RUBIDIUM EFFLUX ASSAY

Assay protocol (adherent cells)

Aliquots of cells in growth medium are plated in quadruplicate onto 24-well or 96-well plates. The plated cells are grown at 37°C for 24-48 hr to reach 70-95% confluence. Cells are then incubated in growth medium containing $^{86}Rb^{+}$ (2 µCi/ml) for 4 hr at 37°C. The labeling mixture is aspirated, and the cells are washed four times with HEPES buffer (15 mM HEPES, 140 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 1.8 mM $CaCl_2$, 11 mM Glucose, pH 7.4; 1 ml/well). Buffer, with or without agonists, is then added to each well. After incubation for 2 min, the assay buffer is collected. Cells are lysed by adding NaOH to each well, and the lysate is then collected. The amount of $^{86}Rb^{+}$ in the medium and in the cell lysates is measured by liquid scintillation counting.

Data analysis

The total amount of $^{86}Rb^{+}$ loaded (cpm) is calculated as the sum of the assay sample and the lysate of each well. The amount of $^{86}Rb^{+}$ efflux is expressed as a percentage of the $^{86}Rb^{+}$ loaded. "Stimulated $^{86}Rb^{+}$ efflux" is defined as the difference between efflux in the presence of nicotinic agonists and basal efflux measured in the absence of agonists. Data is fit using the non-linear curve fitting routines in Prism® (Graphpad Software Inc).