Cell physiology

A real receptor-operated calcium channel?

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SOME of the mechanisms by which activator calcium enters stimulated cells are increasingly well understood as the structure and function of voltage-gated calcium channels are elucidated; others remain more elusive. The existence of receptor-operated calcium channels (ROCCs) was suggested on the basis of smooth muscle responses which seemed to depend on calcium entry, when membrane potential did not change, or when voltage-gated calcium channels were blocked by specific 'calcium antagonists' (ref. 1). ROCCs have also been postulated to explain calcium entry into cells that are not electrically excitable, as in thrombin-stimulated blood platelets. Now Zschauer and colleagues report on page 703 of this issue[2] the demonstration of single channels with the expected properties of ROCCS.

Although ROCCs are an attractive way to explain phenomena such as those mentioned above, it was not clear whether they could be explained by other means, for example, other transduction processes requiring external Ca^{2+} to generate some other intracellular message, or voltage-gated channels not affected by the calcium antagonists. More recently, it has been shown in cells that seem to lack voltage-gated calcium entry, including platelets, parotid-gland and endothelial cells[3-5], that receptor-mediated elevation of cytosolic calcium is greater and more prolonged in the presence of external calcium than in calcium-free medium. This suggests, but does not prove[6], that receptor occupation stimulates calcium entry through some form of ROCC.

Additional support comes from the demonstration[7] of stimulated uptake of radio-labelled $^{45}Ca^{2+}$ and of stimulated entry[8] of another divalent cation, Mn^{2+}. There are many ways that receptor occupation could be coupled to calcium entry, including a direct effect on a subunit of the channel, analagous to the nicotinic acetylcholine receptor–channel complex; a G-protein; the action of Ca^{2+} itself; inositol phosphates; or the action of a protein kinase. Moreover, there are various possible mechanisms for the calcium transport, including a transmembrane channel, or pore, across the plasma membrane; an ionic exchanger; or a specialized pathway direct from the external medium to the interior of an internal organelle (see ref. 5). For many, the only convincing evidence of ROCCs would be the demonstration of calcium currents through single channels that show no voltage dependence in opening and closing.

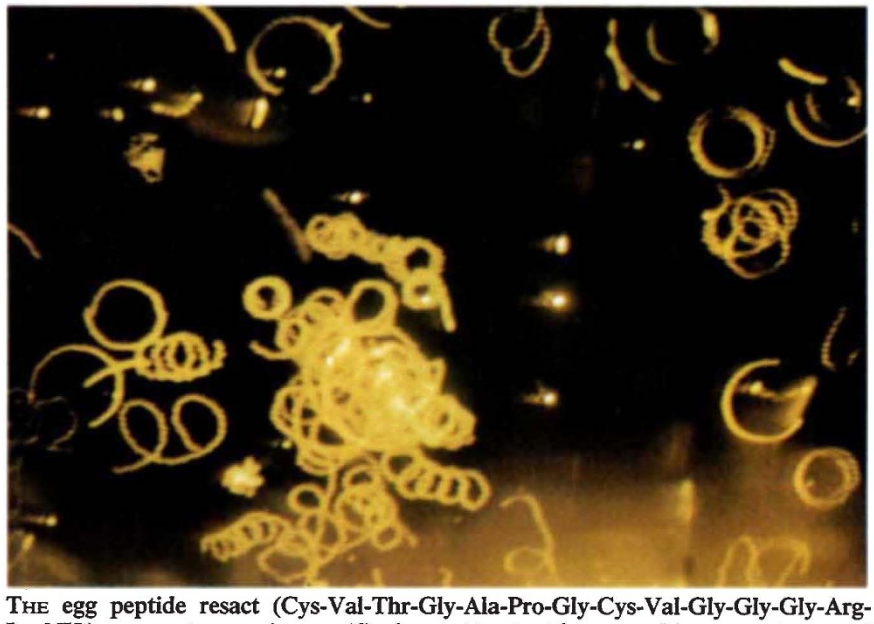
This type of evidence has been difficult to obtain. One of the first convincing examples was the ATP channel examined by patch-clamp in isolated smooth muscle cells, recently reported by Benham and Tsien[8], which is fairly selective for calcium over sodium. In T lymphocytes[9], and now in mast cells[10], evidence has been presented that intracellular inositol trisphosphate ($InsP_3$) can promote calcium-channel activity. A role for inositol tetrakisphosphate ($InsP_4$) in calcium entry remains controversial[10].

Perhaps the most striking demonstration of a ROCC is that reported by Zschauer *et al.* in this issue[1]. The authors have incorporated membrane vesicles from resting and thrombin-stimulated platelets into planar bilayers, and observe single channels 30-fold selective for the divalent cation Ba^{2+} over Na^+ in vesicle-stimulated cells. The conductance, with symmetrical solution containing 150 mM Ba^{2+} (a commonly used surrogate for Ca^{2+} in such experiments) is about 10 pS in the concentration range reported for voltage-gated calcium channels in similar conditions. The membrane fraction clearly contains plasma membrane, but it cannot be excluded that the channel activity comes from contaminating intracellular organelles.

The opening and closing kinetics of the channels derived from platelet vesicles are not influenced by membrane potential or by 10 μM nisolidipine, a potent dihydropyridine antagonist of 'L-type' voltage-gated channels. But these channels are blocked by Ni^+, which is known to block thrombin-stimulated calcium entry into platelets[6]. Thus, the results of Zschauer and co-workers do point to real ROCCs in thrombin-stimulated platelets, as we had earlier proposed from more indirect evidence[3,6].

It is to be hoped that Zschauer and colleagues go on to show that Ca^{2+} itself is permeable through these channels, and to examine the effects of a different class of calcium antagonists. An intriguing and surprising point emerging from their data is that the thrombin-induced channels survive the cell-fractionation procedure. This result suggests that either some covalent modification, perhaps phos-

Chemoattractant for sea urchin sperm



THE egg peptide resact (Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-LeuNH_2) represents a species-specific chemoattractant for sea urchin spermatozoa and is the first natural animal sperm chemoattractant identified. Here, approximately 1 picolitre of a 100-nM resact solution was added to a drop of spermatozoa; within seconds the cells congregate at the point of injection. The tracks represent the movement of the sperm cell over a 2-second exposure. Resact binds to a spermatozoan plasma membrane protein identified as the enzyme guanylate cyclase, whose primary structure is presented by D.V. Goeddel and collaborators on page 708 of this issue. The spiral pattern in the centre of the picture describes the path the sperm cell takes when moving in the gradient. Before it detects the gradient, the sperm cell swims in a circle, seen here on the outside of the picture. (Photo, L.J. Dangott.) □