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TWO TYPES OF BA^{2+} BINDING SITES ON K^+ CHANNELS WITH DIFFERENT SENSITIVITY TO MEMBRANE SURFACE CHARGE

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Previously we showed that Ba^{2+} block of large conductance Ca^{2+}-activated K^+ (BK) channel was larger in the planar lipid bilayer formed with negatively-charged phosphatidylserine (PS) than neutral phosphatidylethanolamine (PE). In this work, have studied the blocking effect of two K^+ channel blockers with different mechanisms of action, Ba^{2+} and tetraethylammonium (TEA), on BK channels of rat skeletal muscle.

External Ba^{2+} induced two types of blocks on the BK channel; long closures lasting several seconds (slow block) and decrease in channel conductance (fast block). The frequency of long closures (0.1-6.0 sec) was dose-dependently increased in both PE and PS membranes. Dwell time histograms of Ba^{2+} induced long closure were well fitted by two time constants (~0.15 sec and ~6 sec) which were not affected by Ba^{2+}. Mean burst time between Ba^{2+} blocks was dose-dependently decreased by Ba^{2+} but there was little difference between the rates of decrease in PE and PS membranes which cannot be explained by simple electrostatic effect. However, the conductance of single BK channel was more effectively reduced by external Ba^{2+} in PS than PE membrane (2x - 4x) and in lower ionic strength than than higher one (1.3x - 2x). In addition, external TEA also reduced BK channel conductance and its blocking effect was similar to that by external Ba^{2+}.

In summary, the association rate of Ba^{2+} for slow block was not different between PE and PS membranes. while BK channel conductance was reduced more effectively in PS membrane. Our data indicate that the presence of two types of Ba^{2+} binding sites in BK channel; one senses the surface charge and the other does not.