Orai1 Channels in Skeletal Myotubes

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The Orai calcium channels have been discovered first in T-lymphocytes [1]. They provide calcium entry into cells in order to replenish the intracellular stores of this cation in the endoplasmic reticulum. Activation of Orai channels occurs after a decrease in the intraluminal concentration of calcium ions induced by the agonists stimulating phospholipase C or due to calcium leakage when SERCA is inhibited by thapsigargin or other inhibitors of this enzyme. Recently high level of Orai1 expression has been demonstrated in skeletal muscles [2] and in cultured skeletal C2C12 and mdx myoblasts and myotubes [3]. In order to determine the role of Orai1 in calcium ion transport via plasma membrane of skeletal cells, its expression was inhibited by the mechanism of the RNA interference. We have demonstrated that the degree of inactivation of mRNA coding Orai1 in multinuclear skeletal myotubes differentiated from mdx myoblasts correlates with the decrease in thapsigargin-induced $^{45}\text{Ca}^{2+}$ influx into these cells [3]. Non-target siRNA did not affect store-operated calcium entry. Transfection of myotubes with siRNA targeted against mRNA of Orai1 partially inhibited $^{45}\text{Ca}^{2+}$ influx into myotubes in response to purinergic receptors activation by ATP, completely eliminated lysophosphatidylcholine-induced $^{45}\text{Ca}^{2+}$ entry and did not affect calcium influx via voltage-operated calcium channels. Suppression of Orai1 results in slowing down of $[\text{Ca}^{2+}]_{\text{cyt}}$ elevation in Fura-2-loaded myotubes and

decrease in maximal $[\text{Ca}^{2+}]_{\text{cyt}}$ increment in response to thapsigargin. Inactivation of mRNA coding Orai1 and suppression of store-operated $^{45}\text{Ca}^{2+}$ influx occurred already 24 h after the transfection. Longer treatment (2–3 days) of myotubes with siRNA targeted against Orai1 caused their death. Thus, the data obtained indicate that Orai1 are responsible for the store-operated calcium entry into skeletal cells. The presence of these channels is important for keeping skeletal cells viability.

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