

Stem Cell-Derived Human Cardiomyocytes: Utility for Risk Evaluation and Determining Complex Mechanisms of Drug Action

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ChanTest Corporation



- **Ph.D. & Postdoctoral work**
 - Vollum Institute, Oregon Health and Science University
 - areas: Neuroscience, Ion Channel Biophysics
- **Expertise:**
 - Patch-Clamp Electrophysiology
 - Ion Channel Structure-Function Relationships
- **Current Position: Study Director, ChanTest**

- Part 1 – ChanTest: background, the hERG assay, and the need for more thorough preclinical safety assessment.
- Part 2 – Why so much “buzz” about stem cell derived human cardiomyocytes (SC-hCMs)?
Methods and validation of the SC-hCM system at ChanTest.
- Part 3 – Two examples of the utility of SC-hCMs for elucidating complex mechanisms of drug action

Part 1: Background



Experience & Capabilities:

- founded 1998; continuous growth; science-based; 18±PhDs, 65±FTEs
 - industry’s largest Ion Channel (IC) portfolio for discovery & safety
 - IC, IC/GPCR, GPCR and Transporter cell-based, **functional** assays
 - validated and optimized on manual and major automated platforms
 - cell lines, SC-derived cells, primary cells
 - introduced hERG assay for cardiac risk assessment
 - 25,000+ compounds tested (safety & efficacy)
 - 250+ clients worldwide
 - successfully audited by FDA & 180+ sponsors
 - 300+ papers published in peer-reviewed journals
-
- **Named “most trusted fee-for-service provider”**
 - **three years running** (*HTStec* Ion Channel Trends Survey)

ChanTest Benefits:

- Accurate Results
- Proven High Value Products
- Fast Response
- Reliable Counsel

Non-Cardiac Drugs Withdrawn for TdP

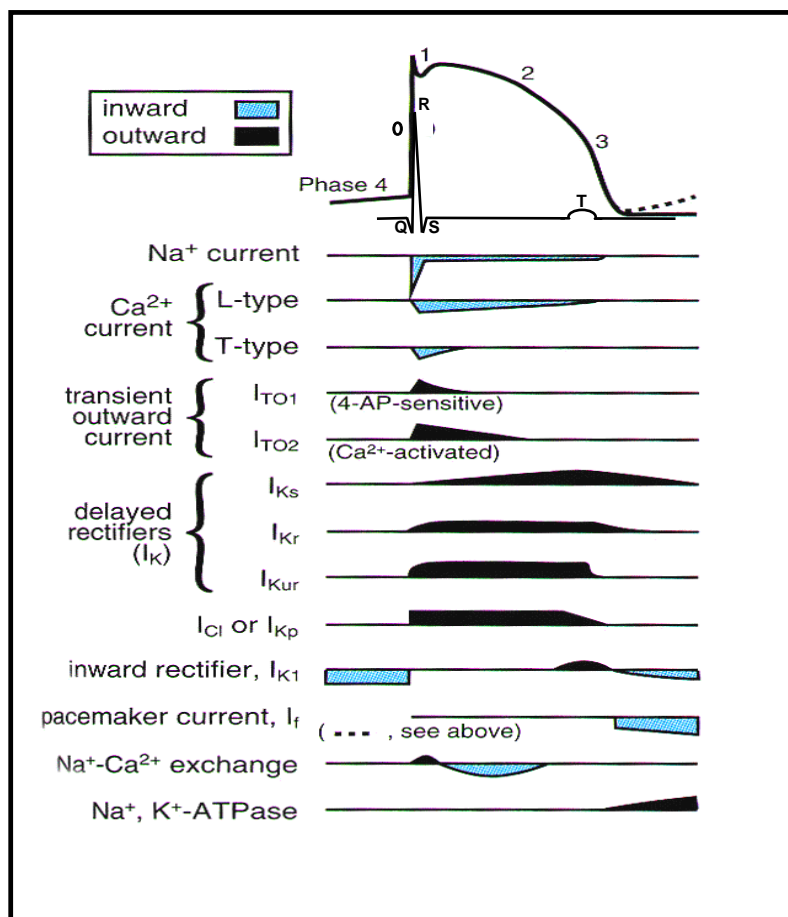
Drug	Class	Date Withdrawn
Terfenadine	Antihistamine	1998
Sertindole	Antipsychotic	1998
Astemizole	Antihistamine	1999
Grepafloxacin	Antibiotic	1999
Cisapride	Prokinetic	2000
Droperidol	Tranquilizer	2001
Levomethadyl	Opiate Dependence	2003

