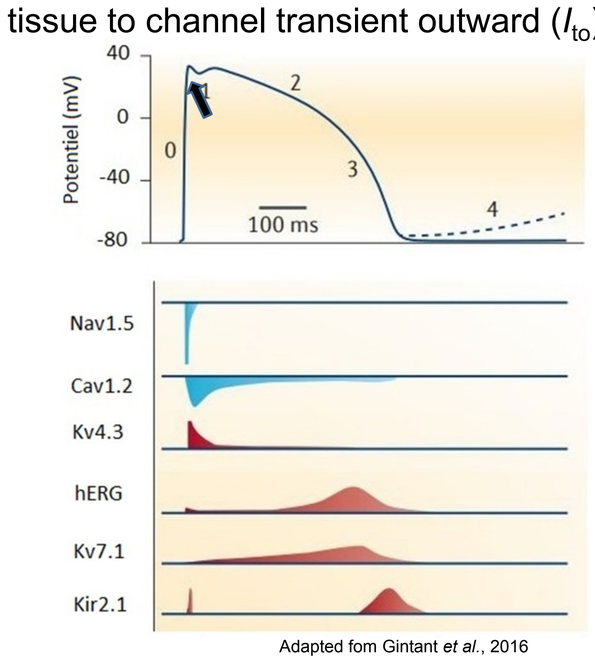
A PHARMACOLOGICAL SYNOPSIS OF SMALL MOLECULES, TOXINS AND CiPA COMPOUNDS TARGETING HUMAN CARDIAC Kv4.3 CHANNELS

Brigitte Schombert, Camille Sanson, Sylvie Houtmann, Michel Partiseti and G. Andrees Böhme, Sanofi-Aventis R&D, Integrated Drug Discovery, High Content Biology, Vitry-sur-seine, France

Introduction

- Kv4.3 α-subunits associate with ancillary β-subunits of the KChIP2 family in heart muscular tissue to channel transient outward (I_{to}) currents.



Adapted fom Gintant *et al.*, 2016

- By virtue of their biophysical properties, I_{to} currents activate and inactivate kinetics,counteract I_{Na} and $I_{Ca,L}$ currents immediately after the upstroke phase 0 of the cardiac action potential to produce the typical "Spike-and-Dome" shape of phase 1 & 2 (arrow).
- Decreased expression or dysfunction of Kv4.3 channels following myocardial infarction or during heart failure can contribute to abnormal repolarization which may result in ventricular arrhythmias.
- Therefore, drug-induced inhibition of Kv4.3/KChIP2-mediated I_{to} exposes to potential cardiosafety liabilities which are important to document early during the drug discovery process.
- Here we have characterized basic electrophysiological properties pertaining to I_{to} currents using an automated patch-clamp station and recombinant cells expressing Kv4.3 and KChIP2.2 subunits.
- The currents obtained were further validated pharmacologically by assessing the efficacy of small molecule and toxin inhibitors known from manual patch-clamp studies to block I_{to}.
- Then, we examined the inhibitory activities of 28 drugs with clinically documented high, medium or low pro-arrhythmic risk encompassing the test- and validation-sets of the Comprehensive *in vitro* Pro-Arrythmia (CiPA) panel.

Methods

Cell line :

- Chinese Hamster Ovary cells stably expressing the human *KCND3* and *KCNIP2* gene products (Charles River, Cat. # CT6171).

Principle of test:

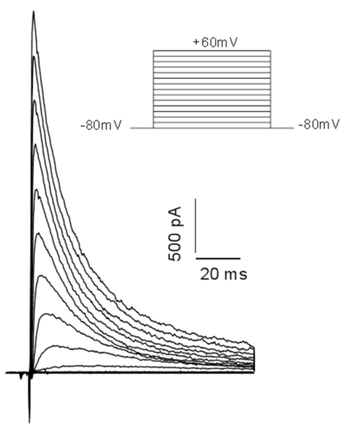
- Whole-cell recording at room temperature on Sophion's 48X planar patch-clamp workstation in single- or multi-hole QPlates®.

Buffers (in mM): *Intracellular*: KF, 120 ; EGTA, 10 ; KCl, 20 ; HEPES, 10. pH adjusted to 7.2 with KOH, osmolarity = 295-300 mOsm ; *Extracellular*: NaCl, 150 ; KCl, 4 ; $CaCl_2$, 2 ; $MgCl_2$, 1 and HEPES, 10. pH adjusted to 7.4 with NaOH, osmolarity = 310 mOsm.

