been identified on chromosome 19q (*18*). The recent demonstration that this locus is close to the gene for apolipoprotein E, which is involved in cholesterol transport into brain, has led to an interesting hypothesis that the apolipoprotein E4 allele may have a causative role in late-onset Alzheimer's disease, both inherited and sporadic forms (*19*). Evidence for a fourth locus for Alzheimer's disease among families of Volga-German descent arises from exclusion of chromosomes 14, 19, and 20 in these pedigrees (*1*). In sum, it seems likely that there are multiple causes of Alzheimer's disease, and perhaps only a minority have defects in the metabolism of APP.

Mutations in the prion protein are now known to explain three neurologic disorders, all inherited as autosomal dominant traits: Gerstmann-Straussler-Scheinker syndrome, familial Creutzfeldt-Jakob disease, and fatal familial insomnia (*20*). These disorders, which occur in mid- to late life, are associated with the rapid onset of neurological signs and symptoms resulting in death within a period of 1 to 3 years. These mutations have important implications for understanding pathologic processes that are both infectious and genetic.

Finally, the causes of neurological diseases are not limited to mutations in nuclear DNA. The identification of mitochondrial DNA mutations, giving rise to maternally transmitted diseases, has opened an entire new area for the analysis of complex multisystem diseases (*1*). Defects in mitochondrial DNA give rise to Kearns-Sayre syndrome, Leigh syndrome, Leber hereditary optic neuropathy, myoclonic epilepsy and ragged red fiber disease, and mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes. Furthermore, recent evidence suggests the possibility that increasing frequency of mutations in mitochondrial DNA with age may contribute to the pathologies of Alzheimer's and Parkinson's disease.

These advances in the diagnosis of genetic neurologic disorders present new challenges for the practicing physician. Presymptomatic genetic testing, developed with great care for HD, has suddenly become possible for a myriad of other disorders. The ethical concerns related to these challenges have not been adequately addressed and will remain a problem for physicians and families. Who will pay for genetic testing? Who can guarantee confidentiality? Can insurance carriers request that genetic testing be accomplished before insurance is underwritten? Who will provide the background evaluation to determine adequate understanding by the patient of the implications of genetic testing? Who will guide the patient through the difficult choices that sometimes must be made about fetal survival after prenatal testing?

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Excitement About Calcium Signaling in Inexcitable Cells

James W. Putney Jr.

Calcium ions act as intracellular messengers that control the functions of cells in many living systems. Traditionally, calcium signaling has been divided into separate categories: studies focusing on electrically excitable cells, such as nerve and muscle, and studies focusing on electrically inexcitable cells, such as epithelial or blood cells. Both excitable and inexcitable cells utilize calcium sequestered in cytoplasmic storage compartments for signaling, but excitable cells rely on a "calcium-induced calcium release" (CICR) mechanism (*1–3*) while, for inexcitable cells, the predominant mechanism for release is triggered by a diffusible messenger, inositol 1,4,5-trisphosphate (IP_3) (*4*). Although signaling in both types of cells is influenced by plasma membrane calcium channels, the channels in the plasma membrane of inexcitable cells apparently are not regulated by membrane potential and their pharmacology is different from that of the voltage-sensitive calcium channels of excitable cells. Recent discoveries, however, have revealed that calcium signaling mechanisms in excitable and inexcitable cells are more similar than previously suspected.

In electrically inexcitable cells, calcium signaling typically is a biphasic process (*5*). Neurotransmitters and hormones stimulate an intracellular organelle to release stored calcium into the cytoplasm, and this release is followed by entry of calcium into the cytoplasm from the extracellular space. The first phase of the calcium signal is attributable to IP_3, a small, polar molecule (*4*). Occupation of plasma membrane receptors activates enzymes that generate IP_3 from plasma membrane phospholipids. The liberated IP_3 then diffuses to specific receptors on an intracellular calcium-storing organelle, either the endoplasmic reticulum or a specialized portion of it called the "calciosome" (*6*). The IP_3 receptor is a ligand-activated, calcium-selective channel. The binding of IP_3 increases the probability of channel opening, which allows calcium to flow into the cytoplasm (*7*). The second phase of the calcium signal likely does not result from the direct action of either a plasma membrane receptor or inositol phosphates, but instead appears to operate through a "capacitative" mechanism (*8, 9*). In capacitative calcium entry, the empty calcium-storing organelle produces a retrograde signal that activates calcium influx across the plasma membrane. An electrical current associated with this entry has been characterized and designated I_{CRAC} (*10, 11*), meaning "calcium release–activated calcium current." The missing link for the capacitative calcium entry theory has been the identification of the signal from the intracellular calcium store.

