Hesperetin preferentially inhibits slow-inactivating currents of an LQT3 syndrome Na^+ channel mutation.

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INTRODUCTION| The citrus flavanone hesperetin (HSP) has been proposed for the treatment of several human pathologies, but its cardiovascular actions remain unexplored. Here we studied the effects of HSP on the human cardiac voltage-gated Na^+ channel ($hNa_V1.5$) and compared it to its effects on a recombinant $hNa_V1.5$ channel baring a mutation (R1623Q) associated with lethal ventricular arrhythmias in the Long QT syndrome type 3 (LQT3). **METHODS|** Whole-cell patch-clamp experiments were used to record Na^+-currents (I_{Na}) in HEK293T cells expressing $hNa_V1.5$ wild type (WT) or mutant channels. **RESULTS |** HSP blocked the $hNa_V1.5$ channels in voltage-dependent manner with an IC_{50} ≈ 100 μM. Its inhibition was decreased by disruption of the F1760 residue. HSP preferentially accelerated the inactivation phase of I_{Na} and decreased the Na^+ net influx into the cell. The effects of HSP on the inactivation phase and voltage-dependent inhibition of I_{Na} were more marked in the LQT3 mutant. **CONCLUSIONS | HSP could be used as a template to develop drugs against cardiac arrhythmias in LQT3.**

