Evidence for direct regulation of the cardiac L-type Ca^{2+} channel during changes in thiol redox state

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It has previously been demonstrated in isolated cardiac myocytes that the current through the L-type Ca^{2+} channel ($I_{Ca\text{-}L}$) can be altered by changes in the redox state of the cell. Mild oxidative stress induced by application of 30 μM hydrogen peroxide (H_2O_2), increases basal $I_{Ca\text{-}L}$ while acute hypoxia (that is associated with a decrease in cellular H_2O_2) decreases basal $I_{Ca\text{-}L}$. However, these effects were demonstrated in mammalian cells where the channel may be regulated by a number of other proteins that can undergo modifications in function as a result of changes in redox state. Therefore, the aim of this study was to determine whether the function of the cardiac L-type Ca^{2+} channel can be altered as a result of direct modification of thiol groups on the channel protein.

HEK cells were transiently transfected with the cDNA for the long N terminal isoform of the α_{1C} subunit of the human L-type Ca^{2+} channel and the purified protein reconstituted into liposomes using the dehydration-rehydration method. Functional examination of channels was performed by patch-clamp method with pipette and external solutions containing (in mM): $BaCl_2$ 100 and KCl 50 (pH adjusted to 7.4). Bay K 8644 (50 μM) was added to facilitate channel opening. The current produced by the reconstituted protein was confirmed as the L-type Ca^{2+} channel current according to the amplitude and slope conductance of the current and sensitivity to the L-type Ca^{2+} channel blocker nisoldipine.

Exposure of liposomes to the thiol-specific oxidising agent 5,5'-dithio-bis[2-nitrobenzoic acid] (DTNB, 200 μM) significantly increased channel open probability (Po) by 98% ($p = 0.033$, $n = 9$) while exposure to the thiol-specific reducing agent dithiothreitol (1 mM) significantly decreased Po by 48% ($p = 0.003$, $n = 7$). Furthermore, addition of H_2O_2 (30 μM) mimicked the effect of DTNB by significantly increasing Po by 67% ($p = 0.049$, $n = 6$). However, exposure of the liposomes to acute hypoxia by decreasing oxygen tension from 150 mmHg to 17 mmHg, had no significant effect on Po ($p = 0.604$, $n = 7$). Of the various thiol redox modifications there is increasing evidence that glutathionylation predominates in cells because glutathione is the most abundant low molecular mass thiol in the human cell. We examined the effect of oxidised glutathione (GSSG) on channel function. Addition of 5 mM GSSG increased the open probability of the channel 5 fold ($p < 0.001$, $n = 5$).

This study indicates that the thiol redox state of the cardiac L-type Ca^{2+} channel is an important determinant of channel function. In addition inhibition of the basal current during hypoxia in cardiac myocytes does not occur as a result of a direct effect of oxygen on the channel protein. Changes in the glutathionylation state of the channel are likely to account for altered channel function during oxidative stress.