hERG potassium channel: Most likely target for drugs that delay repolarization

Drug (IC ₅₀ , μM)	hERG	Kv1.5	Kv4.3	KvLQT1/minK
<i>Antihistamine</i>				
Terfenadine	0.056	0.367	2.70	4.40
Fexofenadine	13.1	389	112	20.4
<i>Antipsychotic</i>				
Risperidone	0.394	9.50	25.5	9.7
Sertindole	0.005	4.00	8.80	0.880
<i>Prokinetic</i>				
Cisapride	0.044	21.2	9.33	3.39

hERG alone is insufficient for risk assessment

- The ECG and cardiac action potential are formed by many different ion channel currents.
- Effects on ion channels can be determined early in development to predict cardiac risk.



Cardiac Channel Panel™	AP Phase
Nav1.5 (I_{Na})	0, 2
Cav1.2/ β_2 , $\alpha_2\delta$, (L-type)	2
Cav3.2 (T-type)	1
Kv4.3 (I_{TO1})	1
KvLQT1/minK (I_{Ks})	2 - 3
hERG (I_{Kr})	2 - 3
Kv1.5 (I_{Kur})	2 - 3
Kir2.1 (I_{K1})	4
HCN2 (pacemaker, I_f)	4
HCN4 (pacemaker, I_f)	4
Kir3.1/3.4 ($I_{K,ACh}$)	4
Kir6.2/SUR2A ($I_{K,ATP}$)	4
NCX1 (Na-Ca exchange)	2

In vitro electrophysiology studies can provide valuable information concerning the effect of a test substance on action potential duration and/or cardiac ionic currents. These assays have an important role in assessing the potential for QT interval prolongation and elucidating cellular mechanisms affecting repolarization. *In vitro* electrophysiology studies employ either single cell (e.g., heterologous expression systems, **disaggregated cardiomyocytes**) or multicellular (e.g., Purkinje fiber; papillary muscle; trabeculae; perfused myocardium; intact heart) preparations.

Tissue and cell preparation for *in vitro* assays are obtained from different laboratory animal species including rabbit, ferret, guinea pig, dog, swine and **occasionally from humans**.

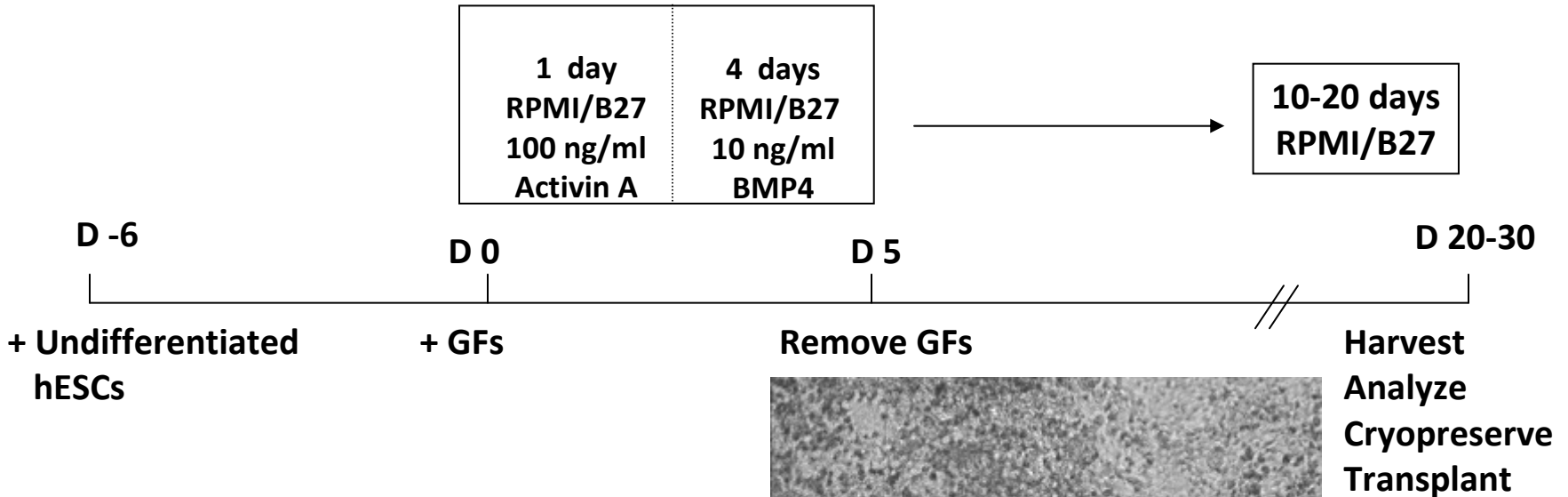
Species differences in terms of which cardiac ion channels contribute to cardiac repolarization and to the duration of the action potential should be considered in selecting a test system.

