & Rinzel, J. 1995. Ca^{2+} excitability of the ER membrane: An explanation for IP_3-induced Ca^{2+} oscillations. *Am J Physiol Cell Physiol* **269,** C1079–C1092.

Li, Y.X. & Rinzel, J. 1994. Equations for $InsP_3$ receptor-mediated $[Ca^{2+}]_i$ oscillations derived from a detailed kinetic model: A Hodgkin–Huxley like formalism. *J Theor Biol* **166,** 461–473.

Lytton, J. & Nigam, S.K. 1992. Intracellular calcium: Molecules and pools. *Curr Opin Cell Biol* **4,** 220–226.

MacKrill, J.J. 1999. Protein–protein interactions in intracellular Ca^{2+}-release channel function. *Biochem J* **337,** 345–361.

MacLennan, D.H., Rice, W.J. & Green, N.M. 1997. The mechanism of Ca^{2+} transport by sarco (endo) plasmic reticulum Ca^{2+}-ATPases. *J Biol Chem* **272,** 28815–28818.

Malviya, A.N. & Rogue, P.J. 1998. 'Tell me where is calcium bred': Clarifying the roles of nuclear calcium. *Cell* **92,** 17–23.

Mao, C., Kim, S.H., Almenoff, J.S., Rudner, X.L., Kearney, D.M. & Kindman, L.A. 1996. Molecular cloning and characterization of SCaMPER, a sphingolipid Ca^{2+} release-mediating protein from endoplasmic reticulum. *Proc Natl Acad Sci USA* **93,** 1993–1996.

McConkey, D.J. & Orrenius, S. 1997. The role of calcium in the regulation of apoptosis. *Biochem Biophys Res Commun* **239,** 357–366.

Meldolesi, J. & Pozzan, T. 1998b. The endoplasmic reticulum Ca^{2+} store: A view from the lumen. *Trends Biochem Sci* **23,** 10–14.

Meldolesi, J. & Pozzan, T. 1998a. The heterogeneity of ER Ca^{2+} stores has a key role in nonmuscle cell signaling and function. *J Cell Biol* **142,** 1395–1398.

Meyer zu Heringdorf, D., Lass, H., Alemany, R. *et al.* 1998. Sphingosine kinase-mediated Ca^{2+} signalling by G-protein-coupled receptors. *EMBO J* **17,** 2830–2837.

Meyer, T. & Stryer, L. 1988. Molecular model for receptor-stimulated calcium spiking. *Proc Natl Acad Sci USA* **85,** 5051–5055.

Mooren, F.C. & Kinne, R.K. 1998. Cellular calcium in health and disease. *Biochim Biophys Acta* **1406,** 127–151.

Nathanson, M.H. & Burgstahler, A.D. 1992. Coordination of hormone-induced calcium signals in isolated rat hepatocyte couplets: Demonstration with confocal microscopy. *Cell Regul* **3,** 113–121.

Nathanson, M.H., Burgstahler, A.D., Mennone, A., Fallon, M.B., Gonzalez, C.B. & Saez, J.C. 1995. Ca^{2+} waves are organized among hepatocytes in the intact organ. *Am J Physiol* **269,** G167–G171.

Nedergaard, M. 1994. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* **263,** 1768–1771.

Neylon, C.B. & Irvine, R.F. 1990. Synchronized repetitive spikes in cytoplasmic calcium in confluent monolayers of human umbilical vein endothelial cells. *FEBS Lett* **275,** 173–176.

Ngezahayo, A. & Kolb, H.-A. 1993. Gap junctional conductance tunes phase difference of cholecystokinin evoked calcium oscillations in pairs of pancreatic acinar cells. *Pflügers Arch* **422,** 413–415.

Niki, I., Yokokura, H., Sudo, T., Kato, M. & Hidaka, H. 1996. Ca^{2+} signaling and intracellular Ca^{2+} binding proteins. *J Biochem (Tokyo)* **120,** 685–698.

