Biological Research
Print version ISSN 0716-9760

Biol. Res. vol.35 no.2 Santiago 2002

http://dx.doi.org/10.4067/S0716-97602002000200008

Biol Res 35: 177-182, 2002

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Calcium signal compartmentalization

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ABSTRACT

Cytosolic calcium signals are produced by suddenly increasing the concentration of free calcium ions (Ca^{2+}). This can occur by opening channels permeable to Ca^{2+} either in the surface cell membrane or in the membranes of intracellular organelles containing high Ca^{2+} concentrations. Ca^{2+} signals can control several different processes, even in the same cell. In pancreatic acinar cells, for example, Ca^{2+} signals do not only control the normal secretion of digestive enzymes, but can also activate autodigestion and programmed cell death. Recent technical advances have shown that different patterns of Ca^{2+} signals can be created, in space and time, which allow specific cellular responses to be elicited. The mechanisms responsible for Ca^{2+} signal compartmentalization are now largely known and will be described on the basis of recent studies of Ca^{2+} transport pathways and their regulation in pancreatic acinar cells. It turns out that the Ca^{2+} handling as well as the structural characteristics of the endoplasmic reticulum (ER) and the mitochondria are of particular importance. Using a variety of Ca^{2+}-sensitive fluorescent probes placed in different sub-cellular compartments in combination with local uncaging of caged Ca^{2+}, many new insights into Ca^{2+} signal generation, compartmentalization and termination have recently been obtained.

Key terms: Calcium signals, Ca^{2+} release channels, Ca^{2+} pumps, endoplasmic reticulum, mitochondria, pancreatic acinar cell, pancreatitis, secretion, apoptosis.

INTRODUCTION

Coordination of the many bodily functions occurs by means of the nervous and hormonal systems. These systems create chemical signals (messages in the form of neurotransmitters and hormones), which are received by specific receptor proteins in the surface cell membranes. When a hormone or a neurotransmitter binds to a specific receptor site on the outside of a cell membrane, a transduction process (often a complex cascade of chemical reactions) occurs, creating an intracellular signal (intracellular messenger) (Burgoyne & Petersen, 1997). In many cases the signal is a rise in the ionized calcium (Ca^{2+}) concentration in the intracellular fluid (cytosol) that surrounds cellular organelles such as the endoplasmic reticulum (ER), the mitochondria and the nucleus (Berridge et al., 2000). One of the perhaps most remarkable features of Ca^{2+} signaling is the ability, in the same cell, to regulate entirely different processes. In this short article, I shall first present briefly the general features of cellular Ca^{2+} handling and thereafter consider a specific example, namely the pancreatic acinar cell, in which Ca^{2+} signals regulate enzyme and fluid secretion. Ca^{2+} signals can also elicit cell division or programmed cell death (apoptosis) and initiate the process of intracellular trypsin activation that leads to autodigestion, which is a central feature of the human disease acute pancreatitis.

Ca^{2+} may have been selected as a signaling molecule early in evolution because of its ability to bind to many proteins and thereby change the conformation of these complex molecules. For the evolution of life in the sea, the presence of Ca^{2+} in seawater was a problem, since Ca^{2+} can form insoluble complexes with phosphates, can cause fatty acids to aggregate into soapy globules and can activate enzymes breaking down proteins (proteases). It was therefore necessary to evolve mechanisms for essentially keeping Ca^{2+} out of the water inside cells.

CALCIUM EXTRUSION AND CALCIUM SIGNAL GENERATION

Powerful mechanisms have evolved to extrude Ca^{2+} from cells. The most important mechanism is the plasma membrane Ca^{2+} pump (Plasma Membrane Ca^{2+}-activated ATPase - PMCA). This pump catalyses the movement of Ca^{2+} from a low to a high concentration (Carafoli 1992). Since the plasma membrane is electrically polarized (about 50 to 90 mV, inside negative), Ca^{2+} also has to be moved against an electrical force. In some cell types there is an additional Ca^{2+} extrusion mechanism that exchanges Ca^{2+} for Na^{+}. The Ca^{2+} extrusion process only works if the cell membrane has a low Ca^{2+} permeability. This is necessary because the normal Ca^{2+} concentration in the extracellular fluid is about 1 mM, which is four orders of magnitude higher than the normal Ca^{2+} concentration in the cytosol, which is about 0.1 µM.

