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The Ca^{2+}-ATPase SERCA3 influences membrane potential and the pattern of cytosolic $[Ca^{2+}]_c$ oscillations in glucose -stimulated mouse β-cells

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Background and aims: The low-affinity Ca^{2+}-ATPase SERCA3 is highly expressed in β-cells. It mediates uptake of cytosolic Ca^{2+} by the endoplasmic reticulum during each period of Ca^{2+} influx from the extracellular medium, whereas the high-affinity SERCA2b controls basal $[Ca^{2+}]_c$. For unknown reasons, β-cells within mouse islets display variable patterns of $[Ca^{2+}]_c$ oscillations during glucose (G) stimulation. These oscillations can be either regular and fast, or mixed with rapid oscillations superimposed on slow ones. We studied the role of the membrane potential (MP) and the possible intervention of SERCA3 in the generation of both types of $[Ca^{2+}]_c$ oscillations.
Materials and methods: All experiments were performed with single islets from normal or SERCA3 KO mice. MP was measured with intracellular microelectrodes. In some experiments, $[Ca^{2+}]_c$ (fura PE3) was monitored simultaneously with MP or insulin release. The expression of SERCA isoforms was studied by semiquantitative RT-PCR and real-time PCR.
Results: Control islets stimulated with 7-15 mmol/l G displayed rapid and regular (3–4.min^{-1}), or mixed (0.2-0.3.min^{-1}) $[Ca^{2+}]_c$ oscillations. Mixed $[Ca^{2+}]_c$ oscillations were observed in more than 50% of the islets stimulated with 8 mmol/l G. Simultaneous recordings of MP in single β-cells within an islet and $[Ca^{2+}]_c$ in the whole islet demonstrated that the mixed pattern did not result from the coexistence of two subpopulations of β-cells with distinct patterns of electrical activity, but from a peculiar electrical activity in each β-cell of the islet: a series of rapid MP oscillations separated by prolonged silent intervals. Each slow $[Ca^{2+}]_c$ increase during mixed oscillations was due to a progressive summation of fast oscillations. Simultaneous measurements of $[Ca^{2+}]_c$ and insulin release revealed that each mixed $[Ca^{2+}]_c$ oscillation triggered a synchronous oscillation of insulin secretion. The role of SERCA3 in the control of MP and $[Ca^{2+}]_c$ was evaluated using islets from SERCA3 KO mice. Ablation of SERCA3 did not alter the expression of other SERCAs as compared with control islets: no expression of SERCA1a or 1b, weak expression of SERCA2a and clear expression of SERCA2b. During stimulation with 7–15 mmol/l G the electrical activity of SERCA3 KO β-cells was different from that of control β-cells. Thus, MP oscillations were characterized by a lower frequency (average -60%) but larger depolarization phase (average +70%). Importantly, MP oscillations were consistently regular. The peculiar pattern with changes in the frequency was never observed, nor was the mixed pattern of $[Ca^{2+}]_c$ oscillations.
Conclusion: The regular or mixed $[Ca^{2+}]_c$ oscillations in G-stimulated islets result from two distinct patterns of electrical activity in all β-cells of the islet. Disruption of SERCA3 abolishes both the periodic electrical activity and the mixed $[Ca^{2+}]_c$ oscillations, and augments β-cell depolarization. These results suggest that the endoplasmic reticulum participates in the control of the β-cell MP during G stimulation.

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Ryanodine receptors and transient receptor potential channels provide a distinct mechanism for depolarization of β-cells

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Background and aims: Depolarization of plasma membrane potential from about -70 to about -40 mV is a critical event in the process of stimulation of insulin secretion by glucose and incretin hormones. Closure of the K_{ATP} channels is widely known to mediate such depolarization. However, closure of the K_{ATP} channels alone cannot fully account for the depolarization in this range unless there is a depolarizing inward current. It has been demonstrated that β-cells have transient receptor potential (TRP) superfamily of cation channels as well as intracellular Ca^{2+} channels like the ryanodine (RY) receptors. It is unclear how these channels contribute to the signalling mechanisms involved in stimulus-secretion coupling. We investigated whether activation of TRP channels could mediate membrane depolarization and we have identified a mechanism that might activate the TRP channels.
Materials and methods: $[Ca^{2+}]_i$ was measured from fura-2 loaded single rat insulinoma cells (S5 cells) derived from INS-1 cells using a microscope-based fluorescence system from PTI. Membrane potential was measured by perforated-patch whole cell configuration of the patch-clamp technique.
Results: We first activated ryanodine receptors by 9-methyl, 5,7-dibromo eudistomin D (MBED) and found that this resulted in a prolonged $[Ca^{2+}]_i$-plateau, in addition to the Ca^{2+} release from the endoplasmic reticulum (ER) and Ca^{2+}-induced Ca^{2+} release. The $[Ca^{2+}]_i$-plateau disappeared on ommission of Ca^{2+} from the extracellular medium suggesting that the plateau represents Ca^{2+} entry across the plasma membrane. We studied properties of the Ca^{2+} entry pathways by studying the effects of pharmacological tools on the $[Ca^{2+}]_i$. These results further suggest that activation of the RY receptors can cause activation of a group of Ca^{2+}-permeable channels located on the plasma membrane. The consequence of activation of these plasma membrane Ca^{2+} channels on the membrane potential was investigated by patch-clamp technique. Activation of RY receptors and consequent activation of the plasma membrane Ca^{2+} channels depolarized membrane potential from -70 mV to -40 mV. The Ca^{2+}entry was blocked by membrane depolarization, SKF 96365, 2-aminoethoxydiphenylborate and $LaCl^{3+}$. Rather surprizingly, it was not altered by $GdCl^{3+}$. Ca^{2+}-entry was not blocked by nimodipine and was not altered by diazoxide. Ca^{2+} entry was observed even when the ER Ca^{2+} pool was emptied by prior treatment with thapsigargin. Replacement of extracellular Na^+ by choline or prior application of carbachol did nor alter Ca^{2+} entry. Ca^{2+} entry deactivated, apparently spontaneously after about five minutes and was activated again in an oscillatory nanner.
Conclusion: The properties of the Ca^{2+} entry demonstrated in this study suggests that it is likely mediated trrough the TRP channels. Such Ca^{2+} entry depolarizes membrane potential even when K_{ATP} channels are opened by diazoxide. Furthermore, we demonstrate that the presumptive TRP channels can be activated as a consequence of activation of RY receptors. This activation is not essentially dependent on the filling state of the ER and could be due mediated by coupling of RY receptors to the TRP channels. Our results suggest that RY receptors and the TRP channels provide a distinct mechanism for membrane depolarization within the critical range of -70 mV to -40 mV. This mechanism may be an important component of signal transduction and stimulus-secretion coupling in β-cells.
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