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Modulation of Cloned T-type Calcium Channels

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The inflow of Ca^{2+} through voltage-activated T-type calcium channels (T-channels) regulates a variety of cellular functions including neuronal excitability, cardiac pacemaker activity, hormone secretion, smooth muscle contraction, and fertilization. Not only are T-channels enormously important for the normal operation of cells, they also play a critical role in pathophysiological conditions such as cardiac hypertrophy and absence epilepsy. Accordingly, T-channels provide important targets for developing novel pharmaceutical drugs. In native tissues, T-channels show unique biophysical properties as follows: activation at low voltage, fast activation, slow deactivation, and tiny unitary conductance. In respect to the kinetic behaviors of T-channel currents, tissue-to tissue variations have been observed. Recently, molecular cloning studies have established that the $\alpha 1$ subunits of the T-channels are encoded by at least three genes called $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$. Thus, it is likely that the diversity of native T-channel currents originates mainly from heterogenous expression of the three isoforms in tissues.

In neuronal tissues, T-channels are under influences of various neurotransmitters. To date, however, it remains unclear which isoform of T-channels is susceptible to modulation by neurotransmitters and whether cellular mechanisms underlying the modulation are membrane-delimited or involve diffusible second messenger and/or phosphorylation. To address these questions, patch-clamp recording of the T-channel currents was made by combining molecular biological tools. In heterologous expression systems, activation of Gq/11, but not Gi/o-coupled receptors selectively inhibited the $\alpha 1I$ T-channels. The $\alpha 1I$ current inhibition was blocked by pretreatment of staurosporine, a broad-spectrum kinase inhibitor and GF109203X, a selective PKC inhibitor. Conversely, application of PMA, a PKC activator mimicked the $\alpha 1I$ current inhibition. Taken together, these data suggest that the $\alpha 1I$ T-channels are regulated by a Gq/11-PKC

signaling cascade. Analysis of protein sequence has revealed many putative phosphorylation sites in the cytoplasmic portions of the cloned T-channels. Accordingly, I am now probing the potential sites for PKC-dependent phosphorylation involved in the current modulation by overexpressing cytoplasmic loops of the α1I T-channels and chimera between α1I and α1G.