Cell, Vol. 83, 679–682, December 1, 1995, Copyright © 1995 by Cell Press

Calcium Ions as Extracellular Messengers

Minireview

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The capacity to regulate their internal ionic composition is a fundamental property of free-living terrestrial organisms (Stewart and Broadus, 1987). This is accomplished by adjusting the movement of ions or water (or both) between cells and various extracellular compartments, including those communicating with the external environment. Of the complement of inorganic ions in such organisms, Ca^{2+} performs an especially large number of intracellular and extracellular functions. It is a well-known intracellular second messenger, controlling numerous vital processes (see minireview by Bootman and Berridge, 1995 [this issue of *Cell*]). The cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) undergoes rapid and often substantial (i.e., severalfold or more) fluctuations in response to extracellular first messengers binding to their cognate receptors on target cells. The extracellular ionized Ca^{2+} concentration ($[Ca^{2+}]_o$), in contrast, is maintained stably at ~1 mM, at least 10,000-fold higher than the resting level of $[Ca^{2+}]_i$ in most cells, providing a seemingly inexhaustible supply of Ca^{2+} for its diverse intracellular functions. Ca^{2+} is also an essential component or cofactor in key extracellular structures or processes (i.e., bone, clotting factors, and adhesion molecules) whose biological functions could be compromised by large changes in $[Ca^{2+}]_o$, with potentially disastrous consequences. It is not intuitively obvious, therefore, that Ca^{2+} serves as an important extracellular first messenger. The recent cloning of an extracellular Ca^{2+}-sensing receptor (CaR), however, has firmly established that $[Ca^{2+}]_o$ serves such an informational role (Brown et al., 1993). This minireview will briefly outline the signaling function of $[Ca^{2+}]_o$, particularly the role of $[Ca^{2+}]_o$-sensing mechanisms within various extracellular compartments in this process.

The $[Ca^{2+}]_o$ Signal

For extracellular Ca^{2+} to serve an informational role, it must undergo sufficiently large perturbations to be recognized by $[Ca^{2+}]_o$-sensing mechanisms (or the responsiveness of the latter must change). In mammals, blood-ionized Ca^{2+} varies by only a few percent over the course of a day or even much of a lifetime. This remarkable stability of $[Ca^{2+}]_o$ results from a homeostatic system with two principal elements: $[Ca^{2+}]_o$-sensing cells (e.g., parathyroid cells) and tissues that respond to either local or systemic signals from these $[Ca^{2+}]_o$-sensing cells by altering their translocation of Ca^{2+} into or out of the extracellular fluid (i.e., kidney, intestine, and bone) (Stewart and Broadus, 1987). The near constancy of the blood-ionized Ca^{2+} concentration underscores the exquisite responsiveness of $[Ca^{2+}]_o$-sensing cells to minute (~2%) fluctuations in $[Ca^{2+}]_o$. Paradoxically, however, it has also deflected attention from the potential informational content of the extracellular ionic composition and appears to have fostered the concept that $[Ca^{2+}]_o$ is, in a broader sense, nearly invariant. It is not. Within the numerous extracellular compartments in complex organisms, some have levels of $[Ca^{2+}]_o$ differing considerably from that measured in blood. Moreover, these compartments can undergo substantial changes in $[Ca^{2+}]_o$ due to alterations in the fluxes of Ca^{2+} into or out of the extracellular space in question.

$[Ca^{2+}]_o$ within bodily cavities communicating with the external environment (the gastrointestinal and genitourinary tracts) undergoes large changes related to the assimilation of nutrients or excretion of wastes. Urinary tonicity, for example, varies over a >10-fold range without accompanying changes in the total amounts of Ca^{2+}, Mg^{2+}, or other ions excreted. At their normal levels of ~1 mM within various extracellular compartments, Ca^{2+} ions are nearly saturated with respect to their phosphate and oxalate salts (e.g., in the urinary tract). Thus, even small increases in urinary Ca^{2+} concentration could entail substantial risk of such salts precipitating. As will be described below, a $[Ca^{2+}]_o$-sensing mechanism has been positioned opportunely within the urinary tract to minimize this risk.

A gradient for $[Ca^{2+}]_o$ has been described in the skin, with generally higher levels in the superficial cellular layers (Menon et al., 1985). This gradient may serve an informational role, being sensed by keratinocytes (Hennings et al., 1980) and promoting their differentiation from the basal layer of continuously proliferating cells to the skin surface, where the superficial, cornified cells die and are sloughed off. There is also a $[Ca^{2+}]_o$ gradient across the placenta, with active transplacental Ca^{2+} transport producing a level of $[Ca^{2+}]_o$ ~10% higher in the fetal than the maternal circulation (Reitz et al., 1977).

