$Na_V1.5$ *big late* : An inactivation deficient mutant of $Na_V1.5$ as screening tool for late sodium currents of the cardiac action potential

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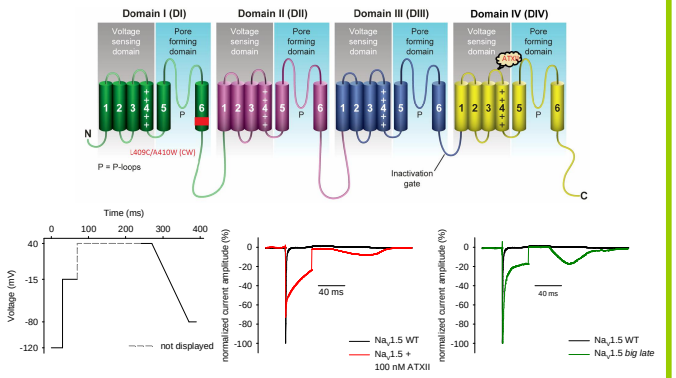
Abstract

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 ($K_V11.1$ or hERG current, I_{Kr}). Therefore, the main focus of pre-clinical *in vitro* tests has been set on detection of I_{Kr} blockade to effectively discard drugs with a propensity to induce TdP. However, not all compounds that block I_{Kr} will eventually induce tachyarrhythmia and, therefore, a detected block of I_{Kr} alone is not specifically predictive for delayed repolarization and TdP. Not all known I_{Kr} blockers cause significant arrhythmia because effects caused by induced reduction of potassium outward currents may be counterbalanced by a reduced calcium inward current (I_{CaL}) or late inward sodium current (I_{NaL}). hERG and L-type calcium currents ($Ca_V1.2$) can easily be assessed in *in vitro* systems using electrophysiology methods (e.g. patch-clamping). Since the physiological late sodium current exhibits only tiny current amplitudes, I_{NaL} needs to be increased for drug screening by decreasing or slowing the inactivation of $Na_V1.5$ channels. This can be pharmacologically achieved by adding a sea anemone toxin II (ATX-II), 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 $Na_V1.5$ (CW) channel was generated and validated using known I_{NaL} blockers. The substitution L409C/A410W was found to lead to a inactivation-deficient mutation. The mutation is localized in D1S6 and presumably prevents access of the intrinsic fast inactivation particle to the inner cavity.

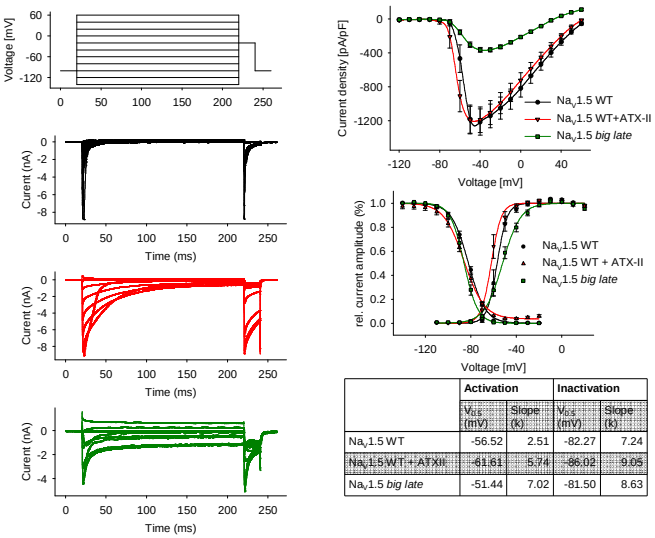
During pharmacological validation using manual and automated (Q-Patch™) patch-clamping, IC_{50} values differed by less than a factor of two between ATXII stimulated and CW mutated $Na_V1.5$ channels. Besides shorter duration of I_{NaL} experiments and larger current amplitudes, also the observed conserved sensitivity to ATXII and overall reduced assay costs are strong arguments to screen late sodium currents in mutated rather than in pharmacologically stimulated $Na_V1.5$ channels.

$Na_V1.5$ *big late*: $Na_V1.5$ L409C/A410W is located in D1S6 and exhibits I_{NaL} currents similar to ATXII stimulated $Na_V1.5$ WT



Steady state I_{NaL} can be recorded using a voltage protocol simulating a cardiac action potential. I_{NaL} is recorded as minimum current amplitude during the voltage ramp from +40 mV to -80 mV (bottom left). $Na_V1.5$ WT channels stimulated with a saturating concentration of 100 nM ATXII (>EC_{100}, bottom middle) show similar late current amplitudes like $Na_V1.5$ *big late* (bottom right). For better comparison, current amplitudes were normalized to the peak current amplitude of $Na_V1.5$ WT.

