versatile tool for direct measurements of local membrane compliances and of forces exerted by single molecules on live cells.

This promises to be an extremely powerful tool for probing molecular interactions at cell surfaces. Although the limitations in the lateral spatial resolution and in the distance resolution normal to the interface do not permit molecular scale definition of the cell surface, the ability 1) to quantify both the static and dynamic properties of single cell surface receptors in their native environments and 2) to characterize local membrane deformations in response to focal attachments is currently unmatched. It is interesting to speculate on the potential information that will be gained from future studies with this method. Perhaps one of the more intriguing questions in cell adhesion research is how the rate of increasing the force on a bond impacts both the measured bond strength and the detachment rate (Dembo et al., 1988; Kaplanski et al., 1993). In addition, what influence does the microenvironment have on cell surface receptor function? Preliminary results suggest that it will also be possible to detect dynamic responses to the formation of receptor-ligand bonds, and this opens up exciting possibilities for elucidating the molecular mechanisms that couple receptor-ligand bond formation to a number of ligand-induced cell responses.

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Burst Busters: Uncovering a New Mechanism in Pancreatic β-Cells

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In this issue of *Biophysical Journal*, Bertram et al. present evidence that a nonspecific cation conductance that is activated by calcium depletion of internal stores plays an important role in bursting electrical activity and insulin secretion in pancreatic β-cells — as if the mechanisms underlying secretion in β-cells were not complex enough already! Located in the pancreatic islets of Langerhans, β-cells are responsible for the synthesis, storage, and exocytosis of insulin granules. The primary endogenous secretagogue for insulin is glucose, and therein lies the complexity. Rather than using a receptor, glucose stimulates secretion only after being taken up through specific glucose transporters (GLUT2) and then metabolized via the glycolytic pathway, the Krebs cycle, and oxidative phosphorylation.

According to the traditional paradigm, increases in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) are responsible for triggering exocytosis. So how does glucose metabolism accomplish this? Two classes of mechanisms are at work here: those that mediate Ca^{2+} influx through the plasma membrane and those involving Ca^{2+} uptake and release from internal compartments. Plasma membrane influx of Ca^{2+} is complicated by the fact that β-cells are electrically excitable. In an islet perifused at low glucose concentrations (≤5 mM), microelectrodes register stable membrane potentials of about −65 mV. This resting potential is determined primarily by large numbers of ATP-sensitive potassium channels (KATP) that are active at resting concentrations of ATP (Satin and Smolen, 1994). At higher concentrations of glucose (10–16 mM), elevated ATP concentrations due to metabolism inhibit these channels, depolarizing the β-cell to such a degree that voltage-activated Ca^{2+} channels now become active, further depolarizing the cell. This self-regenerating depolarization, however, is counteracted by delayed-rectifier K^+ channels that partially repolarize the membrane. This occurs on a time scale of several hundred milliseconds and produces action potential-like spikes of electrical activity that, because the KATP channels remain inactive, repeat over and over again. During this so-called "active phase," each action potential spike (~20 mV in amplitude) brings a small increment of Ca^{2+} into the cell while at the same time the plasma membrane gradually repolarizes over the course of ~15 s.

Although the origins of this slow hyperpolarization have been attributed variously to slow voltage inactivation of Ca^{2+} channels, Ca^{2+}-activated K^+ channels (KCa), and the indirect activation of KATP channels by Ca^{2+} uptake into the mitochondria, the ultimate effect is a sudden drop of membrane potential and a "silent" phase lasting ~10 s. During this period, Ca^{2+} influx is minimal, and simultaneous measurements using fluorescent Ca^{2+} dyes reveal a slow decrease in $[Ca^{2+}]_i$. At the same time, the membrane slowly depolarizes until another volley of action potential spikes occur. These repetitive bursts of electrical activity occur in phase with both an elevated plateau in $[Ca^{2+}]_i$ and increases in the rate of insulin secretion. At even higher concentrations of glucose (20 mM), the silent phase disappears and only continuous spiking is observed. Using extensive data collected by electrophysiologists, a number of realistic kinetic models of bursting electrical activity have been developed (Satin and Smolen, 1994).