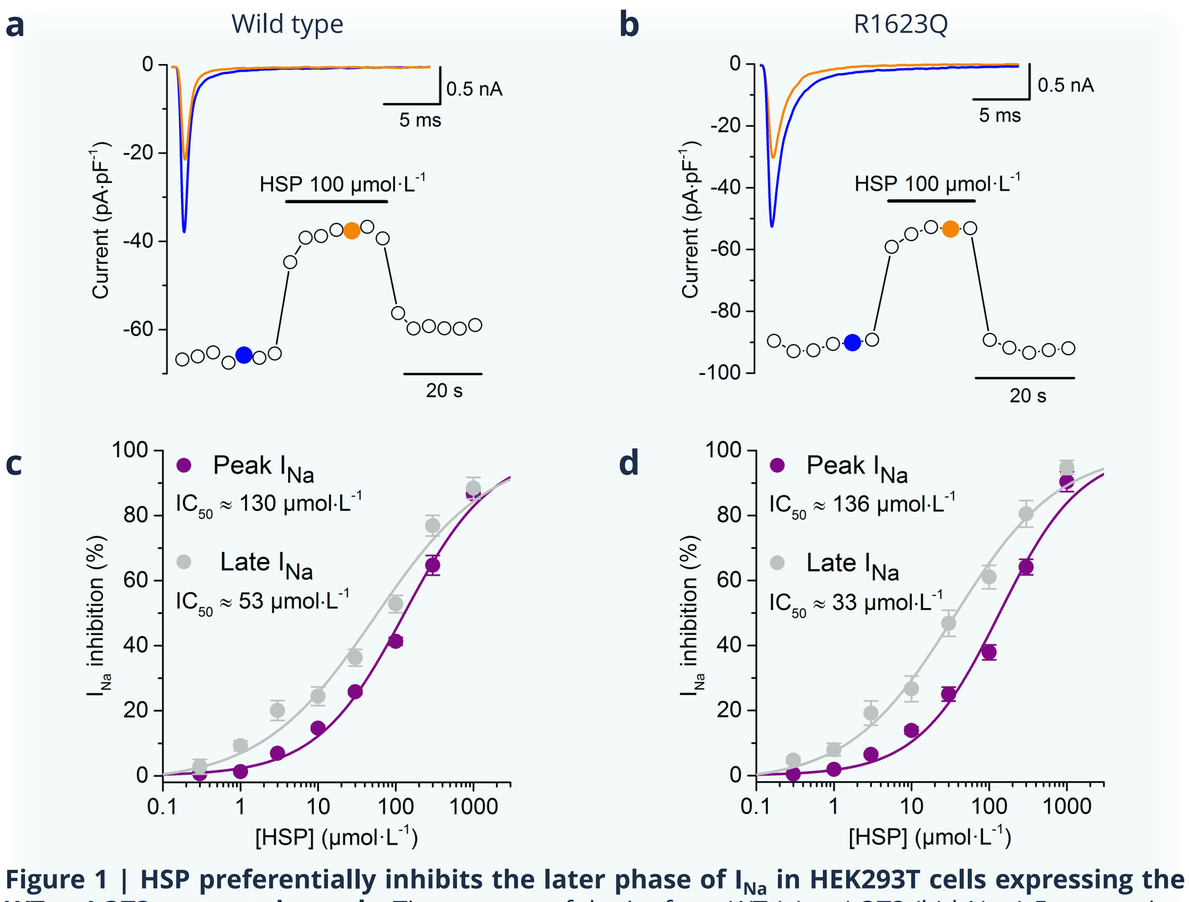

Figure 1 | HSP preferentially inhibits the later phase of I_{Na} in HEK293T cells expressing the WT or LQT3 mutant channels. Time course of the I_{Na} from WT (a) or LQT3 (b) $hNa_V1.5$ expressing HEK293T cells in control condition and in presence of HSP 100 μM. Representative current traces in control (blue) and upon the application of HSP (orange) are shown in the insets and correspond with the coloured data points. Concentration-effect curves for the action of HSP on WT (c) or LQT3 (d) $hNa_V1.5$ currents. The dots represent the mean ± s.e.m. inhibition percentage during the application of different concentrations of HSP (n = 9). The estimated IC_{50} are shown on the insets.