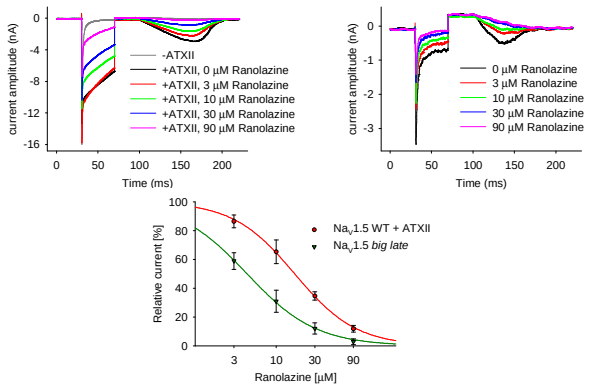
Biophysical characterization of ATXII stimulated $Na_V1.5$, $Na_V1.5$ *big late* vs. $Na_V1.5$ WT



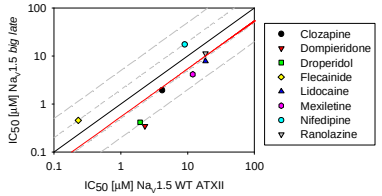
	Activation		Inactivation	
	$V_{0.5}$ (mV)	Slope (k)	$V_{0.5}$ (mV)	Slope (k)
$Na_V1.5$ WT	-56.52	2.51	-82.27	7.24
$Na_V1.5$ WT + ATXII	-61.61	5.74	-86.02	9.05
$Na_V1.5$ *big late*	-51.44	7.02	-81.50	8.63

Cells expressing $Na_V1.5$ WT (black), $Na_V1.5$ WT+ATXII (red) and $Na_V1.5$ *big late* (green) were depolarized to potentials between -140 mV and +60 mV for 200 ms followed by a fixed test pulse to -20 mV. Peak current amplitudes for $Na_V1.5$ *big late* were significantly lower (right top) , while the voltage dependence for activation and inactivation was not significantly affected (right middle and table).

Ranolazine blocks I_{NaL} in ATXII (100 nM) stimulated $Na_V1.5$ and $Na_V1.5$ *big late*

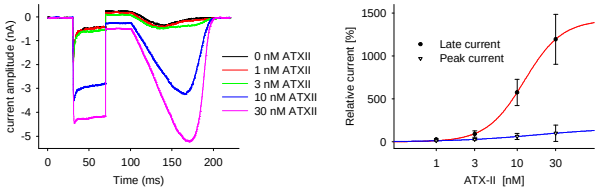


Pharmacological comparison of $Na_V1.5$ *big late* and ATXII stimulated $Na_V1.5$ WT



For a pharmacological validation of I_{NaL}, eight reference compounds were tested on ATXII (100 nM) stimulated $Na_V1.5$ WT and $Na_V1.5$ *big late* channels. Concentration response curves were recorded and IC_{50} values determined using automated patch-clamping (Q-Patch™). Most tested compounds showed an about two to five times higher affinity to $Na_V1.5$ *big late* channels than to ATXII stimulated $Na_V1.5$ WT channels. Short dashed line: two fold, long dashed line: five fold, red line: regression factor 0.54

$Na_V1.5$ *big late* is activated by ATXII in a concentration dependent manner



Summary & Conclusions

- $Na_V1.5$ *big large* shows large I_{NaL} currents, comparable with ATXII stimulated $Na_V1.5$
- The voltage dependence of activation and inactivation is not significantly changed by the L409C / A410W mutation
- Reference compounds show a two fold higher affinity to $Na_V1.5$ *big late* than for $Na_V1.5$
- $Na_V1.5$ *big late* is a suitable test system to screen compounds on their effects on I_{NaL}.

Material & Methods

Codon optimized sequences of $Na_V1.5$ WT and $Na_V1.5$ *big late* ($Na_V1.5$ L409C/A410W) 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-Patch™). The extracellular solution contained (in mM) NaCl (137), KCl (4), $CaCl_2{\cdot}2H_2O$ (1.8), MgCl2·H2O (1), HEPES (10), Glucose (10); intracellular solution: KCl (120), NaCl (10), $MgCl_2{\cdot}6H_2O$ (6), EGTA (5), HEPES (10). All chemicals were purchased from Sigma, ATXII from Alomone Labs. All stock solutions were prepared in DMSO or WFI, stored frozen and diluted in extracellular solution shortly before experimentation. All experiments were carried out at ambient temperature.