Cyclic ADP–Ribose: A New Way to Control Calcium

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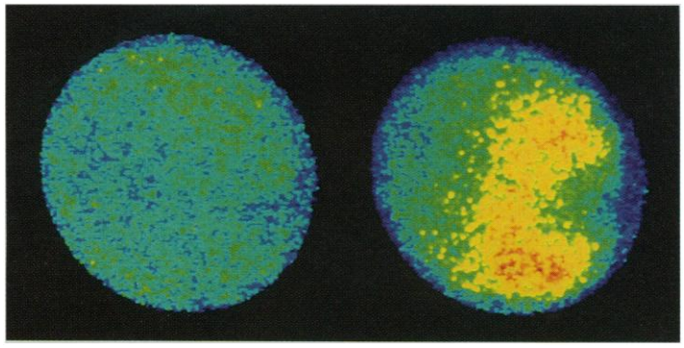
Calcium is released from organelles inside cells into the cellular cytoplasm (*1*), where it triggers such key events as muscle contraction and secretion, and can even regulate metabolic pathways. Calcium mobilization, as this phenomenon is called, is often initiated by signals outside the cell that activate receptors coupled to phospholipases—such signals as hormones, neurotransmitters, and sperm. Then the second messenger inositol 1,4,5 trisphosphate (IP_3), a water-soluble phosphorylated sugar, is generated at the membrane, diffuses to intracellular sites of calcium sequestration such as the endoplasmic reticulum, and causes calcium release (*2*). It now seems that calcium mobilization can also be mediated by another second messenger. A molecule first found in sea urchin eggs—cyclic adenosine diphosphate ribose (cADPR)—is not only more potent than IP_3 in releasing calcium, but is active in a number of mammalian cell types, and furthermore the biochemical machinery for its synthesis and degradation is present in all cells so far examined (*3*). Indeed, in this issue of *Science*, Okamoto and colleagues (*4*) report that cADPR is a second messenger for glucose-induced insulin release from the endocrine pancreas.

Cyclic ADPR, a low molecular weight metabolite of the pyridine nucleotide, nicotinamide adenine dinucleotide (NAD^+), was discovered by Hon Cheung Lee and his colleagues when studying calcium release by microsomes derived from the endoplasmic reticulum of sea urchin eggs (*5*). These vesicles sequester calcium against a large concentration gradient via an adenosine triphosphate–hydrolyzing calcium pump and release calcium after application of IP_3 to the extravesicular space. This result is consistent with the notion that the massive wave of calcium mobilization seen at fertilization of the sea urchin egg is mediated by IP_3 (*6*). However, Lee and his colleagues screened other metabolites for calcium-releasing activity. Among the first to be tested was NAD^+ because pyridine nucleotide concentrations change dramatically after fertilization (*7*). NAD^+ unexpectedly released nearly as much calcium as IP_3, but unlike the immediate IP_3 response there was a lag of 1 to 4 minutes (*8*). In fact NAD^+ itself does not have calcium-releasing activity: the lag was due to the time required for its enzymatic conversion to a metabolite. This compound was later identified by nuclear magnetic resonance and mass spectroscopy as cADPR (*9*), which is synthesized from NAD^+ by the ADP-ribosyl cyclases. ADP-ribosyl cyclase activities are present in many mammalian and invertebrate tissues (*10*), suggesting that cADPR may function widely as a calcium mobilizing agent. Two types of the enzyme have been recognized: the first is a 29-kilodalton cytosolic form that has been purified and cloned from *Aplysia* ovotestis (*11*). The other form is larger and is associated with the plasma membrane of mammalian cells (*12*). How these enzymes are regulated is unclear; however, the *Aplysia* enzyme sequence is homologous to that of the human lymphocyte surface antigen CD38 (*13*). Perhaps there is a family of cell surface receptors that are ADP-ribosyl cyclases and that can be regulated by extracellular ligand binding.



Cyclic ADPR lights up the egg. Microinjection of cADPR into sea urchin eggs results in an increase in intracellular calcium concentration from 100 nM in resting eggs (blue and green) to 900 nM in stimulated eggs (red and yellow). Eggs contain the calcium indicator dye fura-2.