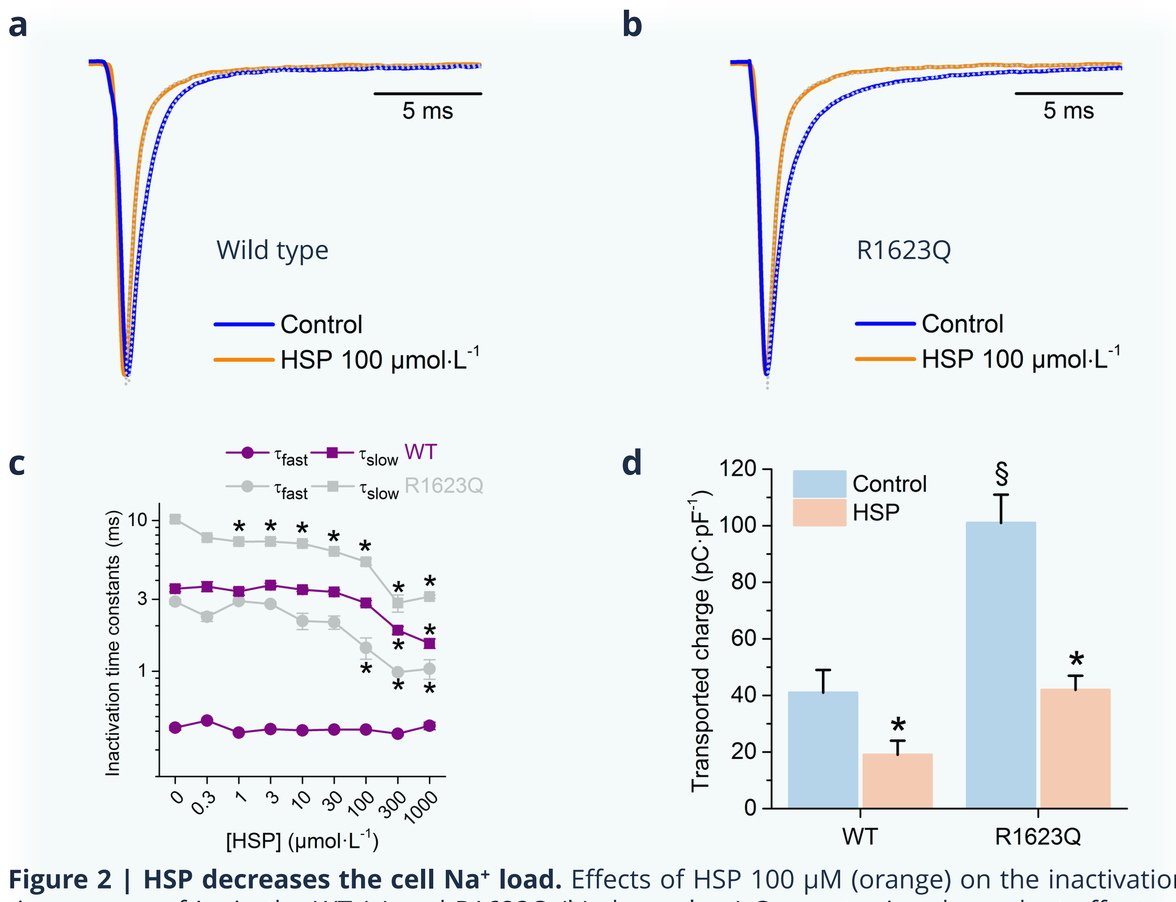

Figure 2 | HSP decreases the cell Na^+ load. Effects of HSP 100 μM (orange) on the inactivation time course of I_{Na} in the WT (a) and R1623Q (b) channels. c) Concentration-dependent effects of HSP on the fast (τ_{fast}) and slow (τ_{slow}) inactivation time constants of I_{Na}. * $P < 0.05$ compared to its own control; n = 12, two-way ANOVA with Tukey's post hoc test. d) HSP 100 μM reduced the amount of transported Na^+ during the I_{Na}. Current traces were integrated and data expressed as charge normalized to membrane capacitance. * $P < 0.05$ with respect to its own control. § $P < 0.05$ with respect to WT; n = 32, two-way ANOVA with a Tukey's post hoc test.

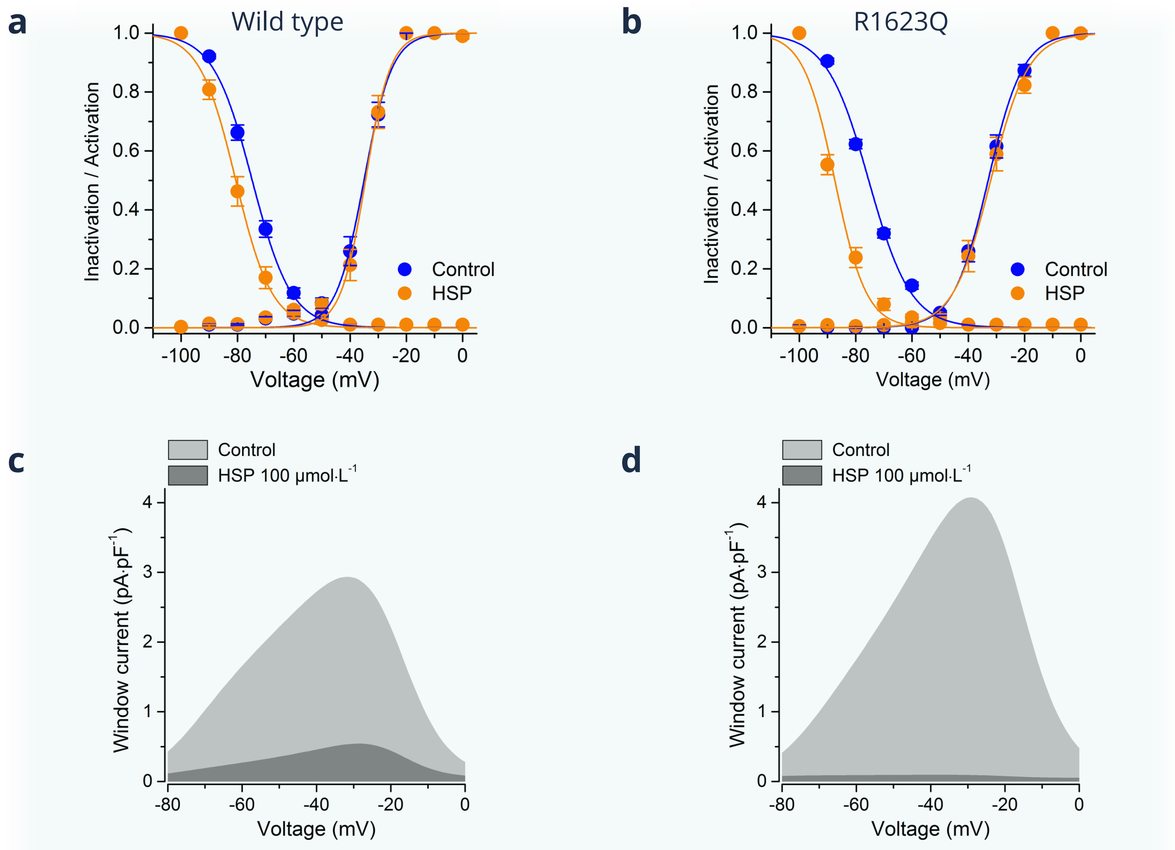

Figure 3 | Effects of HSP on voltage-dependent kinetics of I_{Na} in the WT and the R1623Q mutant. Inactivation and activation curves for WT (a) and R1623Q (b) in control (blue) and in the presence of HSP 100 μM (orange). The dots represent the mean ± s.e.m. of n = 13 normalized peak I_{Na} amplitude (for the inactivation curve) and channel conductance (for the activation curve) values. HSP 100 μM significantly shifted the inactivation curve compared to control in both the WT and R1623Q mutant channels ($P < 0.05$, paired t-test). Consequently, the Na^+ window current is markedly decreased by HSP in both the WT (c) and the R1623Q mutant channels (d).

