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## Effect of KCNE1 mutations on the trafficking of KCNQ1 in CHO-K1 cells

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Mutations of the KCNQ1 and KCNE1 genes have been identified in individuals suffering from the hereditary cardiac long QT (LQT) syndromes. KCNQ1 (pore-forming subunit) and KCNE1 (accessory subunit) encode the potassium channel proteins and mutations in these subunits can lead to a loss or decrease in repolarising current, $I_{Ks}$, via an unknown mechanism. The aim of this study was to examine the effect known KCNE1 mutations (which cause LQT-5) had on the trafficking of the pore-forming subunit, KCNQ1.

The C-terminus of the long isoform of KCNQ1 was tagged with enhanced green fluorescent protein (EGFP) and transiently transfected either alone; with wild type KCNE1 or mutant KCNE1 into CHO-K1 cells. ER co-localisation was determined by co-transfection of pDsRed2-ER (an ER-retained red fluorescent protein, Clontech, UK). Images were acquired with a Bio-Rad Radiance 2000 scanning laser confocal microscope. Co-localisation of green and red fluorescence was quantified using LaserPix software (Bio-Rad, UK). Data are expressed as means±SEM. Statistical analysis was performed using Student's unpaired t-test or one-way ANOVA, as appropriate.

Expression of wild type (WT) KCNQ1-GFP alone resulted in 38.3±2% (n=66) of the protein being ER retained. Co-expression with WT KCNE1 resulted in a reduction of ER retention to 21.6±2% (n=53, P<0.001). Co-expression of KCNQ1 with five of the KCNE1 mutations (G52R, T58P/L59P, S74L, D76N and R98W) did not significantly affect the export of KCNQ1 from the ER in comparison to the WT KCNQ1 control alone (range 34-39%, n=36-46). In contrast, a sixth mutation, T71I, did significantly (P<0.001) reduce the degree of ER retention to a level similar to that of the KCNQ1+WT KCNE1 control (21.0±2% n=49). As five of the mutations did not appear to interact with KCNQ1 in the ER we then investigated whether these mutations had a dominant-negative effect on trafficking of WT KCNQ1 when co-expressed with WT KCNE1. Four of the mutations (T71I, G52R, T58P/L59P and S74L) did not produce significantly different degrees of ER retention compared to the WT controls (range 19-22%, n=14-36). However, two of the mutations (D76N and R98W) did appear to have a significant dominant-negative affect on ER retention (range 36-44%, n=13-23, P<0.001) when compared to the WT controls.

The results from this study suggest that LQT5 mutations in KCNE1 can cause a variety of functional effects including a general failure to promote ER export of the channel complex and a dominant-negative effect on the trafficking of the wild-type channel.

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*Where applicable, the experiments described here conform with Physiological Society ethical requirements.*

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## Direct effects of the specific CFTR blocker $CFTR_{inh}$-172 on the function of isolated rat heart

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The cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel is expressed in the mammalian heart (Hume *et al.* 2000). However, its cardiac function is poorly understood in part due to lack of specific tools to manipulate CFTR activity. Recently, Ma *et al.* (2002) demonstrated that the thiazolidinone $CFTR_{inh}$-172 (C172) is a potent, specific inhibitor of the CFTR $Cl^-$ channel. To investigate the cardiac function of CFTR, we tested the effects of C172 on isolated perfused rat hearts.

Male Wistar rats (250-275 g) were killed by cervical dislocation. The hearts were removed and mounted on a Langendorff apparatus and perfused with Krebs solution (37 °C) at a constant flow rate of 9 ml $min^{-1}$. A balloon was inserted into the left ventricle to monitor ventricular pressure, heart rate and other haemodynamic parameters using a data acquisition system (Chart v4.0.2, PowerLab ADInstruments). Data were analysed offline.

To stimulate CFTR by increasing intracellular cAMP levels, we used forskolin (Fk, 0.5 μM). To inhibit CFTR, we used C172 (10 μM, Asinex Ltd), a concentration that inhibits maximally CFTR-mediated $Cl^-$ secretion in epithelia (Ma *et al.* 2002). Drugs were dissolved in Krebs solution. Individual rat hearts were treated randomly with either Fk, C172 or Fk + C172 with C172 added 2 min after Fk.

Our results are summarised in the Table. Fk (0.5 μM) induced an immediate change in all the parameters within the first 3 min of perfusion. However, at the end of a 10 min perfusion period, HR continued to increase, while other parameters returned to their control levels. In contrast, over a 3 min perfusion period, C172 (10 μM) induced an immediate reduction in LVDP and –dp/dt. However, HR and +dp/dt were not significantly affected by C172. On washing C172 away, we observed a transient increase in LVDP. When administrated to Fk pretreated hearts, C172 abolished the immediate positive inotropic and chronotropic effects of Fk. Vehicle (0.1% DMSO) was without effect (n = 4, data not shown).

Our data suggest that the immediate effects of either the CFTR blocker C172 or the CFTR activator Fk are not consistent with the predicted negative inotropic role of CFTR in the heart (Hume *et al.* 2000). We speculate that the immediate effects of Fk might be due to other mechanisms such as the activation of the L-type $Ca^{2+}$ channel. Further studies are required to determine whether C172 affects cardiac function by CFTR-dependent or independent mechanisms.

| Parameter | Fk (3 min) | Fk (10 min) | C172 (3 min) | Fk + C172 (3 min) |
|---|---|---|---|---|
| n | 16 | 9 | 7 | 4 |
| HR (%) | 116 ± 3[a] | 120 ± 6[a] | 103 ± 2 | 109 ± 2[b] |
| LVDP (%) | 142 ± 11[a] | 103 ± 10 | 80 ± 5[a] | 78 ± 5[b] |
| +dp/dt (%) | 130 ± 10[a] | 110 ± 10 | 73 ± 9 | 91 ± 9[c] |
| -dp/dt (%) | 149 ± 15[a] | 124 ± 17 | 66 ± 10[a] | 88 ± 1[c] |

**Data are mean ± SEM and expressed as the percentage of their control values. a: P < 0.05 compared with control, b: P < 0.05 compared with Fk. Student's paired t-test. c: n = 2**

Hume JR *et. al.* (2000) *Physiol Rev* **80**, 31-81.

Ma T *et. al.* (2002) *J Clin Invest* **110**, 1651-1658

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