Cyclic ADPR mobilizes calcium by a mechanism completely independent of the IP_3 receptor (*5*). Although there is a specific binding site for cADPR on sea urchin egg microsomes, its nature is unknown (*12*). When a second class of intracellular calcium release channel—the ryanodine receptor—was found in sea urchin eggs (*14*), the possibility that cADPR was an endogenous regulator of ryanodine receptors arose. Ryanodine receptors were first characterized as the major calcium release channel of muscle sarcoplasmic reticulum and share substantial similarities with IP_3 receptors in that they are both transmembrane proteins with large cytoplasmic domains and a quatrefoil arrangement of four identical subunits (*15*). A key property of ryanodine receptors, first recognized from studies on isolated sarcoplasmic reticulum and then confirmed by channel isolation and reconstitution in artificial bilayers, is that they can be opened by calcium, that is, they can mediate calcium-induced calcium release (CICR). Ryanodine receptors are widespread in many cell types and have been incorporated in many models to explain the spatial and temporal complexity of cellular calcium signals, which can be oscillatory (*16*) and can propagate as regenerative planar or spiral waves (*17*). On the basis of cross-desensitization studies and experiments with known ryanodine receptor antagonists—ryanodine, ruthenium red, and procaine—cADPR was shown to release calcium via this ryanodine-sensitive CICR mechanism in the sea urchin egg, suggesting that cADPR is an endogenous regulator of CICR through a ryanodine receptor rather than through an IP_3 receptor (*3,14*). This hypothesis has been supported by the demonstration that cADPR at nanomolar concentrations, but not IP_3, mimics caffeine (a ryanodine receptor activator) in inducing oscillations in a calcium-dependent ion current known to reflect intracellular calcium oscillations in rat dorsal root ganglion cells (*18*).

Although cADPR has been shown to have calcium mobilizing activity in a number of cell types including sea urchin eggs (*5*), pituitary cells (*19*), and dorsal root ganglion cells (*18*), it has not been clear whether cADPR functions as a second messenger for extracellular stimuli. Rather, cADPR could be a cofactor for CICR, simply enhancing the sensitivity of its receptor to calcium; this notion would be consistent with the fact that calcium and cADPR potentiate the effects of each other (*18*) and that cADPR itself is present in many mammalian cell types under resting conditions (*20*). However, a major advance in identifying cADPR as a new calcium-mobilizing second messenger has come from the finding that glucose induces a rise in cADPR concentrations in pancreatic β cells, and moreover cADPR stimulates insulin secretion from permeabilized β cells (*4*). Furthermore, cADPR seems to release calcium from β cell microsomes via a ryanodine-sensitive mechanism consistent with the proposed mechanism for this agent's actions in regulating CICR (*3*). Indeed, a CICR mechanism sensitive to caffeine has recently been reported in a β cell line (*21*). The generality

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of cADPR as both a calcium-mobilizing agent and endogenous activator of a ryanodine-like receptor is also indicated by the demonstration by Okamoto and colleagues of cADPR-induced calcium release by an IP_3-insensitive, but ryanodine-sensitive, mechanism in cerebellar microsomes (*4*). No effect of IP_3 was observed either on β cell microsomal calcium release nor insulin secretion, in contrast to previous studies in cultured cells (*22*).

Although many agents have been suggested as important intracellular mediators of nutrient-induced insulin secretion, cADPR is particularly attractive because a role for this molecule in stimulus-secretion coupling in the β cell can explain the mechanisms of a class of diabetogenic drugs, the β-cytotoxins such as streptozotocin. These agents deplete intracellular NAD^+, the precursor of cADPR (*23*). As a result, streptozotocin not only abolishes insulin secretion in response to glucose but also abolishes the glucose-induced synthesis of cADPR, suggesting an essential role for cADPR in β cell function (*4*). It is possible that the well-documented changes in the amounts of pyridine nucleotides induced by glucose in β cells (which precede any changes in intracellular calcium concentrations) (*24*) are required for cADPR synthesis. If so, such changes, which can be followed simply by monitoring pyridine nucleotide autofluorescence (even in concert with calcium measurement by fluorescent indicators), would be a useful initial screen for stimuli that employ the ADP-ribosyl cyclase system to generate calcium signals.