Enticing clues about the identity of this signal come from the recent work of Randriamampita and Tsien (*12*). A diffusible messenger that is released from intracellular compartments in activated Jurkat cells (a T-lymphocyte tumor cell line) stimulates calcium influx across the plasma membrane in macrophages, astrocytoma

The author is at the National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

cells, and a fibroblast cell line. This messenger, calcium influx factor (CIF), has some interesting properties. As expected, CIF is released into the cytoplasm of Jurkat cells when their intracellular stores of calcium are depleted. However, the total amount of cellular CIF does not appear to increase; rather, it too is released from intracellular organelles (presumably, from the same ones that have released calcium) into the cytoplasm. Membranes appear to be permeable to CIF, because Randriamampita and Tsien demonstrated its activity by adding extracts of activated Jurkat cells to the outside of intact cells. Consistent with this property, some CIF activity was found in the medium surrounding the Jurkat cells after exposure to a calcium-releasing stimulus.

Studies of *Xenopus* oocytes by Parekh and co-workers (*13*) also suggest the existence of an internal messenger that regulates calcium entry. Stimulation of phospholipase C by exogenous serotonin receptors (5-HT_{1c}) expressed in the oocytes increased a calcium-selective conductance, as detected with a patch pipette placed over small areas of the oocyte membrane. When these membrane patches were excised from the cell, the measured calcium current in the patches diminished rapidly, and this current could then be recovered by "cramming" the excised patch back into the stimulated oocyte. Parekh *et al.* also showed that the cellular lifetime of this effect was prolonged by the protein phosphatase inhibitor, okadaic acid. This result suggests that removal of a covalently bound phosphate group by an okadaic acid–sensitive phosphatase may inactivate either the messenger or a protein that has become phosphorylated as a result of the action of the messenger. Consistent with these findings is the fact that CIF contains at least one phosphate group and that the activity of CIF is lost when phosphate was removed (*12*).

Two other groups have demonstrated that capacitative calcium entry depends in some fashion on the hydrolysis of guanosine triphosphate (GTP) (*14*, *15*). Either a heterotrimeric or monomeric GTP-binding protein may be involved, but it must be a protein with activity that requires the hydrolysis of GTP and not simply GTP binding. The GTP-dependent step may occur during the generation of the signal from the intracellular organelle or the action of that signal at the plasma membrane.

In electrically excitable cells, the IP_3-triggered mechanism or the CICR mode of signaling or a hybrid pathway may operate. Intracellular calcium release through the IP_3 receptor in excitable cells may in some instances be coupled to capacitative calcium entry, but this is not always the case (*16*–*18*). Rather, virtually all excitable cells have calcium channels in the plasma membrane which are activated by membrane depolarization. In addition to, or instead of, the IP_3 receptor, many excitable cells express another intracellular calcium-releasing channel, the ryanodine receptor (*1*), whose physiological activator is believed to be calcium. Thus, the channel opens when the local calcium concentration increases rapidly, causing CICR. In heart cells, for instance, a rapid entry of a small amount of calcium through voltage-sensitive calcium channels activates the ryanodine receptor and leads to release from intracellular stores of a large amount of calcium (*2*). A similar scenario may occur in neurons, as shown by the work of Friel and Tsien on bullfrog sympathetic ganglia (*19*).

The common conceptual feature of these two calcium signaling pathways (see figure) is the stringent coordination of calcium entry and intracellular calcium release. Both pathways also amplify the calcium signal, but with distinct functional consequences. In excitable cells, CICR amplifies the magnitude and spatial distribution of the transient calcium signal, supplying sufficient calcium for rapid, all-or-none responses. In inexcitable cells, the retrograde signaling provided by capacitative calcium entry amplifies the duration of the calcium signal, providing for sustained or tonic responses.

Yet even these characteristics of excitable and inexcitable cells are not as separate as once thought. For example, capacitative calcium entry contributes to calcium signaling in a number of excitable cell types (*20*). Also, the all-or-none regenerative calcium signals that occur in nerves and muscles occur in inexcitable cells too (*21*). Thus, although the label "inexcitable" appropriately describes the electrophysiological behavior of their plasma membranes, the regenerative calcium spikes that inexcitable cells often exhibit represent excitable behavior of the intracellular membranes. Earlier studies allowed for a ryanodine receptor–mediated CICR in inexcitable cells; now it is clear that the IP_3 receptor itself can fill this role (*22*, *23*). That is, the IP_3 receptor functions as a CICR recep-

