PROCEEDINGS OF THE NUTRITION SOCIETY
A Scientific Meeting was held at the Liverpool Medical Institution, Liverpool on 6/7 April 1989

Symposium on 'Intracellular calcium in the control of metabolism'

Calcium as an intracellular regulator

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All living cells require calcium, to remain alive and to carry out their specialized functions (Campbell, 1983; Nordin, 1988). We need Ca not only to maintain essential skeletal structures, but also to enable us to control the behaviour of our cells and tissues when they respond to a change in environment, be it diet, a stimulus, or a pathogen.

The diverse functions of Ca in the body can be divided into two groups: structural and regulatory. The former involves Ca precipitates in the extracellular matrices forming bones and teeth, as well as non-precipitated Ca in the maintenance of intracellular structures such as organelles and chromatin. The latter, regulatory role, can itself be subdivided into two groups: 'passive' and 'active'. Removal of Ca will prevent blood clotting or the activation of complement in the test-tube. This is because Ca ions are required for at least four enzymes in the blood clotting pathway, and for the first enzyme complex of complement, C1, binding to an antibody–antigen complex. Ca may be considered as a biochemical regulator of these proteins, but is not a regulator in the physiological sense. The Ca is, therefore, 'passive' since changes in plasma Ca neither provoke nor significantly alter these events. In complete contrast, a change in Ca is 'active' inside living cells, enabling the cells to change their behaviour in response to a physiological stimulus, such as a hormone or neurotransmitter.

Disturbances in the structural and regulatory roles of Ca both outside and inside cells play an important part in disease, both its pathogenesis and natural history. But are such disturbances in cell or tissue Ca a cause or consequence of injury? What role does Ca have in determining the threshold between a resting cell and an activated one, or between reversible and irreversible injury and even death?

The quality and indeed the survival of human life requires the right balance between the supply of Ca and its processing within cells. Mobilizable Ca is stored not only extracellularly in bone, but also in specialized compartments within cells. Release of the latter into the cell cytosol is responsible for provoking events such as secretion, movement and division.

CALCIUM BALANCE IN THE CELL

In human cells, the total cell Ca can vary from as little as 0·02 mmol/kg cell water in erythrocytes with no organelles, to more than 5–15 mmol/kg cell water in cells such as

muscle or platelets with large stores of intracellular Ca (Campbell, 1983). The precise distribution of Ca within cells is yet to be defined. However, what is known is that more than 99·9% of the Ca inside cells is bound to organelles such as endoplasmic reticulum, mitochondria, specialized vesicles and the nucleus. The concentration of free Ca^{2+} in the cytosol of a resting cell is about 0·1 μM. Thus, a 10000-fold electrochemical gradient of Ca^{2+} exists across the cell membrane. A small increase in the permeability of the cell membrane, or a small release of Ca^{2+} from an internal store, causes a very large rise in cytosolic Ca^{2+} and will either 'switch' it on or injure it. The electrochemical gradient of Ca^{2+} is maintained by a pump which counterbalances the small passive leakage of Ca^{2+} into the cell.

REGULATORY ROLE

Ca^{2+} is not only required for enzymes in the blood clotting and complement systems, but also for the maximum activity of several extracellular digestive enzymes, including proteases, phospholipases and nucleases. Electrically excitable cells contain channels which are selective for Ca^{2+}, and open when the membrane is depolarized. Thus, Ca^{2+} currents play an important role in the action potential of the heart, provoking contraction, and in provoking transmitter release at nerve terminals. In both cases depolarization of the plasma membrane causes a rise in cytosolic Ca^{2+}, which is the internal signal causing a muscle cell to contract or a nerve terminal to secrete. A vital biological role for Ca^{2+} is, therefore, its ability to trigger events within the cell, enabling us to move, to digest our food and to reproduce.

Events in cells responsible for a change in their behaviour are initiated by a primary stimulus. This may be physical, such as touch or an action potential, or it may be chemical, such as a hormone or neurotransmitter. The stimulus acts at the cell membrane and transmits a signal, through intracellular messengers, to structures and enzymes within the cell (Fig. 1). Three classes of intracellular signal have been identified so far:

(1) Cations: Ca^{2+}, hydrogen, sodium;
(2) Nucleotides: cyclic AMP, cyclic GMP, AMP, GTP;
(3) Phospholipid derivatives: inositol phosphates, diacyl glycerol.

Ca was the first intracellular signal to be discovered (Heilbrunn, 1937, 1943) and is one of the most important and wide ranging. There are six questions central to a full understanding of Ca^{2+} as an intracellular regulator:

(1) Is a rise in free Ca^{2+} in the cytosol or in the appropriate intracellular compartment the trigger for cell activation?
(2) Do secondary regulators which modify the response of the cell to the primary stimulus act on the Ca^{2+} transient or elsewhere?
(3) Where does the Ca^{2+} come from, and how is it released?
(4) How does the Ca^{2+} work?
(5) In cell injury is Ca^{2+} friend or foe?
(6) How did the intracellular Ca^{2+} signalling system evolve?

