Mechanosensitive Na and Ca permeable ion channels in renal tubule cells cultured in a glass micropipette.

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Summary

Cells from the A6 line derived from *Xenopus* kidney were cultured in a glass micropipette. The cells suspended in a culture medium (about 10^3/ml) were seeded in a glass micropipette (1.5 mm in diameter, 25 mm in length). Photograph of a monolayer colony of the A6 cells could be taken by laser scanning differential-interference-contrast microscopy. Second, neonatal rat kidney inner medullary collecting duct (IMCD) cells were cultured on a glass cover slip in a DMEM/F12 medium containing 10% FBS for a short period (6-24 hrs). In order to study a role of the mechanosensitive ion channels for the spread of intracellular calcium, we measured fluorescence intensity (fluo3) of the cells with the confocal laser scanning microscope, before and after mechanical stimulation. IMCD cells were incubated in a standard solution containing 2.5 μM fluo3-AM for 40 min at 24-26 °C. The surface membranes of the IMCD cells were gently prodded and rubbed by a tip of a patch-pipette under another phase difference microscope to avoid cell membrane damage due to vibration from a laser cooling system. At the resting condition, cytosolic and nuclear calcium was low. Therefore, a boundary between the cytoplasm and the nucleus was obscure under both the phase-difference and the fluorescence microscope. After mechanical stimulation the nucleus shrank by 50% in area and its density increased within a minute; then, it slowly moved up toward the apical surface. The fluorescence intensity (index for Ca concentration) of the shrunken nucleus was always increased and was higher than that of the cytoplasm. The ratios of the intensities between in the nucleoplasm and cytoplasm were increased from 1.1 to 1.5. Similar results were also obtained in a zero calcium solution containing 100 μM EGTA. These results indicate that nuclear calcium may be independent of the cytoplasm calcium, and suggest that the nuclear envelope may be a source of calcium for the nucleoplasm. Activation of the Ca-permeable mechanosensitive ion channels may not be required to control gene expression.