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Calcium, an earthy messenger in cells

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Calcium is the major messenger inside cells that is responsible for muscle contraction, secretion and many other processes. This article outlines some of features of these activities and reports on an Academia conference in Heidelberg, Germany.

Calcium is a common element, well known to everybody from chalk and limestone, which are calcium carbonates. Its name is derived from the Latin, *calx* = lime. It is present in sea water but it is maintained there in a rather low concentration because, together with carbon dioxide, it forms calcium carbonate which is deposited as chalk or limestone. A similar concentration of calcium to that in the sea water, 5 mM (0.02%) is found in the blood of animals and man, and is deposited in an equivalent manner to form our bones; in this case the material deposited is a phosphate of calcium, the mineral hydroxyapatite. However, within cells in the body, the concentration of calcium in the cell sap (the cytosol) is kept very much lower, some ten thousand times lower! This is achieved by pumping calcium out of the cells by pumps in the cell membrane as well as by pumping into reservoirs within the cell in membrane systems, notably the endoplasmic reticulum (*er*). The cell membrane itself is relatively impermeable to calcium. The very low calcium concentration in the cell thus depends on the combination of active expulsion by the pumps against the background of the low level of leakage through the cell membrane. Of course, pumps consume energy and they are fuelled by ATP (adenosine triphosphate); a surprising proportion of the energy used by the cell is devoted to these and other ion pumps.

What is the purpose of taking so much trouble to reduce the calcium in the cell? It turns out that calcium is used in cells as a very versatile messenger to turn on a variety of activities, such as muscular contraction, secretion, an increase in

metabolism, and even cell division and differentiation. Recently, it has been found that the cells that control our diurnal rhythms so do through calcium signals.

For such a system of signalling to operate, there need to be mechanisms that can raise the level of calcium inside the cell when required and also mechanisms that can sense the raised level.

The most obvious way of raising the level of calcium in the cell sap is to allow some to leak into the cell from outside and there are specific channels in the cell membrane that can be opened by hormones and neurotransmitters that permit this. There are also channels that respond to changes in the electrical potential across the cell membrane; this is normally negative inside with respect to the outside, but it can be reduced or even reversed, in ways we will consider shortly. We have also noted that calcium is stored in the *er* and it can also be released from these stores in response to changes in the calcium level in the cytosol itself as well as to other messengers within the cell. The most important of these messengers is IP_3 (inositol trisphosphate) which is generated in the cell membrane in response to hormones interacting with receptors on the cell surface. It is active in minute concentrations.

The sensing of calcium level is achieved by proteins that can respond to very low levels of calcium. One of the most ubiquitous of these is calmodulin, whose three-dimensional shape changes on the addition of calcium. Since it forms complexes with other proteins, its change of shape affects them and can cause a change in their activity as enzymes or in other ways.

The basic mechanisms that we have described operate in space and time within the cell as a very potent control system and are very much the subject of intense research activity at the present time. Many aspects of this were presented at a three-day meeting, organized by Ole Petersen for the Academia Europaea and the Tschira Foundation, and held at the Foundation's Conference Centre in Heidelberg in March 2002.

This was an opportune time for such discussions since technological developments have provided powerful new tools for following and manipulating calcium in cells. Firstly, there is the patch clamp technique devised by Erwin Neher and Bert Sakmann, by which a tiny glass tube of 0.001 mm is brought into contact with the cell membrane. In various ways, this can be used for the measurement of the electrical properties of the cell membrane, and can also provide a means of introducing substances into the cell and indeed for replacing some of the normal constituents. One very useful operation is the introduction of dyes that can change their fluorescent character in the presence of calcium and can that can therefore provide a visual measure of changes in calcium level in the cell in both space and time. The results are spectacular, beautiful pictures, and even movies with very fine time resolution. By judicious choice of dyes sensitive to different levels of calcium, the levels in the cytosol, *er*, mitochondria and the cell

nucleus can all be followed. Another powerful tool is provided by substances that contain bound calcium, which can be released in response to a flash of laser light. By this means, calcium can be released in precise locations and times to probe the sensitivity of local structures.