The very low resting Ca^{2+} concentration in the cytosol is ideal for signaling purposes. Sudden addition of a very small amount of Ca^{2+} to the cell interior creates a relatively large rise in the intracellular Ca^{2+} concentration. The simplest way to produce such a Ca^{2+} signal is to open channels in the cell membrane, which allow movement of Ca^{2+}. Since the Ca^{2+} concentration outside the cell is so much higher than inside and since the electrical potential across the cell membrane is such that the interior is negative with respect to the outside, opening of gates to Ca^{2+}-permeable channels results in movement (flow) of Ca^{2+} into the cell. This is indeed one of the important mechanisms for Ca^{2+} signal generation and there are many types of Ca^{2+} channels, which can be regulated by voltage and/or by external or internal messengers (Miller, 2001)

In general, channels can transport many more ions per unit time than pumps. It is therefore easier to produce quickly large intracellular Ca^{2+} signals than to remove quickly the excess Ca^{2+} from the intracellular solution. In order to create sharp and short-lasting Ca^{2+} signals, there are important Ca^{2+} transport mechanisms in the membranes surrounding various intracellular organelles. One of the most important organelles in this respect is the ER, which is a huge system of connected cisterns inside cells that is essential for the production and processing of proteins. With regard to Ca^{2+} signaling, the most important property of the ER is the ability to accumulate Ca^{2+} in its lumen. This is due to the existence in the organelle membrane of a Ca^{2+} pump (Sarco-Endoplasmic Reticulum Ca^{2+}-activated ATPase _ SERCA), which is similar, but not identical to that in the surface cell membrane (Pozzan et al., 1994). The result is a substantial reservoir of Ca^{2+}, held inside the ER. The Ca^{2+} concentration in this compartment is typically about 100-500 µM (Mogami et al., 1998, Alvarez et al., 1999), which is about 1000 - 5000 times higher than in the surrounding cytosol.

The ER could in principle diminish cytosolic Ca^{2+} signals, created by the opening of Ca^{2+} channels in the surface cell membrane, by absorbing some of the Ca^{2+} entering the cell. In the short term this could be more effective than Ca^{2+} extrusion across the surface cell membrane, since the surface area of the ER is much larger (at least by a factor of 10) than the surface area of the cell membrane. On the other hand, the ER can also be used as a source of Ca^{2+} to primarily create intracellular Ca^{2+} signals that do not depend on entry of Ca^{2+} from the outside. Special Ca^{2+} channels in the ER membrane accomplish this. There are several types of such Ca^{2+} channels, which can be regulated by various chemicals produced inside cells in response to neurotransmitter or hormone actions on the outside of the cell. The most important Ca^{2+} release channels in non-muscle cells are activated by inositol 1,4,5-trisphosphate (IP_3) (IP_3 receptors) (Berridge et al., 2000), whereas the most important Ca^{2+} release channels in cardiac muscle cells are activated by a rise in the cytosolic Ca^{2+} concentration (ryanodine receptors) (Petersen & Cancela, 1999; Hidalgo et al., 2002). In skeletal muscle cells there is interaction between the ryanodine receptors and the dihydropyridine receptors in the sarcolemma, so that depolarization of the surface membrane causes opening directly of the Ca^{2+} release channels in the sarcoplasmic reticulum. However, many cell types contain both IP_3 and ryanodine receptors. The IP_3 receptors are also controlled by changes in the cytosolic Ca^{2+} concentration and some subtypes of ryanodine receptors are controlled by the cytosolic messenger cyclic ADP-ribose (cADPR) (Petersen & Cancela, 1999). Recently, the actions of a third type of Ca^{2+} releasing messenger, nicotinic acid adenine dinucleotide phosphate (NAADP), have been characterized (Petersen & Cancela, 1999), but the channel type activated by this messenger is still unknown. The Ca^{2+} sensitivity of the ER Ca^{2+} release channels allows for the interesting phenomenon of Ca^{2+}-induced Ca^{2+} release, originally described by Endo and his collaborators (Endo et al., 1970) in skinned muscle fibres. Very recently this phenomenon has been demonstrated directly and elegantly, by simultaneous

measurements of the Ca^{2+} concentrations in the cytosol and in the ER, in intact voltage-clamped sensory neurones (Solovyova et al., 2002). The Ca^{2+}-induced Ca^{2+} release phenomenon is crucial for the generation of repetitive Ca^{2+} spiking.

