The measurement of inactivation in the human ether-á-go-go related gene channel

M.J. Perrin,[1,2] T.J. Campbell[2] and J.I. Vandenberg,[1] [1]Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst, NSW, 2010, Australia and [2]St Vincent's Clinical School, Level 5 DeLacey Building, St Vincent's Hospital, Victoria Street, Darlinghurst, NSW 2010, Australia.

The human ether-á-go-go related gene (hERG) encodes the α-subunit of the rapid delayed rectifier K^+ channel (IK_r) in the heart. IK_r displays marked inward rectification due to a very fast rate of channel inactivation at positive membrane potentials. The correct measurement of inactivation is of fundamental importance in the characterisation of any hERG mutation. We hypothesised that the standard method for measuring inactivation - a 3 pulse voltage clamp protocol - was flawed giving a Boltzmann distribution of steady-state inactivation (SSinact) left-shifted compared to the true value. To test this hypothesis, whole cell patch-clamping was performed on CHO-K1 cells stably expressing wild-type hERG. Standard 3 and 2 pulse voltage clamp protocols were employed and analysed. A virtual model of hERG gating was used to assess the reliability of the experimental data. The SSinact of hERG measured with a 3 pulse protocol was significantly left shifted compared with a 2 pulse protocol. V0.5 for 3 pulse: -90.6mV +/- 3.3mV. V0.5 for 2 pulse: -74.6mV +/- 3.3mV, (p = 0.04). Virtual modelling confirmed the validity of these findings and provided evidence that the 2 pulse protocol was a better representation of the true distribution. The standard 3 pulse voltage protocol to measure SSinact results in a SSinact curve left-shifted compared to a 2 pulse protocol. Theory and virtual modelling evidence suggests greater accuracy with a 2 pulse protocol. Almost all papers published to date on hERG inactivation utilise a 3 pulse voltage clamp protocol. There is now evidence that published values of steady-state inactivation in hERG differ significantly from the true value.