Prior to birth, $[Ca^{2+}]_o$ within the fluid-filled alveoli of the developing lungs of the fetal sheep is ~0.5 mM. Postnatally, the fluid lining of the alveoli is reduced to a thin layer, the hypophase, with a $[Ca^{2+}]_o$ that has increased 3- to 4-fold (to 1.6–2 mM) (Eckenhoff, 1989). The hypophase contains the phospholipid surfactant, which is essential for preventing alveolar collapse. Optimal deposition of surfactant on the alveolar lining cells takes place at levels of $[Ca^{2+}]_o$ of 1–2 mM. The surfactant-containing, lamellar bodies of the type 2 pneumocytes contain abundant Ca^{2+} (as much as 75 mM). Therefore, exocytosis of the lamellar bodies provides not only surfactant but also Ca^{2+} that is essential for this phospholipid to function properly at this critical air–fluid interface.

$[Ca^{2+}]_o$ in narrow intercellular spaces within the brain can be surprisingly labile given the ~10,000-fold higher level of $[Ca^{2+}]_o$ relative to resting levels of $[Ca^{2+}]_i$. Evoking neuronal activity ranging from physiological activation (stimulating sensory neurons within the somatosensory cortex by stroking a cat's paw [Nicholson et al., 1977]) to

induction of seizure-like activity in the cerebellum of an anesthetized rat (Heinemann et al., 1977), decreases $[Ca^{2+}]_o$ reversibly by anywhere from 20% to as much as 90%. Stimulation of glutamate receptor channels in rat hippocampal pyramidal neurons likewise decreases $[Ca^{2+}]_o$ (Stabel et al., 1990) owing to activation of Ca^{2+} influx through the plasma membrane. The stimulation of cation-nonselective (e.g., NMDA and AMPA/kainate) channels may contribute to concomitant increases in $[K^+]_o$ and reductions in $[Na^+]_o$ in this circumstance. The latter can secondarily affect $[Ca^{2+}]_o$. For example, decreasing $[Na^+]_o$ in hippocampal slices from 154 to 114 mM (which also reduces osmolality proportionately) substantially reduces $[Ca^{2+}]_o$, probably through a mechanism involving the Na^+/Ca^{2+} exchanger (Chebabo et al., 1995). The latter effects raise the intriguing possibility that osmosensing in mammalian species could involve, in part, the sensing of osmolality-elicited changes in $[Ca^{2+}]_o$. There are also large fluxes of Ca^{2+} into and out of the restricted synaptic space. Synaptic vesicles can contain abundant Ca^{2+} (up to ~10 mmol/kg) that is released exocytotically from presynaptic neurons along with neurotransmitters. Subsequent activation of Ca^{2+}-permeable channels on postsynaptic neurons results in a large influx of Ca^{2+}, which could produce substantial reductions in $[Ca^{2+}]_o$ within the synaptic cleft.

Like secretory vesicles, the lumen of the endoplasmic reticulum accumulates considerable (millimolar) quantities of Ca^{2+} that are released by intracellular messengers such as inositol trisphosphate, cytosolic Ca^{2+} per se, and cyclic ADP-ribose (Bootman and Berridge, 1995). Could Ca^{2+} sensors monitor the extent of filling of the endoplasmic reticulum or other membrane-enclosed compartments that accumulate Ca^{2+}, thereby contributing to "spontaneous" release of Ca^{2+}?

How Cells Sense $[Ca^{2+}]_o$

Parathyroid cells and other cell types express a CaR that provides an illustrative example of how specific cells recognize and respond to the $[Ca^{2+}]_o$ signal. The CaR recognizes not only $[Ca^{2+}]_o$ and other divalent cations (Mg^{2+}, Ba^{2+}, and Sr^{2+}), but also trivalent cations of the lanthanide series (e.g., Gd^{3+}) and even organic polycations such as protamine, polylysine, and neomycin. The surprising promiscuity of the receptor may be of biological significance, as the CaR probably functions as a $[Mg^{2+}]_o$ sensor in vivo (see below) and could potentially also recognize other endogenous polycationic ligands present at concentrations appropriate for interacting with the receptor. By Northern blot analysis, the CaR is expressed most abundantly in mammalian parathyroid and kidney but also at lower levels in other tissues, including thyroid (specifically the $[Ca^{2+}]_o$-sensing thyroidal C cells), intestine, lung, and various regions of brain (Brown et al., 1993; Ruat et al., 1995). In parathyroid and C cells, it may represent the principal mediator of $[Ca^{2+}]_o$-elicited changes in parathyroid hormone (PTH) and calcitonin secretion. It is unclear how the parathyroid cell reverses the usual stimulatory effect of Ca^{2+} on exocytosis, producing the atypical, inverse relationship between both $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ on the one hand and PTH secretion on the other.