Finally the role of another important organelle, with respect to cellular Ca^{2+} handling, should be mentioned. The mitochondria are the cellular power generators. The chemical energy is produced in the form of adenosine trisphosphate (ATP), which is used to drive very many cellular processes including contraction and secretion. It turns out that three dehydrogenases in the Krebs cycle are modulated by the intramitochondrial Ca^{2+} concentration in the µM range (Denton & McCormack, 1990). Furthermore, it has now become clear that cytosolic Ca^{2+} signals result in uptake of Ca^{2+} into the inner mitochondrial space via a special Ca^{2+} transporter in the inner mitochondrial membrane known as the Ca^{2+} uniporter (Pozzan et al., 1994; 2000). When an external signal activates a cell, for example to secrete, by creating a cytosolic Ca^{2+} signal, this results in mitochondrial Ca^{2+} uptake, which subsequently stimulates mitochondrial Ca^{2+}-dependent dehydrogenases and leads to ATP production (Jouaville et al., 1999, Voronina et al., 2002a). Ca^{2+} signaling in this way controls both the physiological end product, namely in this case secretion, but also the necessary energy production. We can use the terms `stimulus-secretion coupling' and `stimulus-metabolism coupling' as headings under which to discuss these events. Mitochondria also need to have an exit pathway for Ca^{2+}; this is a so-called ion exchanger, which takes up Na^{+} in return for Ca^{2+}.

CALCIUM SIGNALING IN PANCREATIC ACINAR CELLS

The pancreas is an organ in the abdominal cavity, which mainly consists of exocrine cells involved in producing the pancreatic juice, which is secreted into the gut. The pancreatic juice contains many different enzymes produced in and secreted from the acinar cells, which break down the food products. The acinar cells are highly polarized. The apical pole is tightly packed with vesicles, normally referred to as secretory or zymogen granules. They contain the digestive (pro)enzymes. The basal part of the cell contains the nucleus surrounded by the very densely packed ER which, as already explained, contains the major mobilisable internal Ca^{2+} store. There are functionally important extensions of the ER into the granular pole. The mitochondria are principally located on the border between the granular apical pole and the basal part of the cell (Tinel et al., 1999, Park et al., 2001a).

There are two important stimulants of pancreatic enzyme secretion: the neurotransmitter acetylcholine (ACh) and the circulating hormone cholecystokinin (CCK). It is well established that both CCK and ACh evoke enzyme secretion in a Ca^{2+}-dependent manner and that both these stimulants elicit primarily release of Ca^{2+} from the ER (Petersen & Cancela, 1999, Petersen et al., 2001). Both ACh and CCK elicit Ca^{2+} signals in the pancreatic acinar cell. At the lowest concentrations of the stimulants, the signal consists of repetitive short-lasting rises in the Ca^{2+} concentration, which are confined to the granular apical pole (local spiking). These signals are due to repetitive and co-ordinated openings of Ca^{2+} release channels located in the ER extensions in the granular pole (Thorn et al., 1993). These local Ca^{2+} signals in the apical granular pole are fully sufficient to elicit the secretory response (Maruyama & Petersen, 1994, Park et al., 2001b). The confinement of the physiological Ca^{2+} signals to the apical granular pole is essentially due to two factors: the concentration of the Ca^{2+} release channels in the granular ER extensions (Petersen et al., 2001) and the perigranular mitochondrial Ca^{2+} buffer barrier (Tinel et al., 1999, Park et al., 2001b). The granular apical pole is by far the most sensitive part of the cell to all Ca^{2+} signal generating stimuli, for example, IP_3, cADPR, NAADP and Ca^{2+} itself (Ashby et al., 2002, Cancela et al., 2000; 2002,).

The rise in the local cytosolic Ca^{2+} concentration in the apical pole evokes secretion by exocytosis. Exocytosis requires ATP, which is produced locally from neighbouring mitochondria, stimulated by the local Ca^{2+} signals (Voronina et al., 2002a). A single shortlasting Ca^{2+} spike in the granular apical pole is able to elicit such an exocytotic response (Maruyama & Petersen, 1994). In addition to the secretion of enzymes there is also a need for fluid to be secreted, so that the enzymes can be washed into the duct system and from there into the gut. Fluid secretion is principally activated by opening of channels permeable to Cl^{-} in the luminal plasma membrane. It has recently been demonstrated directly that a local elevation of the cytosolic Ca^{2+} concentration in the apical granular pole elicits opening of the Cl^{-} channels in the luminal plasma membrane (Park et al., 2001b).

