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Activity-dependent inhibition of N-type calcium channels: a novel PKC-independent effect of phorbol esters

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Multiple second-messenger pathways and intrinsic channel properties such as inactivation precisely regulate calcium entry through N-type calcium channels. However, it remains unclear how these different mechanisms interact. To begin to address this question we expressed N-type channels ($Ca_v2.2$ (α_{1B}), $\alpha_2\delta$, and β_{1b}) in HEK293 cells and used barium as the charge carrier to isolate voltage-dependent inactivation. Inactivation was produced by an individual step depolarization lasting 1-15s, trains of brief step depolarizations, or trains of action potential-like waveforms (APW). Recovery from inactivation was tracked using brief steps at given intervals following the stimulus step / train. Recovery following “short” (<2s) stimuli was clearly biphasic, while after longer duration stimuli (>10s) the fast component was minimal and the slow component increased. Acute application of the phorbol ester PMA (50-200nM) had little effect on the resting amplitude of I_{Ba} or inactivation during 5-50Hz stimulus trains. However, the slow phase of I_{Ba} recovery (but not the fast phase) was significantly reduced/slowed by PMA. This was not blocked by inhibitors of PKC that target the catalytic domain of the enzyme (bisindolylmaleimide I, Go6983, PKC inhibitory peptide 19-36), but was blocked by calphostin-C which targets the diacylglycerol binding C1 domain of PKC and other proteins. Our data identify a novel PKC-independent mechanism by which PMA (and perhaps diacylglycerol signaling) inhibits N-type calcium channels. The inhibition is dependent on the frequency and duration of stimulation and might involve stabilization of a “slow inactivated state” of the channel. *Supported by NIH (NS052446) and a VUMC Discovery Grant.*