A rise in cytosolic Ca^{2+} is responsible for all forms of muscle contraction and vesicular secretion, as well as certain examples of cell aggregation, cell transformation, cell division and activation of intermediary metabolism (Campbell, 1983, 1988*a*; Nordin, 1988). Crucial to the experimental verification of this concept has been the ability to monitor changes in free Ca^{2+} in living cells. This is based on incorporating into the cell

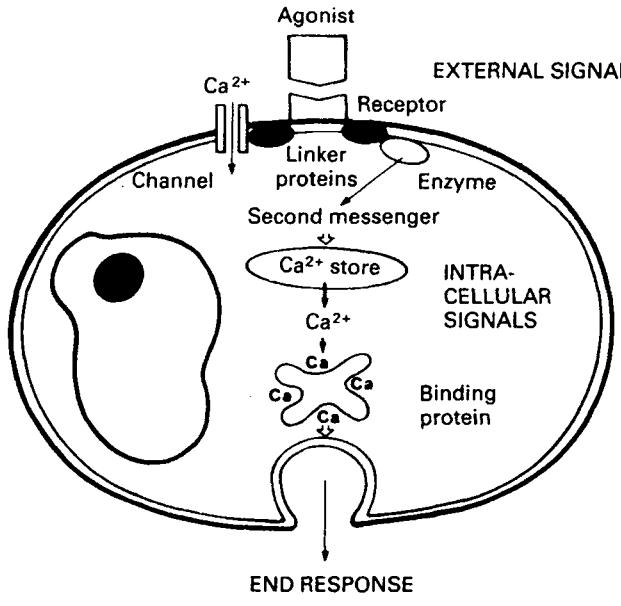

Fig. 1.

dyes or indicators whose colour, fluorescence, chemiluminescence or nuclear magnetic resonance change when they bind Ca^{2+} (Ashley & Campbell, 1979; Tsien, 1980; Grynkiewicz *et al.* 1985; Rink & Cobbold, 1987; Campbell, 1988*a*,*b*). An interaction, or chemisymbiosis, exists between intracellular Ca^{2+} and the other intracellular signals, e.g. H^+ and Na^+, cyclic nucleotides and diacyl glycerol. This chemisymbiosis determines the thresholds for activation in each cell (Campbell, 1988*b*). The number of cells switched on at any point in time or by a particular concentration of stimulus, as well as the magnitude of the response, can be modified by secondary regulators which may alter the Ca^{2+} transiently, how Ca^{2+} acts, or independently of Ca^{2+} via another signal. Thresholds in individual cells may be controlled by oscillations in intracellular free Ca^{2+} (Woods *et al.* 1986). Two questions arise: first, what is the source of this Ca^{2+} for cell activation, and second, how does the Ca^{2+} act to switch on the cell?

SOURCES AND ACTION OF INTRACELLULAR CALCIUM

An increase in cytosolic free Ca^{2+} arises from a combination of release from an internal store and an increase in the permeability of the cell membrane to Ca^{2+}. In heart muscle and neutrophils respectively voltage-dependent or receptor-operated Ca^{2+} channels open. This is followed by release from an internal Ca^{2+} store and a further increase in membrane permeability to maintain the rise in intracellular Ca^{2+} (Hallett & Campbell, 1982; Lew *et al.* 1985). In contrast, in skeletal muscle and liver, the action potential or hormone respectively cause a release of Ca^{2+} from the internal store, which is followed by an increased flux of Ca^{2+} into the cell to maintain activation. The main mobilizable intracellular Ca^{2+} store in all cells is the endoplasmic reticulum. In skeletal muscle this is released directly by an electrical signal. In heart muscle it is released by the small amount of Ca^{2+} which moves into the cell through the action potential, and in all other cells Ca^{2+}

https://doi.org/10.1079/PNS19900008 Published online by Cambridge University Press

is released by inositol trisphosphate (Berridge, 1984). This substance is produced at the cell membrane as a result of activation of a phospholipase (*EC* 3.1.4.3) which catalyses the cleavage of a minor membrane phospholipid, phosphatidyl inositol-4, 5-bisphosphate, to diacyl glycerol and inositol trisphosphate. Diacyl glycerol remains within the membrane and activates an enzyme known as protein kinase C (*EC* 2.7.1.37). The water-soluble inositol trisphosphate causes a 'Ca^{2+} cloud' within the cell. Recovery of the cell after removal of the stimulus involves replenishment of the Ca^{2+} store, and pumping some Ca^{2+} out of the cell via a Ca^{2+}, Mg^{2+}-ATPase or Na^{+}–Ca^{2+} exchange.