Osipchuk, Y. & Cahalan, M. 1992. Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. *Nature* **359,** 241–244.

Parekh, A.B. & Penner, R. 1997. Store depletion and calcium influx. *Physiol Rev* **77,** 901–930.

Parekh, A.B., Terlau, H. & Stümer, W. 1993. Depletion of $InsP_3$ stores activates a Ca^{2+} an K^+ current by means of a phosphatase and a diffusible messenger. *Nature* **364,** 814–818.

Patels, S., Joseph, S.K. & Thomas, A.P. 1999. Molecular properties of inositol 1,4,5-trisphosphate receptors. *Cell Calcium* **25,** 247–264.

Pedersen, P.S. 1991. Separate agonist-specific oscillatory mechanisms in cultured human sweat duct cells. *J Physiol (Lond)* **433,** 549–559.

Penner, R., Fasolato, C. & Hoth, M. 1993. Calcium influx and its control by calcium release. *Curr Opin Neurobiol* **3,** 368–374.

Petersen, C.C. & Petersen, O.H. 1991. Receptor-activated cytoplasmic Ca^{2+} spikes in communicating clusters of pancreatic acinar cells. *FEBS Lett* **284,** 113–116.

Pettit, E.J. & Fay, F.S. 1998. Cytosolic free calcium and the cytoskeleton in the control of leukocyte chemotaxis. *Physiol Rev* **78,** 949–967.

Pickles, R.J., Brayden, D.J. & Cuthbert, A.W. 1991. Synchronous transporting activity in epithelial cells in relation to intracellular calcium concentrations. *Proc R Soc Lond [Biol]* **245,** 53–58.

Pralong, W.F., Wollheim, C.B. & Bruzzone, R. 1988. Measurement of cytosolic free Ca^{2+} in individual pancreatic acini. *FEBS Lett* **242,** 79–84.

Putney, J.W. Jr 1990. Capacative calcium entry revisited. *Cell Calcium* **11,** 611–624.

Putney, J.W. Jr 1993. Excitement about calcium signaling in inexcitable cells. *Science* **262,** 676–678.

Putney, J.W. Jr 1999. "Kissin' cousins": Intimate plasma membrane–ER interactions underlie capacitative calcium entry. *Cell* **99,** 5–8.

Randriamapita, C. & Tsien, R.Y. 1993. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Nature* **364,** 809–814.

Rasmussen, H. 1970. Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* **170,** 404–412.

Rhee, S.G. & Bae, Y.S. 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. *J Biol Chem* **272,** 15045–15048.

Rink, T.J. & Merritt, J.E. 1990. Calcium signalling. *Curr Opin Cell Biol* **2,** 198–205.

Ritter, M. & Lang, F. 1991. Effect of bradykinin, ATP and adrenaline on cell membrane resistances of Madin–Darby canine kidney cells. *J Physiol (Lond)* **443,** 45–54.

Rizo, J. & Sudhof, T.C. 1998. C_2-domains, structure and function of a universal Ca^{2+}-binding domain. *J Biol Chem* **273,** 15879–15882.

Rizzuto, R., Brini, M., Murgia, M. & Pozzan, T. 1993. Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. *Science* **262,** 744–746.

Robb-Gaspers, L.D., Rutter, G.A., Burnett, P., Hajnoczky, G., Denton, R.M. & Thomas, A.P. 1998. Coupling between cytosolic and mitochondrial calcium oscillations: Role in the regulation of hepatic metabolism. *Biochim Biophys Acta* **1366,** 17–32.

Robb-Gaspers, L.D. & Thomas, A.P. 1995. Coordination of Ca^{2+} signaling by intercellular propagation of Ca^{2+} waves in the intact liver. *J Biol Chem* **270,** 8102–8107.

Røttingen, J.-A., Camerer, E., Mathiesen, I., Prydz, H. & Iversen, J.-G. 1997. Synchronized Ca^{2+} oscillations induced in Madin Darby canine kideny cells by bradykinin and thrombin but not by ATP. *Cell Calcium* **21,** 195–211.

