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Involvement of K_{Ca} Channels and Stretch-activated Channels in Calcium Influx Triggering Membrane Fusion of Chick Embryonic Myoblasts

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Calcium influx is known to be prerequisite for membrane fusion of myoblasts. However, little is known about the channels that are responsible for the entry of calcium ions into the cells. Here we show that K_{Ca} channels and stretch-activated channels are involved in the calcium influx. Upon analysis of single channel recordings, calcium sensitivity of K_{Ca} channels in myoblasts was found to be about 6-fold higher than that in myotubes. Their density in myoblasts (1.68 μm^{-2}) was also about 6-fold higher than that in myotubes (0.27 μm^{-2}). In addition, opening of the calcium-permeable cationic channels in myoblasts was found to increase with membrane stretch and could be blocked by gadolinium. The density of stretch-activated channels was 0.22 μm^{-2} for myoblasts, and the relative permeability of calcium to potassium was P_{Ca}/P_K = 3.6. The channels could generate inward calcium currents in physiological solution. Furthermore, the activation of K_{Ca} channels by phloretin dramatically hyperpolarized the resting membrane potential of myoblasts and this effect could be reversed upon treatment of tetraethylammonium. While phloretin induced precocious fusion, tetraethylammonium or gadolinium blocked not only the phloretin-induced precocious fusion but also the spontaneous fusion of myoblasts. These results suggest that stretch-activated channels and K_{Ca} channels are involved in the calcium influx that triggers myoblast fusion.

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Protein kinase C Regulates Spreading of HeLa Cells on a Gelatin Substratum by inducing F-actin formation and the Upregulation of β1 Integrins

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Spreading of HeLa cells on a gelatin substratum requires the activation of protein kinase C (PKC) which occurs as a results of arachidonic acid release upon cell-substratum attachment. This study examines how PKC activation initiates cell spreading. Cell spreading was accompanied by an increase in the relative F-actin content. Inhibition of PKC by calphostin C blocked the increase of F-actin content and cell spreading. Conversely, treating cells with PKC activating phorbol ester not only increased the relative F-actin content but also enhanced cell spreading in various conditions. The increase of relative F-actin content by PKC activation was also observed in suspension cells. The kinetics of F-actin formation was similar to that of PKC activation in suspension cells. Activation of PKC also induced the upregulation of β1 integrins from an internal pool to the cell surface in a microtuble-dependent manner. The upregulation of β1 integrins appears to be required for optimum cell spreading since inhibition of the upregulation partially blocked cell spreading. The results are consistent with a dual role of PKC in regulating HeLa cell spreading. One is through the formation of F-actin and the other is by deliverying collagen receptors to the cell surface where they can bind to the ECM molecules.