CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

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Project Number

S0409

Project Title

Ion Channel Composition of Human Mesothelioma Cells

Abstract

Objectives/Goals

This study was conducted in order to determine whether human mesothelioma cells express ion channels of the TRPM and SOCE families. I specifically tested these cells for the TRPM-2, TRPM-7, and I-CRAC currents, utilizing the whole-cell patch clamp technique. The TRPM-2 (Transient-Receptor-Potential Melastatin 2) channel is involved in cell death and lysosomal calcium ion release. TRPM-7 is a Magnesium conducting ion channel, which has been shown to affect cell proliferation in both normal and cancer cells. The I-CRAC channel is a primary pathway for calcium entering the cell.

Methods/Materials

For the experiments, REN mesothelioma cells were kept in standard external solution (in mM): 140 NaCl, 2.8 KCl, 1 CaCl2, 2 MgCl2, 11 glucose, 10 HEPES-NaOH (pH 7.2 adjusted with NaOH). Standard internal pipette-filling solutions contained (in mM): 120 or 140 Cs-glutamate, 8 NaCl, 1 MgCl2, 10 HEPES-Cs/KOH (pH 7.2 adjusted with CsOH/KOH). 20 µM IP3 and 10 mM Cs-BAPTA or 1 mM ADPR was added to its final concentrations as appropriate. Patch-clamp experiments were performed in the whole-cell configuration at 21 to 25 degrees C. All data were acquired with "PatchMaster" software. Voltage ramps of 50 ms spanning the voltage range from -100 to +100 mV were delivered from a holding potential of 0 mV at a rate of 0.5 Hz over a period of 300 s. For analysis, current amplitudes were extracted at +80 mV.

Results

I did not detect I-CRAC in any of the cells that I tested; yet I consistently found a large current that may be TRPM-7. This current was not inhibited when I applied TEA-Cl to the external solution, which is a characteristic of the TRPM-7 current. When testing for TRPM-2, the cell was perfused with 1mM ADPR in the internal solution. The TRPM-2 current was not present in any of the REN mesothelioma cells that I tested; yet I did find a different, unidentified current when ADPR was present in the solution of the patch pipette. This novel current was not inhibited by TEA Cl, but did not develop even in the presence of ADPR when Lanthanum was applied to the external solution.

Conclusions/Discussion

From these results I conclude that human mesothelioma cells may not express I-CRAC nor TRPM-2 channels, yet consistently develop TRPM-7-like currents as well as another, unidentified ionic current, which is not inhibited by TEA-Cl, but is not present when Lanthanum-Cl is applied.

Summary Statement

This experiment utilized the whole cell patch clamp technique in order to determine whether human mesothelioma cells exhibit ion channels of the TRPM and SOCE families.

Help Received

Dr. Andrea Fleig and George Myers supervised my research at the Laboratory for Cell and Molecular Signaling, QCBR, University of Hawaii.