The first papers presented concerned the release of the transmitter molecule, acetylcholine from the nerve endings. The acetylcholine is held in tiny vesicles within the terminals of the nerve fibre, many of which are in close apposition, indeed attached to the cell membrane. The arrival of the nerve impulse with a reduction or reversal of the membrane potential opens voltage sensitive calcium channels and the raised calcium level that results causes the vesicles to fuse with the cell membrane and release the transmitter to the outside in a short explosive burst. The process is normally difficult to follow since the nerve endings are very small; however, there are some synapses in the central nervous system in which giant nerve endings virtually enclose the post-synaptic cell. While these endings release another transmitter, glutamate, the process is the same and the intervention of calcium in the release process could be studied in detail.

In the central nervous system, a surprisingly large proportion of its volume is taken up by non-neuronal cells, the glia, whose function remains rather unclear but has something to do with nourishing the neurones. It was reported that a glial cell type, the astrocyte, was activated by glutamate released in synaptic activity and, in turn, through calcium-activity released NO (nitric oxide), which caused relaxation of neighbouring blood vessels and thus increased local blood flow in an area of neuronal activity. This is a particularly interesting finding because the widely used magnetic resonance imaging (fNMR) and other methods for registering local nervous activity in the brain all rely on measuring changes in blood flow and this looks like a mechanism for it.

Calcium is released inside the cell by the action potential in both skeletal and cardiac muscle and this is potentiated by calcium-activated calcium release from the *er*. It then interacts with a relative of calmodulin called troponin C, which is coupled to another protein tropomyosin, which in the resting state inhibits the already charged contractile complex of the proteins actin and myosin together with a power source, ATP. A calcium combination with troponin C changes its shape, this changes that of the coupled tropomyosin and this now allows the contractile complex to become active. In a cardiac muscle, a rhythmic wave of calcium sweeps down the fibre in each period of activity. A cardiac muscle in disease is liable to develop an irregular electrical and contractile activity, called fibrillation. This was produced experimentally in the rat heart and was seen to be accompanied by a chaotic calcium release.

Calcium also plays a pivotal role in the activity of secreting glands. The pancreas produces a secretion that has two parts, it discharges the proteolytic enzyme trypsin for digestion, but it also secretes an alkaline fluid in which the

enzyme is dissolved. The fluid is formed as a result of the transport of chloride across the apical membrane, and the enzyme which was stored in vesicles in the vicinity of the apical membrane is discharged as a result of their fusion with the membrane (see Figure 1). Both of these events can be produced if calcium is delivered near the apical membrane, but not if it is delivered to the base or the sides of the cell. This differentiation has been elegantly shown by the use of the laser-directed release from caged calcium mentioned previously. However, the physiological process of secretion in the pancreas depends on either acetylcholine released from nerves or of the hormone cholecystokinin coming through the blood stream. In both cases these substances interact with receptors located on the cell base, which then causes the formation of $IP_{3,}$ which in turn releases calcium from the *er*. The calcium release channels are concentrated in parts of the *er* that penetrate into the apical region. IP_3 diffuses easily within the cell and reaches the apical granule region where it releases the calcium. On the other hand, calcium does not diffuse easily in cells because it is readily bound to rather immobile proteins. The released calcium is pumped out of the cell apex and is replaced by calcium that enters the cell base and moves through special channels in the *er* to the apical region. The release of calcium also causes uptake into neighbouring mitochondria, which stimulates their metabolic activity. This serves to restore the energy stores of the cell, which are also needed for the synthesis of new secretory products. The calcium release occurs in bursts and this rhythmic nature of the calcium elevation is important in physiological activation of the cell. By contrast, a global maintained rise in the level causes the death of the cell by activating a group of enzymes, the caspases, which destroy the cell; this can also follow excessive depletion of the calcium stores in the cell. The origin of the rhythmic calcium waves is an interesting theoretical problem in dynamics and there were several papers presented at the end of the conference dealing with it. In one approach, the model was based on the situation that calmodulin molecules occur in clusters and are coupled to kinases that can cause phosphorylation of proteins. This can set up a positive feedback process that could generate waves of calcium with some features common to those found experimentally.