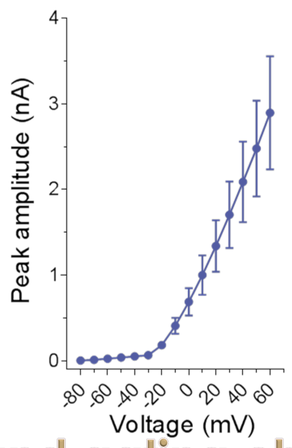
Pharmacology:

- Treatments were applied in 6 cumulative concentrations in buffer containing 0.3% DMSO and 0.06% Pluronic F-68 as surfactant to decrease precipitation if any. Phrixotoxin-2 (PaTx2) was applied in buffer containing 0.1 % Bovine Serum Albumin to decrease peptide adherence to labware and microplates fluidics.
- Inhibitions were quantified as change in the normalized area under the current curve (*i.e.* integral charge transferred) except for PaTx-2 which was quantified as change in peak amplitude.

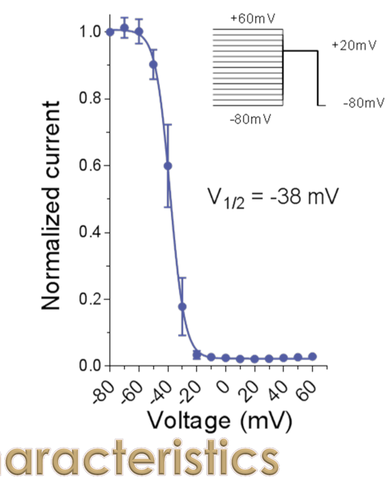
Traces



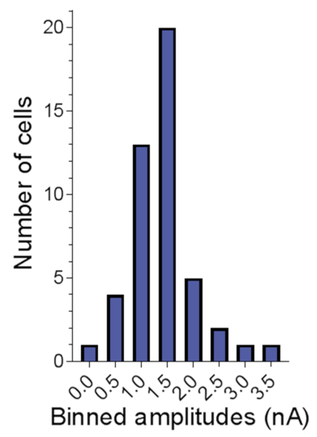
Activation



Inactivation



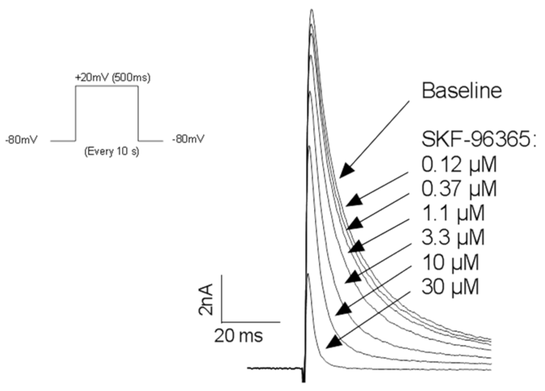
Frequency distribution

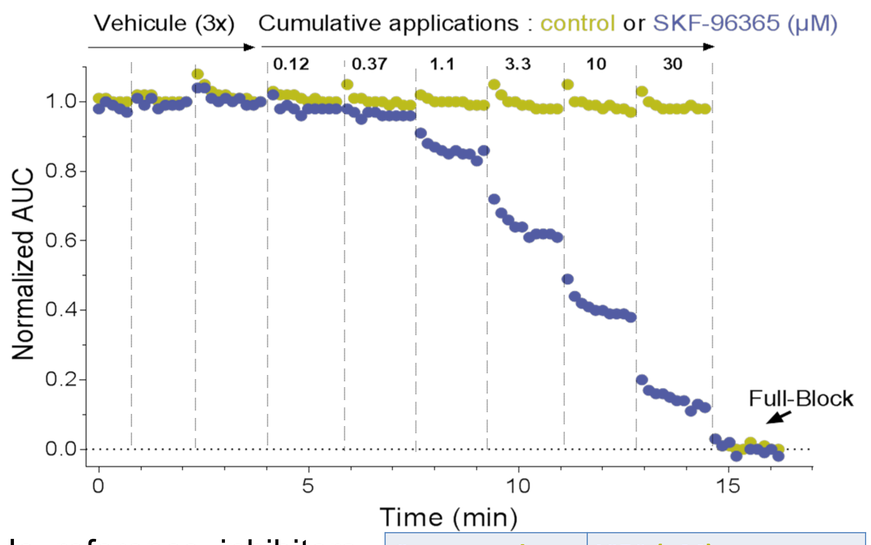


Current properties and gating characteristics

Currents displaying fast activation and inactivation kinetics developed at membrane potentials above -20 mV, reaching peak outward amplitude within tens of ms and rapidly extinguishing well before the end of 500 ms depolarizing pulses. The median peak current amplitude at +20 mV was 1.4 nA (inter-quartile range : 0.98 nA to 1.7 nA, *N* = 47).