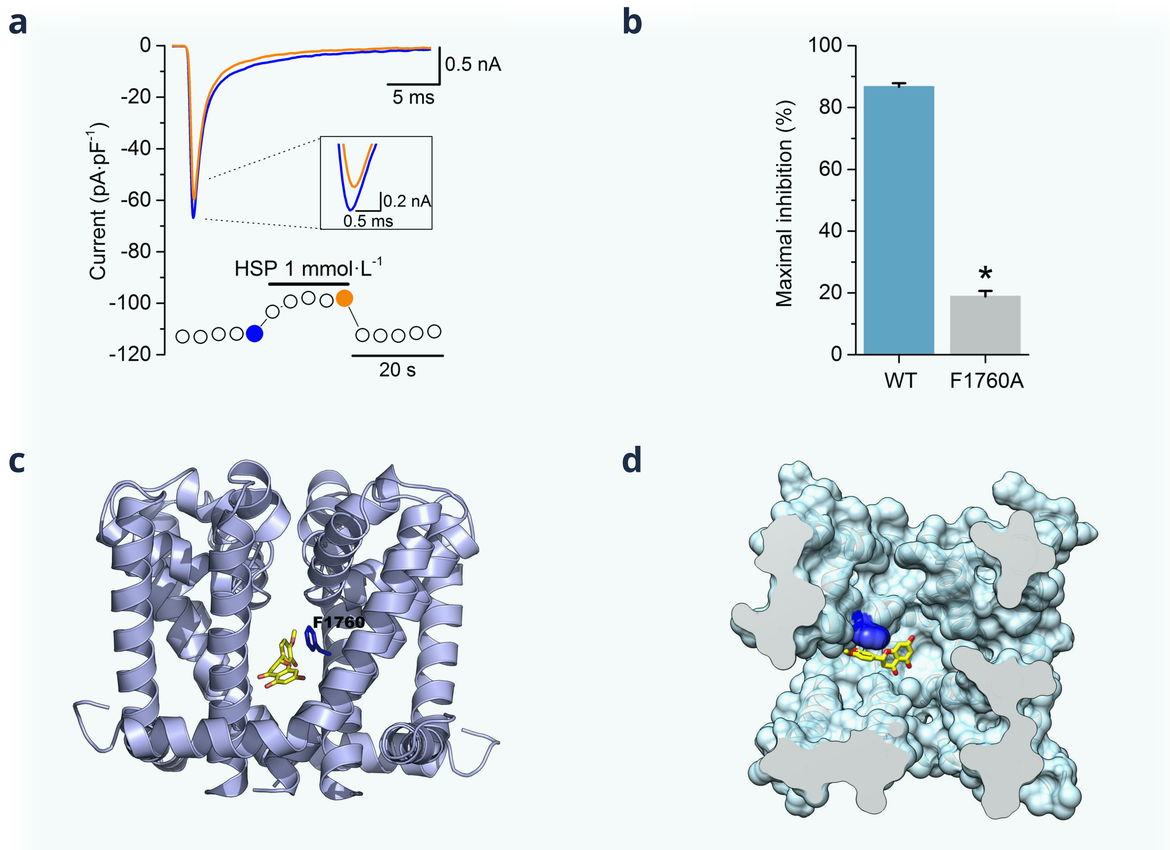

Figure 4 | HSP interacts with the $hNa_V1.5$ channel local anaesthetic (LA) binding site. a) Time course of the I_{Na} in a HEK293T cells expressing the $hNa_V1.5$ channel with the mutation F1760A. Representative current traces in control (blue) and upon the application of HSP 1 mM (orange) are shown in the insets and correspond with the coloured data points. b) Bar graph comparing the mean ± s.e.m. maximal inhibition of peak I_{Na} by HSP in WT and F1760A mutated channel (n = 9, * $P < 0.05$, two-sample t-test). c) and d) Molecular docking experiments in a model of the $hNa_V1.5$ channel. HSP (yellow) was bound at the $hNa_V1.5$ LA binding site (blue).