Single β cells show a pronounced spatiotemporal complexity in calcium signals, with large oscillations that propagate as waves and mirror oscillatory changes in membrane potential (*25*). The frequency of calcium spiking, but not the amplitude of the spikes, is increased by raising the extracellular glucose concentrations, leading to the possibility that the calcium signal in β cells is frequency modulated, as proposed for other cells (*16*). Because calcium influx is required for β cell calcium spiking and wave generation (*25*), but such calcium excitability is thought to be a property of the calcium release mechanisms of intracellular calcium stores (*16,17*), it is possible that influx of calcium through voltage-gated channels of the plasma membrane and the increase in cADPR (both stimulated by glucose) together trigger CICR. Such synergy could in turn produce calcium spikes, as in dorsal root ganglion cells (*18*), and amplify the calcium signals as intracellular waves. This scheme is consistent with the finding that streptozotocin abolishes glucose-induced waves and oscillations (*26*) by perhaps reducing cADPR synthesis.

The structural analogies between the two classes of intracellular calcium release channel, IP_3 receptors and ryanodine receptors, have now been mirrored by functional similarities. IP_3 receptors can also mediate CICR (*27*). In fact, an underlying characteristic of intracellular calcium release channels seems to be that they mediate calcium-activated calcium release that can be modulated by specific cytoplasmic agonists like IP_3 or cADPR and can alone or together give rise to the rich diversity of calcium signalling profiles seen in cells. Their location and relative abundance, together with local concentrations of modulators and calcium, will determine which channel operates at a particular spatial and temporal coordinate. The indication by the work of Okamoto and colleagues (*4*), that cADPR may function as a second messenger for calcium release in β cells, strengthens the notion that there is at least a duality in signalling pathways controlling calcium fluxes across the endoplasmic reticulum. Improvement in techniques for measuring cADPR in cells will help to identify other physiological processes regulated by this provocative calcium signalling pathway.

References

1 M F Rossier and J W Putney, Jr , *Trends Neurosci* **14**, 310 (1991)
2 M J Berridge and R F Irvine, *Nature* **341**, 197 (1989)
3 A Galione, *Trends Pharmacol Sci* **13**, 304 (1992)
4 S. Takasawa, K Nata, H Yonekura, H Okamoto, *Science* **259**, 370 (1993)
5 P J Dargie, M C Agre, H C Lee, *Cell Regul* **1**, 279 (1990)
6 M J Whitaker and R F Irvine, *Nature* **312**, 636 (1984)
7. D Epel, *Sci Am.* **237**, 128 (November 1977)
8 D L Clapper, T F Walseth, P J Dargie, H C Lee, *J Biol Chem* **262**, 9561 (1987)
9 H C Lee *et al* , *ibid* **264**, 1608 (1987)
10 N Ruskino and H C Lee, *ibid* , p 11725
11 H C Lee and R Aarhus, *Cell Regul* **2**, 203 (1991), M R Hellmich and F Strumwasser, *ibid* , p 193, D L Glick *et al* , *ibid* , p 1211
12 H C Lee, *J Biol Chem* **266**, 2276 (1991)
13 D J States, T F Walseth, H C Lee, *Trends Biochem Sci* **17**, 495 (1992)
14 A Galione, H C Lee, W B Busa, *Science* **253**, 1143 (1991)
15 S Fleischer and M Inui, *Annu Rev Biophys Chem* **18**, 333 (1989)
16 M J Berridge and A Galione, *FASEB J* **2**, 3074 (1988)
17 J Lechleiter, S Girard, E Peralta, D Clapham, *Science* **252**, 123 (1991)
18 K. Currie, K Swann, A Galione. R H Scott, *Mol Biol Cell* **3**, 1415 (1992)
19 H Koshiyama, H C Lee, A H Tashjian, *J Biol Chem* **266**, 16985 (1991)
20 T F Walseth, R Aarhus, R J Zelznikar, Jr , H C Lee, *Biochim Biophys Acta* **1094**, 113 (1991)
21 M S Islam, P Rorsman, P -O Berggren, *FEBS Lett* **296**, 287 (1991)
22 M Prentki *et al* , *Nature* **309**, 562 (1984)
23 H Yamamoto, Y Uchigata, H Okamoto, *ibid* **294**, 284 (1981)
24 W -F Pralong, C Bartley, C B Wollheim, *EMBO J* **9**, 53 (1990)
25 E Gylfe, E Grapengiesser, B Hellman, *Cell Calcium* **12**, 29 (1991)
26 E Grapengiesser, E Gylfe, B Hellman, *Toxicology* **63**, 263 (1990)
27 M Iino and M Endo, *Nature* **360**, 76 (1992)