Røttingen, J.-A., Enden, T., Camerer, E., Iversen, J.-G. & Prydz, H. 1995. Binding of human factor VIIa to Tissue factor induces cytosolic Ca^{2+} signals in J82 cells, transfected COS-1 cells, Madin-Darby Canine Kidney cells and in human endothelial cells induced to synthesize Tissue factor. *J Biol Chem* **270,** 4650–4660.

Saez, J.C., Connor, J.A., Spray, D.C. & Bennett, M.V. 1989. Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. *Proc Natl Acad Sci USA* **86,** 2708–2712.

Sage, S.O. 1992. Three routes for receptor-mediated Ca^{2+} entry. *Curr Biol* **2,** 312–314.

Sage, S.O., Adams, D.J. & Breemen, C. 1989. Synchronized oscillations in cytoplasmic free calcium concentration in confluent bradykinin.stimulated bovine pulmonary artery endothelial cell monolayers. *J Biol Chem* **264,** 6–9.

Sandberg, K., Bor, M., Ji, H., Markwick, A., Millan, M.A. & Catt, K.J. 1990. Angiotensin II-induced calcium mobilization in oocytes by signal transfer through gap junctions. *Science* **249,** 298–301.

Sandberg, K., Ji, H., Iida, T. & Catt, K.J. 1992. Intercellular communication between follicular angiotensin receptors and Xenopus laevis oocytes: Medication by an inositol 1,4,5-trisphosphate-dependent mechanism. *J Cell Biol* **117,** 157–167.

Sanderson, M.J. 1995. Intercellular calcium waves mediated by inositol trisphosphate. *Ciba Found Symposium* **188,** 175–189.

Sanderson, M.J., Charles, A.C., Boitano, S. & Dirksen, E.R. 1994. Mechanisms and function of intercellular calcium signaling. *Mol Cell Endocr* **98,** 173–187.

Sanderson, M.J., Charles, A.C. & Dirksen, E.R. 1990. Mechanical stimulation and intercellular communication

increases intracellular Ca^{2+} in epithelial cells. *Cell Regul* **1,** 585–596.

Sanderson, M.J., Chow, I. & Dirksen, E.R. 1988. Intercellular communication between ciliated cells in culture. *Am J Physiol* **254,** C63–C74.

Santella, L. & Carafoli, E. 1997. Calcium signaling in the cell nucleus. *FASEB J* **11,** 1091–1109.

Schlegel, W., Winiger, B.P., Mollard, P. *et al.* 1987. Oscillations of cytosolic Ca^{2+} in pituitary cells due to action potentials. *Nature* **329,** 719–721.

Schlosser, S.F., Burgstahler, A.D. & Nathanson, M.H. 1996. Isolated rat hepatocytes can signal to other hepatocytes and bile duct cells by release of nucleotides. *Proc Natl Acad Sci USA* **93,** 9948–9953.

Shuttleworth, T.J. 1999. What drives calcium entry during $[Ca^{2+}]_i$ oscillations? – challenging the capacitative model. *Cell Calcium* **25,** 237–246.

Sjaastad, M.D. & Nelson, W.J. 1997. Integrin-mediated calcium signaling and regulation of cell adhesion by intracellular calcium. *Bioessays* **19,** 47–55.

Sneyd, J., Keizer, J. & Sanderson, M.J. 1995a. Mechanisms of calcium oscillations and waves: A quantitative analysis. *FASEB J* **9,** 1463–1472.

Sneyd, J., Wetton, B.T.R., Charles, A.C. & Sanderson, M.J. 1995b. Intercellular calcium waves mediated by diffusion of inositol trisphosphate: A two-dimensional model. *Am J Physiol Cell Physiol* **268,** C1537–C1545.