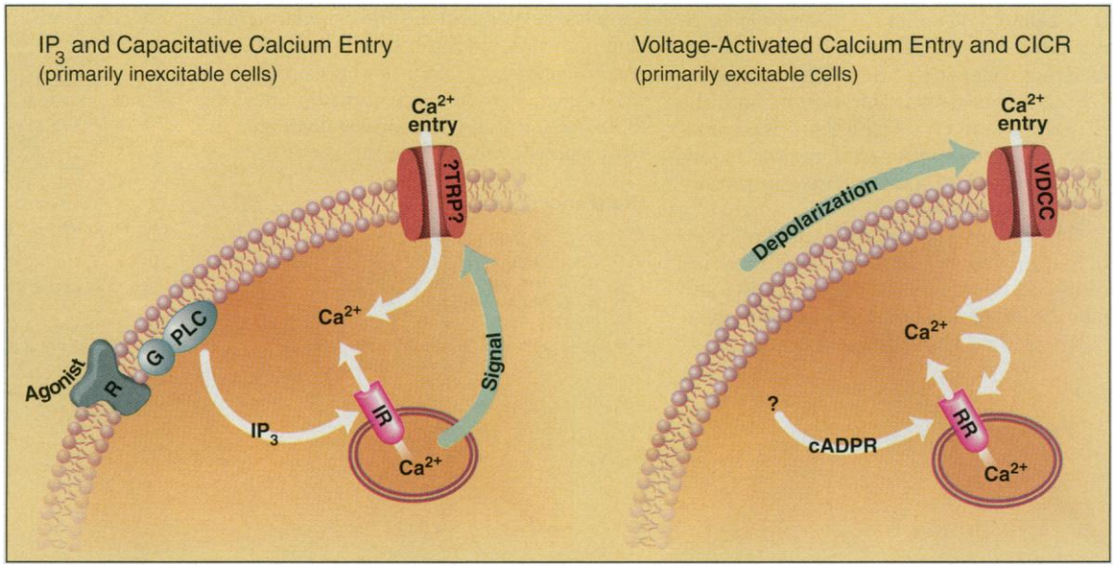

ILLUSTRATION: KATHARINE SUTLIFF

Calcium signaling motifs. In electrically inexcitable cells (**left**), signaling is generally initiated when an agonist activates a surface membrane receptor (R) that, usually through a G protein (G), activates phospholipase C (PLC), which degrades phosphatidylinositol 4,5-bisphosphate, releasing the soluble messenger, inositol 1,4,5-trisphosphate (IP_3). The IP_3 activates an IP_3 receptor (IR) and thus releases calcium from an intracellular organelle to the cytoplasm. The release of calcium from the organelle causes a signal to be generated, which activates a plasma membrane calcium entry pathway (capacitative calcium entry), perhaps through a channel similar to the *trp* gene product in *Drosophila*. In electrically excitable cells, patterns of calcium signaling are somewhat more variable. Calcium may enter cells when voltage-dependent calcium channels (VDCC) are activated by the depolarization associated with action potentials. This calcium can cause further release of intracellularly stored calcium by activating a CICR mechanism associated with the ryanodine receptor (RR) calcium channel. This ryanodine receptor calcium channel may, in some instances, be regulated by cyclic adenosine diphosphate ribose (cADPR). By analogy with IP_3, there may be mechanisms regulating the cellular levels of cADPR.

tor with a sensitivity to calcium that is regulated by the binding of IP_3, and vice versa. Perhaps this is not so surprising in light of the similarity between the amino acid sequences of the IP_3 and ryanodine receptors (*24*). It appears that electrically inexcitable cells may contain only a single, homogenous pool of intracellular calcium that is sensitive to IP_3 (*25*), and that electrically excitable cells may have a more complex arrangement of intracellular calcium pools regulated by different mechanisms (*26*).

Although the ryanodine receptor has been thought to be regulated primarily through a CICR pathway, a small, water-soluble molecule that can function as a regulatory ligand for at least some forms of the ryanodine receptor has been discovered: cyclic adenosine diphosphate ribose (cADPR) (*27*). The cADPR molecule functions similarly to IP_3; it increases the probability of channel opening of the ryanodine receptor by increasing its calcium sensitivity. It is tempting to speculate that calcium signaling in neurons may be regulated by cADPR, and there is recent evidence suggesting that this may be so (*28, 29*). In the sea urchin oocyte, both the IP_3 receptor and a cADPR-sensitive ryanodine receptor appear to function redundantly to produce a regenerative intracellular calcium signal (*30, 31*).

The plasma membrane calcium channels regulated by the capacitative mechanism and by membrane depolarization also may be more similar than thought previously. A blind *Drosophila* mutant, *trp*, is incapable of maintaining a sustained photoreceptor potential (*32*). This phenotype is mimicked by lanthanum, a blocker of calcium channels, suggesting that the deficit is related to a failure of calcium entry. The photoreceptor signaling mechanism in insects utilizes an IP_3 signaling system, and thus it may be that the normally sustained photoreceptor potential depends on capacitative calcium entry. Interestingly, when the gene encoding *trp* was cloned and sequenced, sequence similarity was detected between this protein and the $\alpha 1$ subunit of the voltage-sensitive calcium channel (dihydropyridine receptor) (*33*), which is believed to contain the ion-conducting portion of the channel. As might be expected, *trp* does not contain the charged residues in the S4 transmembrane segment that are believed to provide the membrane potential sensor for voltage-sensitive channels.

Recognition of diversity and similarity in calcium signaling mechanisms becomes especially important in view of recent findings on the relation between patterns of gene expression and specific calcium signaling pathways (*34, 35*). It will be useful for neuroscientists, as well as those interested in calcium signaling in electrically inexcitable cells, to take note of developments in this rapidly evolving field.

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