Several inherited diseases of extracellular Ca^{2+} homeostasis result from CaR mutations, and these "experiments in nature" have provided valuable insights into the normal functions of the receptor. Familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT) are the phenotypes resulting from inactivating mutations of the receptor when present in the heterozygous and homozygous states, respectively (reviewed by Brown et al., 1995). It is also clear, however, that mutations in the CaR, which resides on chromosome 3, are not the only genetic basis for the FHH phenotype. A locus on chromosome 19, as well as one linked to neither chromosome 3 nor 19, has also been identified, but the responsible genes have not yet been identified (Heath et al., 1993; reviewed by Brown et al., 1995). Higher than normal concentrations of $[Ca^{2+}]_o$ are present in affected individuals with inactivating CaR mutations, with mild hypercalcemia (~10%–20% above the upper limit of normal) in heterozygotes and severe hypercalcemia (1.5- to 2-fold elevations in $[Ca^{2+}]_o$) in homozygotes. The hypercalcemia results in large part from a gene dose–dependent "resistance" of the parathyroid glands to $[Ca^{2+}]_o$, but affected individuals with FHH and NSHPT also manifest diminished responsiveness to $[Ca^{2+}]_o$ in other tissues. The CaR normally regulates renal tubular reabsorption of Ca^{2+} in the thick ascending limb of the nephron, with increased levels of $[Ca^{2+}]_o$ on the basal (i.e., blood) side of the tubule inhibiting Ca^{2+} reabsorption. Therefore, the reduced responsiveness of the renal CaR to $[Ca^{2+}]_o$ in FHH and NSHPT leads to excessive renal tubular Ca^{2+} reabsorption of Ca^{2+} ions that contributes to the maintenance of hypercalcemia (Brown et al., 1995).

The CaR is also present in the renal collecting ducts, where it antagonizes the stimulatory action of vasopressin on water permeability under normal circumstances, presumably through CaR-mediated changes in water channel function or availability in the apical membrane of the collecting duct. Patients with FHH, on the other hand, concentrate their urine normally despite being hypercalcemic (because of resistance of the collecting duct CaR to $[Ca^{2+}]_o$) (Brown et al., 1995). CaRs in the thick ascending limb and collecting duct, therefore, may function coordinately to balance the homeostatic needs of free-living terrestrial organisms with respect to Ca^{2+} and water. With Ca^{2+} excess, the CaR in the thick limb reduces renal tubular Ca^{2+} reabsorption, resulting in increased urinary Ca^{2+} loads. If urinary concentration continued unabated, the resultant increase in urinary Ca^{2+} concentration might cause precipitation of Ca^{2+}-containing salts. Therefore, inhibition of urinary concentrating ability under these circumstances could serve to reduce the risk of kidney stones or tissue Ca^{2+} deposition.

Patients harboring activating mutations of the CaR, in contrast, exhibit a generally benign form of autosomal dominant hypocalcemia. In these patients, the CaR has an increased basal (i.e., $[Ca^{2+}]_o$-independent) activity, increased affinity for $[Ca^{2+}]_o$, or both (reviewed by Brown et al., 1995), such that the level at which $[Ca^{2+}]_o$ is maintained by the homeostatic system is "reset" downward. As might

be expected from the preceding discussion on inactivating mutations, some affected individuals with activating mutations show marked increases in renal Ca^{2+} excretion and impaired urinary concentrating ability as $[Ca^{2+}]_o$ is increased from its resting hypocalcemic level, presumably because of increased responsiveness of CaRs within the kidney to $[Ca^{2+}]_o$.

Several general statements can be made about the functions of the CaR as they relate to maintenance of $[Ca^{2+}]_o$ homeostasis (Figure 1). First, the CaRs in parathyroid cells and C cells sense levels of $[Ca^{2+}]_o$ that reflect systemic Ca^{2+} homeostasis and adjust calciotropic hormone secretion appropriately to normalize $[Ca^{2+}]_o$. The CaR on renal epithelial cells, on the other hand, senses $[Ca^{2+}]_o$ locally within the kidney and modulates the actions of other hormones (e.g., PTH or vasopressin) on solute and water flow to achieve the desired level of $[Ca^{2+}]_o$ in blood and urine. Second, the polarity of the CaR (e.g., whether it is apical or basal—facing the external or internal environment of the organism, respectively) is critical in determining the compartment in which $[Ca^{2+}]_o$ is sensed and the resultant modifications in ion transport, water flow, or other CaR-regulated processes.