What makes β-cell Ca^{2+} handling really complicated is that plasma membrane influx is strongly coupled to uptake and release by internal stores,

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especially the mitochondria and the endoplasmic reticulum (ER). Recent measurements using the fluorescent dye, aequorin, specifically targeted to the mitochondrial matrix in INS-1 cells (Rutter et al., 1993) suggest that as much as 20% of the Ca^{2+} that enters during depolarization is taken up rapidly into the mitochondria, where it remains for a minute or so. Similarly, experiments using thapsigargin, a specific inhibitor of the ER Ca^{2+}-ATPase pump, have shown that the dip in $[Ca^{2+}]_i$ after stimulation with glucose is due to Ca^{2+} uptake into the ER. Furthermore, the work of Ämmälä et al. suggests that muscarinic agonists, which potentiate insulin secretion via parasympathetic inputs, can induce a distinct type of $[Ca^{2+}]_i$ oscillation that involves inositol 1,4,5-trisphosphate (IP_3). Computer simulations by Keizer and DeYoung that coupled a kinetic model of agonist-induced Ca^{2+} handling and glucose-induced electrical activity revealed a strong influence of membrane potential on IP_3-dependent Ca^{2+} oscillations. Those simulations also showed that the apamine-insensitive KCa conductance found by Ämmälä et al. could explain quantitatively the observed agonist-induced interruptions in continuous electrical activity observed at high glucose concentrations.

Evidence that a cation current activated by Ca^{2+} depletion of the ER also couples the plasma membrane and ER Ca^{2+}-handling mechanisms has surfaced recently (Worley et al., 1994). Depletion-activated currents that are selective for Ca^{2+} have been reported in a number of electrically inactive cell types (Hoth and Penner, 1992). Loosely referred to as I_{crac} (for Ca^{2+} release-activated Ca^{2+} current), the maximum value of these currents is typically 10–20 pA and, if carried by ion channels, they would have the smallest unitary conductance yet known (~25 fS).

In their joint theoretical/experimental collaboration, Bertram et al. (1995) provide evidence that an I_{crac}-like activity in β-cells can explain the puzzling effects of muscarinic agonists on bursting. When applied to an islet in the presence of 11.1 mM glucose, 100 μM carbachol triggers a rapid hyperpolarization and after 2–3 min leads to "muscarinic bursts." These bursts have a more depolarized silent phase than with glucose alone, and only a few spikes in the active phase. Measurements of $[Ca^{2+}]_i$ after carbachol stimulation show a rapid rise, followed by a slow decrease upon which brief increases in $[Ca^{2+}]_i$ caused by the muscarinic bursts are superimposed. Because carbachol activates IP_3-induced Ca^{2+} release from the ER, the authors reasoned that these observations could be explained by 1) a rapid loss of Ca^{2+} from the ER that leads to 2) hyperpolarization via the apamine-insensitive KCa conductance and, finally, to 3) depolarization via I_{crac} into the voltage range where muscarinic bursts can occur.

This explanation was explored quantitatively using a kinetic model that relies on slow voltage inactivation of a Ca^{2+} current for glucose-induced bursting to which a nonselective I_{crac}-like cation current was added along with a model for IP_3-induced ER Ca^{2+} oscillations. In combination, the two models provide a quantitative description of muscarinic bursting and the effects of thapsigargin (Tg) and diazoxide on the bursts. In the model, as in the experiments, Tg transforms the normal (11.1 mM) glucose-bursting pattern in a dose-dependent manner: at low concentrations (1 μM), lengthening the active and shortening the silent phase and, at higher concentrations (5 μM), producing continuous spiking. Diazoxide, an enhancer of the KATP channel, changes Tg-induced continuous spiking into muscarinic bursting.

There are two provocative suggestions in the paper. First, that repolarization during the silent phase in muscarinic bursts is due to apamine-insensitive KCa channel activation, predominately via Ca^{2+} released from the ER; and second, that the long transient phase of continuous spiking preceding glucose-induced bursting can be explained by changes in the filling state of the ER. The idea here is that the ER store is relatively empty in the resting state when Ca^{2+} influx is suppressed but fills in the presence of glucose, which then attenuates I_{crac}. This produces the additional hyperpolarization required to initiate glucose-induced bursting.

Although it is too early to judge whether these suggestions are correct, they seem quite plausible at this stage. Furthermore, they are compatible with earlier observations of cation currents associated with muscarinic stimulation of β-cells. If this scenario holds up to further scrutiny, it will provide yet another example of the complex checks and balances that β-cells have evolved to regulate $[Ca^{2+}]_i$.

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