Mutation of cysteines on the α interacting domain of the long NT isoform of the $Ca_v1.2$ channel protein alters the biophysical properties of the channel

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Oxidative stress is associated with persistent glutathionylation of the L-type calcium channel that results in an increase in intracellular calcium and protein synthesis consistent with the development of myocyte hypertrophy. In search of the critical cysteines, we have shown previously that mutation of cysteines on the α interacting domain (AID) of the $Ca_v1.2$ protein alters the response of the channel to thiol modifying agents. In this study we further characterised the effect of the mutations on the biophysical properties of the channel and the folding of the channel protein.

The human long N terminal (NT) isoform of $Ca_v1.2$ (α subunit) was expressed in HEK293T cells. Cysteines were mutated to a serine or an alanine. The channel protein was purified by histidine tag purification and incorporated in liposomes for functional analysis by patch-clamp technique. Consistent with our previous results, exposing the long NT isoform to 2mM oxidised glutathione (GSSG) increased the open probability (Po) of the channel 1.95±0.09 fold (n=5) while in separate experiments exposing the NT isoform to 1 mM reduced glutathione (GSH) decreased Po of the channel to 0.46±0.07 (n=10; $P<0.05$ *vs* control solution in the same patch, paired t-test) without altering the current-voltage relationship or ion conductance of the channel. However addition of 2mM GSSG to the long NT isoform with cysteines mutated at C519S, C543S and C547A in the AID region attenuated the effects of GSSG and GSH on the Po of the channel. In the absence of GSH and GSSG Po was significantly smaller in the AID mutant protein (Po 0.008±0.001, n=10 *vs* 0.020±0.003, n=33; $P<0.05$). This suggests that the AID region regulates the open probability of the channel. Consistent with this argument, we found that the temperature at which the AID mutant protein folded when assessed by thermal shift assay was significantly less than the long NT isoform (33.67±1.37°C n=11, *vs* 37.69±0.68°C, n=11; $P<0.05$).

We conclude, that in addition to regulating the function of the channel during oxidative stress, C519, C543 and C547 on the α interacting domain of $Ca_v1.2$ may be responsible for regulating the open state of the channel and posttranslational folding of the channel protein.