Insights into adrenergic stimulation of calcium channels

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In the heart, activation of β-adrenergic receptors increases calcium influx through the L-type calcium channel and leads to positive inotropic and chronotropic effects. We have demonstrated that under hypoxic conditions this leads to the development of early afterdepolarisations and spontaneous arrhythmia as a result of an increase in sensitivity of the L-type calcium channel to β-adrenergic receptor stimulation. It is generally accepted that cAMP-dependent protein kinase A (PKA) can phosphorylate the pore forming and voltage sensing α-subunit ($Ca_v1.2$) of the heterotetramer channel. However the site of phosphorylation remains controversial. We directly tested the effect of PKA phosphorylation on the function of purified human $Ca_v1.2$ reconstituted in liposomes. We find that direct phosphorylation of $Ca_v1.2$ subunit is sufficient for PKA-dependent increase in channel activity and an auxiliary protein is not required. We demonstrate that the N terminal and C terminal regions of $Ca_v1.2$ do not contain the critical sites and mutation of Ser1928 does not attenuate the effect of PKA. We examined whether the cytoplasmic regions contained the critical sites. We mutated four serine residues to alanine at S436, S754, S834, and S1458 by site directed mutagenesis. *In vitro* phosphorylation studies demonstrated a decrease in the phosphorylation level of the mutant protein *vs* wt intracellular loop regions after densitometry analysis (n=3; $P<0.05$). In addition mutation of the 4 serines attenuated the effect of PKA on open probability of the purified channel protein reconstituted in liposomes (PKA+Na+ATP n=6 *vs* inactivated PKA n=5; *P*=NS). We are now determining whether phosphorylation of all 4 serines are necessary for the increase in channel activity.