Na\textsubscript{V}1.5 \\big late
\textbf{: An inactivation deficient mutant of Na\textsubscript{V}1.5 as screening tool for late sodium currents of the cardiac action potential}
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\textbf{Abstract}
Torsades de pointes (TdP) is a potentially fatal type of a ventricular tachycardia associated with delayed repolarization of the cardiac action potential. The major reason for pharmacologically induced TdP is the blockade of the voltage-gated potassium channel (\(K\textsubscript{+}\)1.1 or hERG current, \(I\textsubscript{K}\)). Therefore, the main focus of pre-clinical in vitro tests has been set on detection of \(I\textsubscript{K}\), blockade to effectively discard drugs with a propensity to induce TdP. However, not all compounds that block \(I\textsubscript{K}\) will eventually induce tachyarrhythmia and, therefore, a detected block of \(I\textsubscript{K}\) alone is not specifically predictive for delayed repolarization and TdP. Not all known \(I\textsubscript{K}\) blockers cause significant arrhythmia because effects caused by induced reduction of potassium outward currents may be counterbalanced by a reduced calcium inward current (\(I\textsubscript{Ca}\)) or late inward sodium current (\(I\textsubscript{Na}\)). hERG and L-type calcium currents (\(I\textsubscript{Ca}\)) can be easily assessed in vitro systems using electrophysiology methods (e.g. patch-clamping). Since the physiological late sodium current exhibits only tiny current amplitudes, \(I\textsubscript{Na}\) needs to be increased for drug screening by decreasing or slowing the inactivation of Na\textsubscript{V}1.5 channels. This can be pharmacologically achieved by adding a sea anemone toxin II (AHAII), which binds to the extracellular linker of segments S3-S4 of domain IV or by using inactivation modifying mutations. For this study a cell line stably expressing a mutated Na\textsubscript{V}1.5 (\(I\textsubscript{Na}\)) channel was generated and validated using known \(I\textsubscript{Na}\) blockers. The substitution L409C/A410V was found to lead to a inactivation-deficient mutation. The mutation is located in D155S and presumably prevents access of the intrinsic fast inactivation particle to the inner cavity. During pharmacological validation using manual and automated (Q-PatchTM) patch-clamping, IC\textsubscript{50} values differed by less than a factor of two between ATXII stimulated and CW mutated Na\textsubscript{V}1.5 channels. Besides shorter duration of \(I\textsubscript{Na}\) experiments and larger current amplitudes, also the observed sensitivity to ATXII and overall reduced assay costs are strong arguments to screen late sodium currents in mutated rather than in pharmacologically stimulated Na\textsubscript{V}1.5 channels.

\textbf{Summary \\& Conclusions}
• Na\textsubscript{V}1.5 \big late\textsuperscript{\textsuperscript{large}} shows larger \(I\textsubscript{Na}\) currents, compared with ATXII stimulated Na\textsubscript{V}1.5
• The voltage dependence of activation and inactivation is not significantly changed by the L409C / A410V mutation
• Reference compounds show a two fold higher affinity to Na\textsubscript{V}1.5 \big late\textsuperscript{\textsuperscript{large}} than for Na\textsubscript{V}1.5
• Na\textsubscript{V}1.5 \big late\textsuperscript{\textsuperscript{large}} is a suitable test system to screen compounds on their effects on \(I\textsubscript{Na}\).

\textbf{Material \\& Methods}
Cotont optimized sequences of Na\textsubscript{V}1.5 WT and Na\textsubscript{V}1.5 \big late\textsuperscript{\textsuperscript{large}} (Na\textsubscript{V}1.5 L409C/A410V were cloned into suitable expression vectors and verified by sequencing. HEK293 cells were stably transfected and selected by antibiotic selection and FACS sorting. Clones were electrophysiologically tested and after biophysical characterization the assay was optimized for automated patch-clamping (Q-PatchTM). The extracellular solution contained (in mM) NMD (137), KCl (4), Ca\textsubscript{2+} (1.8), Mg\textsubscript{2+} (2.0), HEPES (10), Glucose (10), intracellular solution: KCl (120), NaCl (10), MgCl\textsubscript{2} (4.0) (5), EGTA (5), HEPES (10). All chemicals were purchased from Sigma, ATXII from Alomone Labs. All stock solutions were prepared in DMSO or DMSI, stored frozen and diluted in extracellular solution shortly before experimentation. All experiments were carried out at ambient temperature.