The ability of Ca^{2+} to trigger the cell depends on the Ca^{2+} cloud reaching the appropriate Ca^{2+}-binding protein. These Ca^{2+}-binding proteins include calmodulin, found in all cells, troponin C in muscle, and a family of cytoskeletal proteins, for example gelsolin. Binding of Ca^{2+} either triggers the event directly or leads to phosphorylation of other proteins which cause the event. The precise molecular basis of these effects remains to be established, but a key feature of Ca^{2+}-binding proteins is co-ordination by oxygen which provides selectivity for Ca^{2+} over Mg^{2+}.

PATHOLOGY

Exogenous factors such as toxins, or viruses and bacteria responsible for infectious diseases, as well as endogenous factors such as T cells and components of the immune system, can cause an increase in the permeability to, and content of, Ca^{2+} in cells. This is relevant to the molecular mechanisms underlying diabetes, rheumatoid arthritis and multiple sclerosis. Disturbances in cell Ca^{2+} also occur in several genetic abnormalities, for example sickle cell anaemia and cystic fibrosis. Malfunctioning of Ca^{2+}-dependent processes occurs in heart disease, muscle disease and many endocrine disorders. Furthermore, tissue calcification is a feature not only of disorders of whole-body Ca^{2+} metabolism, but also in many types of severe tissue injury. Necrotic cells contain some ten to 100 times their normal Ca^{2+} content. The questions which now need to be answered are first, are these abnormalities in cell and tissue Ca^{2+} a cause or consequence of injury, and second, are they altered by Ca^{2+} supply either from the diet or skeletal stores?

However, a rise in intracellular Ca^{2+} in cell injury may not necessarily be disadvantageous. Gap junctions are sealed by a rise in cytosolic Ca^{2+}, thereby protecting neighbouring cells (Rose & Loewenstein, 1975). We have shown that membrane-pore family proteins, such as the membrane attack complex of complement, induce a rise in cytosolic free Ca^{2+} within a few seconds of binding the terminal component (Campbell *et al.* 1979; Campbell & Luzio, 1981). This rise in Ca^{2+} activates reactions within the cell, including a protection mechanism enabling the cell to remove the potentially lethal complexes by endocytosis or budding. Before this cells may undergo a second permeability threshold to molecules of 500–1000 molecular weight (Patel & Campbell, 1987). Those cells which do not protect themselves in time die. The reversible injury of cells by complement and other membrane-pore formers such as T-cell perforins (Poinie *et al.* 1987), bacterial toxins and viral proteins may play a key role in the release of respective oxygen metabolites in the joints of patients with rheumatoid arthritis, and in demyelination in multiple sclerosis (Scolding *et al.* 1989). Furthermore, attacking-agents such as complement or toxins can be removed via budding or endocytosis, thereby enabling the cell to recover from attack.

PHARMACOLOGY

Many therapeutic agents work by interfering with the various biological roles of Ca^{2+}. For example, receptor and channel blockers can prevent Ca^{2+} entering the cell, whilst some drugs can alter the distribution of Ca^{2+} within the cell or its mode of action.

THE FUTURE

In spite of the wide acceptance of Ca^{2+} as an intracellular regulator we still do not understand precisely how it works, nor precisely how it interacts with other intracellular signals such as cyclic nucleotides. In order to solve these problems it will be necessary to measure, locate and manipulate other intracellular signals besides Ca^{2+}, energy supply and covalent modification of proteins in single cells. Cloning of bioluminescent proteins (Inouye *et al.* 1985; Prasher *et al.* 1985), the development of transgenic luminous cells (DeWet *et al.* 1987) and image intensification (Poinie *et al.* 1987) of colour shifts involved in indicators by energy transfer (Campbell & Patel, 1983), offer exciting prospects for the future.

A further intriguing problem is how this universal feature of all eukaryotic cells, apparently rare or absent in prokaryotes and archaebacteria, evolved. It is proposed that the intracellular Ca^{2+} signalling system originated some 2·5 thousand million years ago as a response to a chemically- and biologically-induced rise in the free-Ca^{2+} bathing cells. In order to protect themselves from the potentially lethal effects of this Ca^{2+} on chromatin condensation, protein cleavage and precipitation, and anion co-ordination and precipitations, cells had to evolve Ca^{2+} pumps, and a means of sequesting the Ca^{2+} internally. Only then could cells begin to exploit the advantages arising from the resulting Ca^{2+} gradients across the organelle and plasma membranes.

PERSPECTIVES

Ca fulfils many functions crucial to the survival of the human body, in addition to its role in the skeleton. It is well known that severe dietary changes in Ca, either deficiency or excess, together with hormonal and vitamin imbalance, will cause skeletal abnormalities both in the developing child and the adult. The question the nutritionist now needs to answer is a subtle one. How does the dietary supply of Ca^{2+}, and its processing by the body, affect the efficiency of cell and tissue function through interactions with the regulatory role of Ca and, thus, determine the efficiency of performance of the whole body in health and disease?

The author thanks the many members of his research group over the past 15 years for much hard work, and the MRC, SERC, Arthritis and Rheumatism Council and Multiple Sclerosis Society for financial support.

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Printed in Great Britain