Sneyd, J., Wilkins, M., Strahonja, A. & Sanderson, M.J. 1998. Calcium waves and oscillations driven by an intercellular gradient of inositol (1,4,5)-trisphosphate. *Biophys Chem* **72,** 101–109.

Somogyi, R. & Stucki, J.W. 1991. Hormone-induced calcium oscillations in liver cells can be explained by a simple one pool model. *J Biol Chem* **266,** 11068–11077.

Stauffer, P.L., Zhao, H., Luby-Phelps, K., Moss, R.L., Star, R.A. & Muallem, S. 1993. Gap junction communication modulates $[Ca^{2+}]_i$ oscillations and enzyme secretion in pancreatic acini. *J Biol Chem* **268,** 19769–19775.

Streb, H., Irvine, R.F., Berridge, M.J. & Schulz, I. 1983. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306,** 67–69.

Taylor, C.W. 1998. Inositol trisphosphate receptors: Ca^{2+}-modulated intracellular Ca^{2+} channels. *Biochim Biophys Acta* **1436,** 19–33.

Thomas, A.P., Bird, G.S., Hajnoczky, G., Robb-Gaspers, L.D. & Putney, J.W.J. 1996. Spatial and temporal aspects of cellular calcium signaling. *FASEB J* **10,** 1505–1517.

Thomas, A.P., Renard, D.C. & Rooney, T.A. 1991. Spatial and temporal organization of calcium signalling in hepatocytes. *Cell Calcium* **12,** 111–126.

Thorn, P. 1996. Spatial domains of Ca^{2+} signaling in secretory epithelial cells. *Cell Calcium* **20,** 203–214.

Topham, M.K. & Prescott, S.M. 1999. Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. *J Biol Chem* **274,** 11447–11450.

Tordjmann, T., Berthon, B., Claret, M. & Combettes, L. 1997. Coordinated intercellular calcium waves induced by noradrenaline in rat hepatocytes: Dual control by gap junction permeability and agonist. *EMBO J* **16,** 5398–5407.

Tordjmann, T., Berthon, B., Jacquemin, E. *et al.* 1998. Receptor-oriented intercellular calcium waves evoked by vasopressin in rat hepatocytes. *EMBO J* **17,** 4695–4703.

Toyofuku, T., Yabuki, M., Otsu, K., Kuzuya, T., Hori, M. & Tada, M. 1998. Intercellular calcium signaling via gap junction in connexin-43- transfected cells. *J Biol Chem* **273,** 1519–1528.

Trump, B.F. & Berezesky, I.K. 1992. The role of cytosolic Ca^{2+} in cell injury, necrosis and apoptosis. *Curr Opin Cell Biol* **4,** 227–232.

Tsien, R.W. & Tsien, R.Y. 1990. Calcium channels, stores, and oscillations. *Annu Rev Cell Biol* **6,** 715–760.

Weissberg, P.L., Little, P.J. & Bobik, A. 1989. Spontaneous oscillations in cytoplasmic calcium concentration in vascular smooth muscle. *Am J Physiol* **256,** C951–C957.

Wilkins, M. & Sneyd, J. 1998. Intercellular spiral waves of calcium. *J Theor Biol* **191,** 299–308.

Williams, R.J.P. 1992. Calcium and calmodulin. *Cell Calcium* **13,** 355–362.

Woods, N.M., Cuthbertson, K.S.R. & Cobbold, P.H. 1986. Repetetive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature* **319,** 600–602.

Ying, X., Minamiya, Y., Fu, C. & Bhattacharya, J. 1996. Ca^{2+} waves in lung capillary endothelium. *Circ Res* **79,** 898–908.

Yule, D.I., Stuenkel, E. & Williams, J.A. 1996. Intercellular calcium waves in rat pancreatic acini: Mechanism of transmission. *Am J Physiol* **271,** C1285–C1294.

Zimmermann, B. & Walz, B. 1997. Serotonin-induced intercellular calcium waves in salivary glands of the blowfly Calliphora erythrocephala. *J Physiol (Lond)* **500,** 17–28.