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The Role of the Transient Receptor Potential Channels and the Intracellular Ca^{2+} Channels in Ca^{2+} Signaling in the β-cells

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Thesis abstract

Previous studies from our group reported that pancreatic β-cells express ryanodine receptors (RyRs) that can mediate Ca^{2+}-induced Ca^{2+} release (CICR). The full consequences of the activation of RyRs on Ca^{2+} signaling in these cells, however, remained unclear. An important open question was whether activation of the RyRs leads to activation of any Ca^{2+} channels in the plasma membrane, and thereby depolarizes membrane potential. One main aim of the thesis was to address this question. As a corollary, we have also looked for the existence of functional TRPV1 channels, and have elucidated the molecular mechanisms that underlie the $[Ca^{2+}]_i$-elevating effect of ADP ribose in these cells.

We used methods such as measurement of the $[Ca^{2+}]_i$ in single cells loaded with fura-2, patch clamp technique, Western blot analysis, immunohistochemistry, a variety of pharmacological tools, and a series of carefully designed protocols. In most experiments, we used S5 cells, derived from the rat insulinoma cell line INS-1E, but we also used primary β-cells from mice, rat, and human.

Activation of the RyRs by 9-methyl 5,7-dibromoeudistomin D (MBED) increased the $[Ca^{2+}]_i$ with an initial peak, followed by a decline to a plateau phase, and regenerative spikes superimposed on the plateau. The initial $[Ca^{2+}]_i$ increase was due to the activation of the RyRs in the ER, since it was abolished by thapsigargin, but was present when extracellular Ca^{2+} was omitted or when Ca^{2+} entry was blocked by SKF 96365. The plateau phase was due to Ca^{2+} entry across the plasma membrane, since it was abolished by omission of extracellular Ca^{2+}, and blocked by SKF 96365. The plateau phase was not solely dependent on the filling state of the ER, since it was not abolished by thapsigargin. Inhibition of the voltage-gated Ca^{2+} channels by nimodipine did not inhibit the plateau phase. Several agents that block TRP channels, e.g. La^{3+}, Gd^{3+}, niflumic acid, and 2-APB, inhibited the plateau phase. It was also inhibited by membrane depolarization. We conclude that the plateau phase was due to activation of some TRP-like channels. Activation of RyRs by MBED also induced membrane depolarization. The spikes required Ca^{2+} entry through the L-type voltage-gated Ca^{2+} channels, as they were abolished by nimodipine. The spikes resulted from CICR, since they were inhibited in a use-dependent way by ryanodine, and abolished after depletion of the ER by thapsigargin. Thus, activation of RyRs activated TRP-like channels, depolarized the plasma membrane, activated L-type voltage-gated Ca^{2+} channels and triggered CICR.

During the course of this thesis we reported that TRPM2 is present in the INS1-E cells and the human β-cells. We studied whether TRPM2 was involved in the Ca^{2+} entry triggered by the activation of RyRs. N-(p-amylcinnamoyl) anthranilic acid (ACA), an inhibitor of TRPM2, did not inhibit the MBED-induced $[Ca^{2+}]_i$ entry. ADP ribose (ADPr), when applied intracellularly, is an agonist of TRPM2. We found that extracellularly applied ADPr increased $[Ca^{2+}]_i$ in the form of an initial peak followed by a plateau that depended on extracellular Ca^{2+}. EC_{50} of ADPr was ~30 μM. NAD^+, cADPr, a phosphonate analogue of ADPr (PADPr), 8-bromo-ADPr or breakdown products of ADPr did not increase $[Ca^{2+}]_i$. Inhibitors of TRPM2, e.g. flufenamic acid, niflumic acid, and ACA did not affect the ADPr-induced $[Ca^{2+}]_i$ increase. Two specific inhibitors of the purinergic receptor P2Y1, e.g. MRS 2179 and MRS 2279 completely blocked the ADPr-induced $[Ca^{2+}]_i$ increase. The $[Ca^{2+}]_i$ increase by ADPr required activation of PI-PLC, since the PI-PLC inhibitor U73122 abolished the $[Ca^{2+}]_i$ increase. The ADPr-induced $[Ca^{2+}]_i$ increase was through the IP_3 receptors, since it was inhibited by 2-APB, an inhibitor of the IP_3 receptors. ADPr increased $[Ca^{2+}]_i$ in the transfected human astrocytoma cells that expressed the P2Y1 receptors, but not in the wild type astrocytoma cells. We conclude that extracellular ADPr is an endogenous and specific agonist of P2Y1 receptors.

Capsaicin and AM404, two specific agonists of TRPV1, increased $[Ca^{2+}]_i$ in the INS-1E cells. Capsazepine, a specific antagonist of TRPV1, completely blocked the capsaicin-induced $[Ca^{2+}]_i$ increase. Capsaicin elicited inward currents that were abolished by capsazepine. TRPV1 protein was detected in the INS-1E cells and human β-cells by Western blot. However, no TRPV1 immunoreactivity was detected in the human islet cells and human insulinoma by immunohistochemistry. Capsaicin did not increase $[Ca^{2+}]_i$ in primary β-cells from rat or human. We conclude that INS-1E cells express functional TRPV1 channels.

In summary, we have shown that (1) RyR activation leads to activation of TRP-like channels in the plasma membrane, membrane depolarization, activation of L-type voltage-gated Ca^{2+} channels and CICR. (2) ADPr is a specific and endogenous low affinity ligand for the P2Y1 receptors. (3) Functional TRPV1 channels are expressed in the INS-1E cells, but not in the primary β-cells.

Keywords: Ca^{2+} signaling, signal transduction, islets of Langerhans, β-cells, ryanodine receptors, Ca^{2+}-induced Ca^{2+} release, TRP-channel, TRPV1, capsaicin, P2Y1 receptors, and ADP ribose.