ACh and CCK both elicit the same type of secretory response but CCK, in addition to the local Ca^{2+} signals, also occasionally induces the appearance of global and much longer lasting Ca^{2+} elevations (Thorn et al., 1993). The global Ca^{2+} signals, which also involve the nucleus (see Jaimovich & Carrasco, 2002), can elicit mitosis (cell division) and it is known that CCK evokes substantial growth of the pancreas (Petersen et al., 1994).

TOXIC CALCIUM SIGNALS AND DISEASE

When high, and unphysiological, concentrations of ACh or CCK are used, it is possible to elicit prolonged (sustained) global elevations of the cytosolic Ca^{2+} concentration. Whereas the initial rise in the cytosolic Ca^{2+} concentration induced by such toxic stimulation is due to release of Ca^{2+} from the ER, the subsequent sustained phase is entirely dependent on entry of Ca^{2+} from outside the cell through special channels in the surface cell membrane (Raraty et al., 2000).

The pancreatic acinar cell contains, as already mentioned, high concentrations of very powerful digestive enzymes capable of breaking down all ingested food products including proteins. These enzymes can also digest the pancreatic acinar cells themselves, as well as other cells in the pancreas and other organs. This happens in the human disease Acute Pancreatitis, which often has a fatal outcome. Recent work has shown that a toxic CCK concentration (10 nM as compared to the physiological range of 1-10 pM), activates the protein degrading enzyme trypsin inside the granules in the pancreatic acinar cell. This never happens when physiological stimulation is applied. The toxic CCK concentration also elicits structural changes similar to those encountered in the disease Acute Pancreatitis, in which secretory granules are replaced by vacuoles (Raraty et al., 2000). The dangerous enzyme activation inside the acinar cell can be prevented simply by removal of external Ca^{2+} (Raraty et al., 2000). These and other findings indicate that the inappropriate enzyme activation in the granular pole is dependent on entry of Ca^{2+} from the outside (Raraty et al., 2000). A specific blocker of the store-operated Ca^{2+} channel in the acinar cell membrane could therefore be effective in preventing the inappropriate enzyme activation.

Although CCK hyperstimulation is effective in producing a condition that resembles acute pancreatitis, this disease is not caused by excessive CCK stimulation. One of the classical causes of acute pancreatitis is reflux of bile into the pancreas. Very recently we have been able to show that several bile acids, applied in pathophysiologically relevant concentrations, induce prolonged cytosolic Ca^{2+} signals (Voronina et al., 2002b). Since prolonged cytosolic Ca^{2+} signals generated pharmacologically by blocking the SERCA pumps with thapsigargin induce intracellular trypsin activation and vacuole formation in a Ca^{2+} -dependent manner, it is likely that the bile acids would do the same. Further investigations are needed, but probably the ability of bile acids to induce acute pancreatitis (Voronina et al., 2002b) is due to excessive and prolonged cytosolic Ca^{2+} signals.

CALCIUM AND CELL DEATH

Ca^{2+} signals play an important role in the regulation of apoptosis, or programmed cell death (Ferri & Kroemer, 2001, Gerasimenko et al., 2002). Our recent work indicates that Ca^{2+} signals can elicit apoptosis in the acinar cells only if there is a simultaneous reduction in the electrical potential difference across the inner mitochondrial membrane (mitochondrial depolarisation). This can occur by opening a special channel known as the permeability transition pore (Ferri & Kroemer, 2001). The control of apoptosis is very complex and a detailed account is outside the scope of this article, but one important event is the release of the protein cytochrome c from the mitochondria which, via various steps, leads to activation of enzymes known as caspases. Caspases are the `executioners' of apoptosis (Ferri & Kroemer, 2001; see also Razik and Cidlowski, 2002). In general, various oxidants can induce inappropriate programmed cell death. In pancreatic acinar cells, it has been established that the oxidant menadione is able in a consistent and reproducible manner to elicit apoptosis. Menadione elicits global Ca^{2+} signals and also depolarises the mitochondrial membrane by opening the permeability transition pore. Our recent work shows that both global Ca^{2+} signals and opening of permeability transition pores in mitochondria are absolutely required for induction of apoptosis in the pancreatic cells. A twin-track model, in which Ca^{2+} signal generation and co-operative oxidant and Ca^{2+} actions on the mitochondria induce the apoptotic signal (Gerasimenko et al., 2002), best explains this.

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Received: July 24, 2002. In revised form: July 24, 2002. Accepted: July 29, 2002

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