What is the function of the CaR in tissues that are not involved in systemic $[Ca^{2+}]_o$ homeostasis? The CaR in brain is present in several regions, including hippocampus, hypothalamus, and cerebellum, where it may sense local rather than systemic levels of $[Ca^{2+}]_o$ (or perhaps some other endogenous CaR ligand) within the brain or cerebrospinal fluid. Ruat et al. (1995) have used immunohistochemistry to localize the CaR to nerve endings, including those on blood vessels. In view of the nerve cell–dependent changes in $[Ca^{2+}]_o$ within the brain discussed above, it is plausible to suggest (Ruat et al., 1995) that the neuronal CaR recognizes and responds to local changes in $[Ca^{2+}]_o$ resulting from varying levels of synaptic and neuronal activity. Figure 2 illustrates schematically

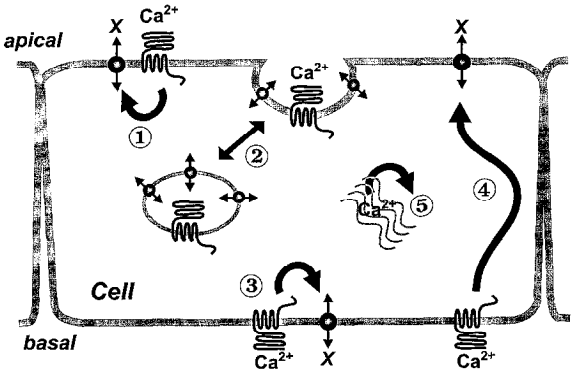

Figure 1. Schematic Representation of Possible Ways in Which CaRs (or Other Ion-Sensing Receptors) Might Regulate Epithelial Cell Function

The Ca^{2+} sensor (shown for the sake of argument with the CaR serpentine motif) could regulate the following processes: apical transport in responses to signals from apical (1) or basal (4) CaRs, basal transport in response to basal CaRs (3), trafficking of transporter-containing vesicles (2), or loading of intracellular Ca^{2+} stores (5).

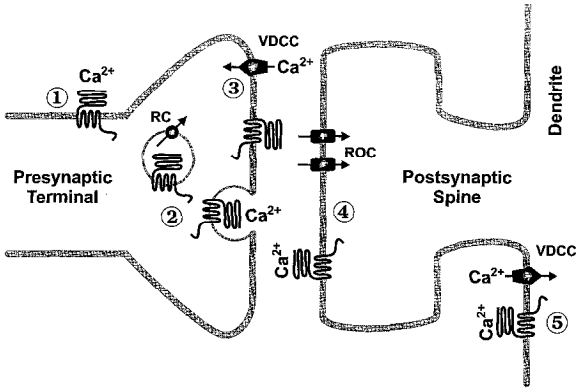

Figure 2. Hypothetical Ways in Which CaRs Could Regulate the Function of Hippocampal Pyramidal Neurons Presynaptically or Postsynaptically

The CaR (or another sensor for Ca^{2+} or some other ion) is shown as regulating presynaptic, voltage-dependent Ca^{2+} channels (VDCC) via receptors either within (3) or outside (1) of the synaptic cleft or postsynaptic VDDC or glutamate receptor–operated channels (ROC) through receptors inside (4) or outside (5) of the synaptic space. The CaR might also regulate exocytosis of synaptic vesicles or the loading of such vesicles with Ca^{2+}.

how neuronal CaRs might function depending on their location on the cell.

It is intriguing that the CaR is present within the hippocampus, which also contains the metabotropic glutamate receptors (mGluRs), to which the CaR bears a striking similarity in its overall topology (Brown et al., 1993; Nakanishi, 1992). Did the two receptors arise from a common ancestor by fusion of a "sensing" domain for small charged molecules (which perhaps originated from the bacterial periplasmic binding proteins [Conklin and Bourne, 1993]), to a serpentine signal-transducing domain? Could the CaR, like the mGluRs, be involved in long-term potentiation and other processes in hippocampal pyramidal neurons related to learning and other cognitive functions?

