# Pharmacological activation of defective hERG potassium channels in the treatment of long QT type 2 syndrome

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**Introduction:** Congenital long QT syndrome is an electrical, pro-arrhythmic disorder of the heart that is associated with an increased risk of arrhythmia and sudden cardiac death. Long QT syndrome type 2 (LQTS2) is caused by any one of >500 different mutations in the human ether-à-go-go related gene (hERG) potassium channel. A subset of LQTS2 mutations cause dysfunction by accelerating the closure (deactivation) of hERG channels at the end of each heartbeat. Slow channel closure is a signature of hERG channels and is critical for allowing the generation of a protective current against the Torsade de Pointes tachyarrhythmia in response to premature stimuli. In the last decade, several pharmacological activators of hERG channels have been identified, some of which act by slowing channel closure. Our aim was to investigate whether activators which slow hERG channel closure, such as Rottlerin, could restore WT-like function to fast closing mutants and thus have therapeutic potential for the treatment of LQTS2.

**Methods:** Currents were recorded from Chinese hamster ovary (CHO) cells expressing wild type (WT) or mutant hERG channels using the whole-cell patch clamp technique. We investigated the effect of the activator Rottlerin on three LQTS2 mutations, all of which exhibit fast channel closure, located in disparate regions of the channel protein: R56Q (N-terminus), T421M (voltage sensor) and E788K (C-terminus). All data are reported as mean ± SEM.

**Results:** We first characterized the effect of Rottlerin on WT hERG channels. Using the time constant of the deactivation kinetics as our measure of choice, the EC50 value for Rottlerin was 1.2μM. At 3μM, the effect of Rottlerin was almost maximal, slowing the deactivation time constant for WT hERG from 62.8 ± 3.1 ms (n=12) in control, to 113.7 ± 9.4 ms (n=12) in 3μM Rottlerin ($P$<0.0001, paired t-test). Next we examined the effect of Rottlerin on the fast deactivating N-terminal mutation R56Q. Rottlerin (3 μM) slowed the deactivation of R56Q channels from 27.1 ± 3.0 ms (n=5) to 105.3 ± 12.5ms (n=5; $P$<0.0001, ANOVA). As a consequence, Rottlerin also restored the protective current, elicited in response to premature stimuli, passed by R56Q channels to a value greater than that of WT channels (n=5; $P$<0.0001, ANOVA). In contrast to its restorative effects on R56Q channels, Rottlerin failed to restore WT-like function to another LQTS2 mutation, T421M, located in the voltage sensor region. The tau for deactivation of T421M channels was 43.8 ± 1.3 ms (n=4) in control, and 64. 0± 8.6 ms (n=4) with 3 μM Rottlerin ($P$>0.05, ANOVA). The response to premature stimuli for T421M was virtually non-existent (<10% of WT) and only increased minimally with Rottlerin. Similarly, Rottlerin produced only small effects on the final LQTS2 mutation studied, E788K, which is located in the C-terminus of the channel. In E788K channels, deactivation was 30.7 ± 3.0 ms (n=6) in control and 38.1 ± 3.0ms (n=6) in 3 μM Rottlerin ($P$>0.05 ANOVA). Rottlerin also had minimal effects on the protective current elicited by E788K channels in response to premature stimuli.

**Discussion:** Rottlerin restored function to some but not all enhanced deactivation mutants. R56Q was the only mutant where function was restored to levels similar to WT. Although the T421M mutant had normal rates of deactivation restored back to WT, the response to premature stimuli was not able to be rescued. For the E788K mutant, Rottlerin failed to slow deactivation in a similar magnitude to R56Q with the rates of deactivation still significantly faster than in WT channels. We suggest that the interaction of Rottlerin with the hERG channel is site dependent and hence can only restore function in some mutants but not all. In conclusion, these studies provide proof-of-principal evidence that pharmacological activators may be able to rescue function in at least some mutant hERG channels. There are several more activators and mutants that will be evaluated in future studies.