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PKC and calcium channel trafficking

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The L-type calcium channel (Cav1.2) regulates cardiac contraction and is modulated by different hormones and neurotransmitter via G-proteins and protein kinases. PKC-activating hormones mediated by the α_1 adrenergic, angiotensin I, muscarinic or endothelin receptor enhance the cardiac Cav1.2 via Gq and PKC in vivo and in vitro.[1] The molecular mechanism underlying the PKC-dependent upregulation is unclear. *In vitro* studies showed that only the cardiac α_{1C} subunit containing the long amino terminus[2] is upregulated by PKC.[3] Essential for the upregulation are the first 46 amino acids of the long amino terminus.[3] This part of the α_{1C} subunit is not phosphorylated by PKC. PKC-dependent channel enhancement is decreased by coexpression of the β_2 subunit suggesting that the β_2 subunit interferes with the binding of PKC to the N-terminus. *In vitro* and *in vivo* phosphorylation of various other residues have been reported including Ser1928.[4] Phosphorylation of Ser1928 has been implicated in the upregulation of Cav1.2 by PKC and PKA.

Alternative molecular mechanisms for the regulation of Cav1.2 by PKC were suggested: i) upregulation of the channel by PKC-dependent phosphorylation of the α_{1C} subunit; ii) binding of PKC to the "cardiac" N-terminus of the α_{1C} subunit and interruption of the interaction of the N-terminus with other parts (i.e. the I-II loop or the C-terminus) of the α_{1C} subunit that inhibits channel function; iii) binding of PKC to the α_{1C} subunit and improved trafficking of the channel protein to the plasma membrane. The last mechanism was partially supported by the consistent finding that upregulation of the Cav1.2 channel required some time that might be caused either an intracellular relocalization of PKC to the channel or by increased trafficking of the channel protein. The relocalization hypothesis was less likely, since a constitutive macromolecular complex between classical PKCs and cardiac Cav1.2 was reported.[4]

Raifman and colleagues from Dascal's group investigated the possible mechanism in detail expressing the α_{1C} subunit with the long "cardiac" N-terminus in *X. oocytes*.[5] PKC was either activated by PMA (phorbol ester) or through stimulation of the coexpressed muscarinic receptor M3. Plasma membrane expression of the α_{1C} subunit was quantified either by immunocytochemistry in giant membrane patches (GMP)[6] or by surface biotinylation followed by western blotting. Both methods reported an increased plasma membrane expression of the α_{1C} subunit, although the extend of the increase varied with the used methods being 126% with GMP and 36% with biotinylation. The authors interpreted their results as clear evidence that PKC increased the trafficking of the channel protein to the plasma membrane in *X. oocytes*. Moreover, they could show that the increased plasma membrane localization depended on the long "cardiac" N-terminus and required the phosphorylation of Ser1928.

The question arising immediately was, is this finding specific for *X. oocytes* or does it show the general mechanism of the PKC enhancement. To answer in part this question they used the same protocol in HL-1 cells. HL-1 cells are derived from AT-1 mouse atrial cardiomyocyte tumor cells, which retain some of the features of a mature atrial cardiomyocytes. PKC enhanced also in the HL-1 cell the plasma membrane

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expression of the α_{1C} subunit with some preference for the full length α_{1C} subunit.

These results suggest now that the PKC-dependent enhancement of the Cav1.2 current might be caused by an increased trafficking of the translated α_{1C} subunit to the plasma membrane in the cardiomyocyte. Transfer of these results to the living animal raises some problems. Mutation of serines and threonines implicated as phosphorylation sites of PKA and PKC to alanine including Ser1928, Ser1700 and Thr1704, and 22 potential phosphorylation sites[7] showed that upregulation of the Cav1.2 channel by isoproterenol was not affected by the alanine mutation. It is possible but unlikely that PKC and PKA use different mechanisms to upregulate the channel, because both phosphorylate Ser1928. Raifman et al report that mutation of Ser1928 to alanine in their α_{1C} subunit prevented the PKC-dependent trafficking of the channel in *X. oocytes*. Qian et al.[8] reported similar findings for the beta-adrenergic regulation of Cav1.2 in neurons and smooth muscle. In both cell types, the mutation Ser1928Ala prevented beta-adrenergic upregulation, whereas beta-adrenergic upregulation was preserved in the cardiomyocytes. It is therefore likely that regulation of the Cav1.2 channels by protein kinases involves different components in distinct tissues. It is further possible that phosphorylation of Ser1928 is a mandatory step in *X. oocytes* but can be surpassed in cardiomyocytes.

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