Small molecule inhibitors

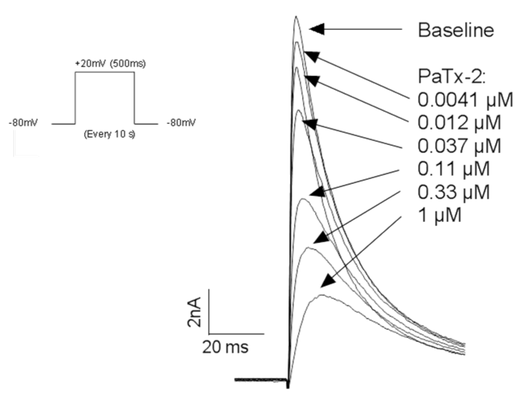


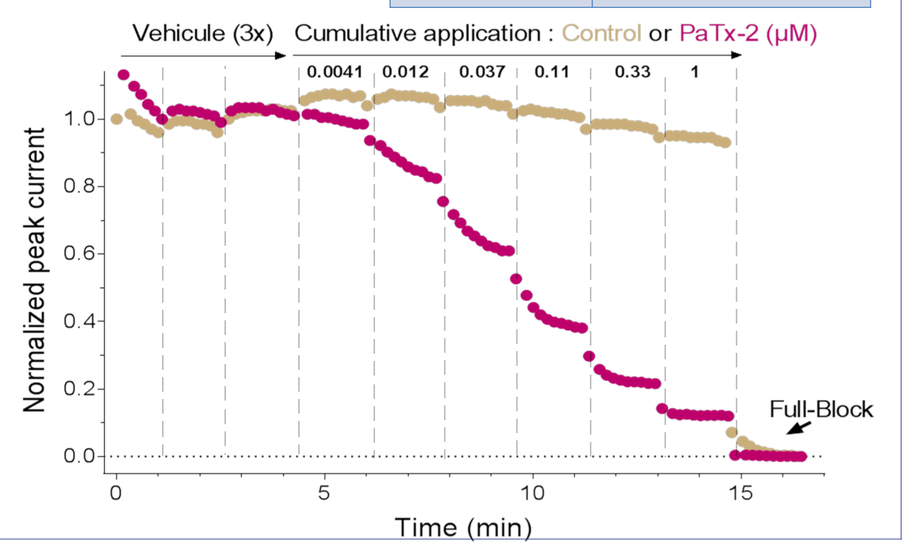


SKF-96365 and the other small molecule reference inhibitors tested (HP-184, Sibutramine and Dapoxetine) affected the apparent rate of current decay more strongly than the peak current. In contrast, the peptide toxin phrixotoxin-2 (PaTx-2) isolated from the venom of the Chile tarantula *Phrixotrichus auratus* preferentially decreased the current size.

Compound	IC50 (µM)
HP-184	3.3 ± 0.3
Sibutramine	6.5 ± 0.7
Dapoxetine	14 ± 1.8
SKF-96365	5.2 ± 0.5
PaTx-2	0.09 ± 0.008

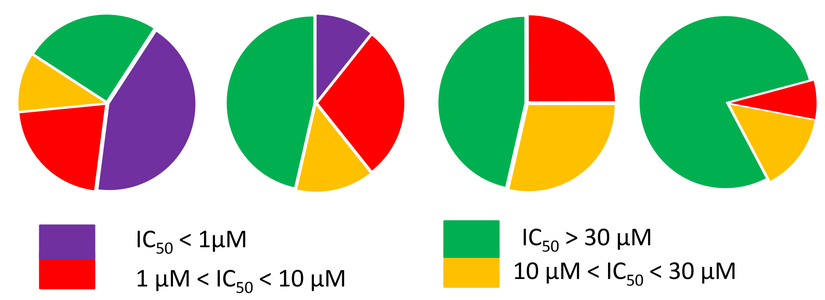
Phrixotoxin-2





Pharmacology of CiPA compounds

IC_{50}(µM)				
Compound	hERG	CaV1.2	NaV1.5	KV4.3
Bepridil	0.19	6	3.1	13
Dofetilide	0.047	204	94	> 300
Quinidine	1.3	2.9	35	15
d-Sotalol	644	> 300	> 300	>300
Vandetanib	3.2	19	39	91
Disopyramide	79	114	> 90	> 90
Azimilide	1.1	42	18	> 90
Ibutilide	0.016	83	110	68
Chlorpromazine	1.7	5.8	5.4	16
Cisapride	0.015	33	> 300	> 300
Terfenadine	0.17	2.2	3.3	68
Ondansetron	0.42	227	86	> 300
Astemizole	0.017	0.59	2.8	22
Clarithromycin	295	103	> 90	> 90
Clozapine	4.8	9.2	23	62
Domperidone	0.17	38	9.3	111
Droperidol	0.1	20	19	73
Pimozide	0.045	1.1	4.7	292
Risperidone	0.41	138	102	43
Diltiazem	17	8.2	15	84
Mexiletine	53	47	53	147
Ranolazine	25	156	101	> 300
Verapamil	0.6	1.5	29	58
Loratadine	11	17	20	9.3
Metoprolol	316	> 300	> 300	> 300
Nifedipine	92	0.051	23	31
Nitrendipine	38	0.37	4.4	7.8
Tamoxifen	6.7	24	20	93



Conclusion

While the majority of CiPA compounds are strong hERG inhibitors, half of them are CaV1.2 inhibitors and, to a lesser extent, NaV1.5 peak inhibitors. Only a minority affect Kv4.3 at concentrations < 30 µM.

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