In chromaffin cells in the adrenal gland, the total cell Ca concentration is very high and so is the cytosol level after hormonal stimulation, but the restoration of resting levels occurs in the mitochondria, which have a very high temporary Ca storage capacity.

Other papers dealt with the role of calcium in mast cells in antibody release and with many details of IP_3 actions. It is not surprising, in view of its central importance in cell function, that there are several variant forms of calcium pumps and of the receptors for the various substances controlling the calcium concentrations.

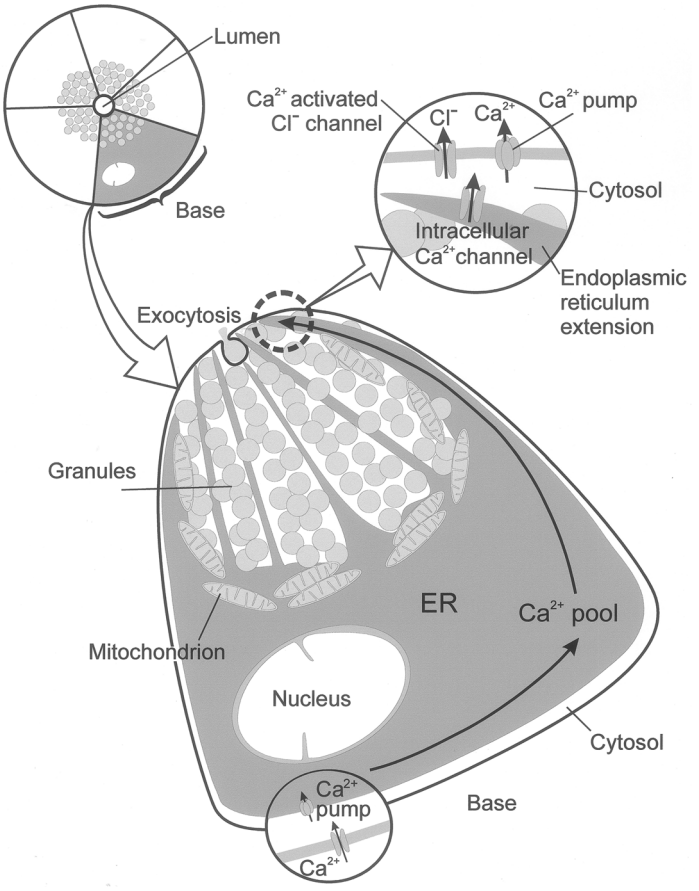

Figure 1. The pancreatic acinar cell. The inset in the upper left corner shows an acinar unit. Five cells surround a small lumen. The secretory material is delivered into this lumen, which in a three-dimensional model would be seen connected to a small duct. Several ducts would converge and finally the secretory material would be delivered into the gut, where the digestion of the food occurs. The main part of the figure shows the organization of an individual acinar cell. The apical pole, near the lumen, is tightly packed with granules containing the digestive enzymes. A single secretory event is shown. A granule has fused with the luminal cell membrane and, at the point of fusion, an opening has been created through which the secretory material escapes. The nucleus is surrounded by the densely packaged endoplasmic reticulum (*er*), which essentially takes up all the space outside the nucleus and the granular pole. *er* strands penetrate into the granular pole all the way to the luminal membrane. The calcium release channels are clustered in these *er* extensions and the calcium pumps are concentrated in the luminal cell membrane (inset in upper right corner). Below the nucleus, the calcium entry process is shown. Calcium enters via a channel in the basal cell membrane and is thereafter taken up into the nearby *er* via its Ca pump (courtesy of Ole Petersen).

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About the Author

Arnold Burgen is Editor-in-chief of *European Review*.