It seems likely that there are additional forms of CaRs or sensors that mediate the numerous effects of extracellular Ca^{2+} that have been described on various cell types (reviewed by Brown, 1991). For instance, elevations in $[Ca^{2+}]_o$ reduce PTH gene expression and directly or indirectly inhibit parathyroid cellular proliferation. $[Ca^{2+}]_o$ also inhibits osteoclastic bone resorption, in association with changes in $[Ca^{2+}]_i$ reminiscent of those in parathyroid cells. Furthermore, raising $[Ca^{2+}]_o$ inhibits the proliferation and promotes the differentiation of keratinocytes (Hennings et al., 1980) and also modulates the growth, differentiation, or both of cultured mammary cells and various other epithelial cells. It is not yet clear which of these actions of $[Ca^{2+}]_o$ are mediated by the recently cloned CaR and which reflect the actions of additional $[Ca^{2+}]_o$ sensors. It is likely that other forms of $[Ca^{2+}]_o$ sensors exist, as the pharmacologic profile for the inhibitory actions of $[Ca^{2+}]_o$ and other polyvalent cations on osteoclasts, for instance, differs distinctly from that of the parathyroid CaR. cDNAs encoding a putative CaR related to the low density lipoprotein superfamily, which is expressed on parathyroid cells, placental cyto-

trophoblasts, and kidney proximal tubules, have recently been isolated but have not yet been characterized functionally in terms of their $[Ca^{2+}]_o$-sensing properties (Lundgren et al., 1994; Saito et al., 1995). Much additional work is required, therefore, to define the nature of the $[Ca^{2+}]_o$ sensors mediating the effects of $[Ca^{2+}]_o$ on epithelial cells and other cell types and the signal(s) to which such $[Ca^{2+}]_o$ sensors respond in situ. Presumably, diseases of $[Ca^{2+}]_o$ sensing in addition to those described to date for the CaR may result from dysfunction of such $[Ca^{2+}]_o$ sensors.

Are There Sensors for Additional Ions?

Changes in the concentrations of extracellular ions other than Ca^{2+} modulate the functions of various cell types in ways that suggest the presence of specific ion-sensing mechanisms (reviewed by Brown, 1991). The CaR in the thick ascending limb mediates inhibitory actions of elevated levels of not only $[Ca^{2+}]_o$ but also $[Mg^{2+}]_o$ on tubular reabsorption of both ions. Patients with FHH can have mild hypermagnesemia, while those with untreated NSHPT may show more substantial increases in $[Mg^{2+}]_o$ (Brown et al., 1995). Thus, direct actions of $[Mg^{2+}]_o$ on the CaR in the renal thick limb (and perhaps on the parathyroid) may play a key role in "setting" normal levels of $[Mg^{2+}]_o$, and the CaR may act as an important (if not the principal) $[Mg^{2+}]_o$-sensing receptor in tetrapods. In addition, cells of the adrenal zona glomerulosa are exquisitely sensitive to changes in $[K^+]_o$, responding with alterations in the production of the mineralocorticoid hormone, aldosterone, which regulates renal and gastrointestinal handling of K^+. Fibroblasts and other cells respond to $[Ni^{2+}]_o$, $[Cd^{2+}]_o$, and other transition metals (but not to $[Ca^{2+}]_o$ or $[Mg^{2+}]_o$) with alterations in phosphoinositide turnover and $[Ca^{2+}]_i$ similar to CaR-mediated effects on parathyroid cells (Smith et al., 1989). Extracellular phosphate ions regulate the function of bone cells and kidney cells through an unknown mechanism(s). Are there receptors for $[K^+]_o$, $[Ni^{2+}]_o$, and phosphate ions, either G protein–coupled receptors analogous to the CaR or perhaps distinct forms of receptors regulating a variety of cellular effector systems?

Conclusions

The cloning of a G protein–coupled CaR from parathyroid, kidney, and brain and the identification of human diseases resulting from gain-of-function or loss-of-function mutations in the CaR have firmly established that extracellular Ca^{2+} (and probably Mg^{2+}) can serve as a first messenger. The CaR enables cells to sense changes in $[Ca^{2+}]_o$ that reflect systemic changes in $[Ca^{2+}]_o$ homeostasis (i.e., as sensed by the parathyroid gland), alterations in local cellular activity that are related to mineral ion or water metabolism (e.g., in the kidney), or local cellular functions unrelated to systemic fluid and electrolyte homeostasis (i.e., in the vicinity of brain cells). Further studies may reveal the presence of additional CaRs or sensors that mediate specific, direct actions of extracellular Ca^{2+} (as opposed to indirect actions due solely to associated changes in $[Ca^{2+}]_i$) on particular cell types. Receptors/sensors for other extracellular ions may also exist that represent mechanisms through which various cells recognize the informational content of their local ionic environment and respond in ways that produce autocrine, paracrine, or endocrine/systemic effects on cellular function.

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