- Question: Can stem cell-derived human cardiomyocytes (SC-hCMs) improve cardiac risk assessment?
 - “Off the shelf” availability of human cardiomyocytes in large quantities is very appealing. Potential to overcome shortcomings:
 - Recombinant cell lines may not recapitulate physiology
 - Native human tissue sporadically available, expensive and often compromised
 - Non-human models may not reflect human physiology
 - Electrophysiological and pharmacological validation required. Data is limited, but datasets growing as pharma industry is actively evaluating and adopting SC-hCM technology.

Part 2: Methods and validation of the SC-hCM system at ChanTest

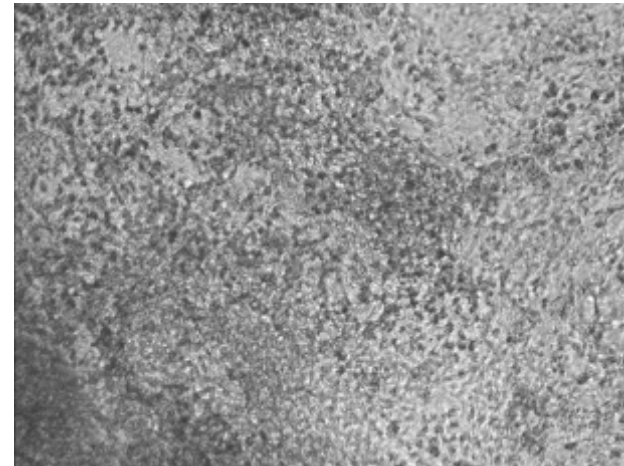


GE Healthcare Cardiomyocytes



Eliminate undesirable raw materials:

MEF-CM replaced with defined media system for hESC expansion



Slide Courtesy of Geron Corporation

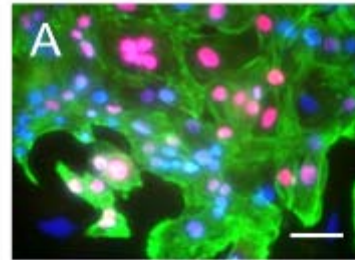
Scale-up manufacturing process:

Method sufficiently scaled for FTC ($>10^{10}$ cells/lot)

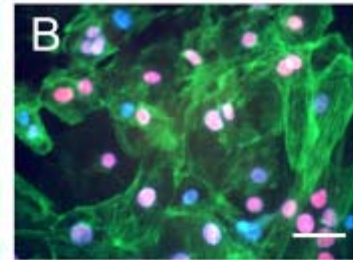
Initial POC obtained for method to support Phase 3 and commercial scale

SC-hCMs Express Cardiac Proteins

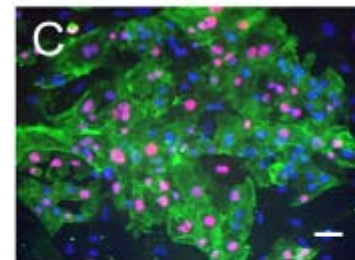
NKX2.5
Tropomyosin



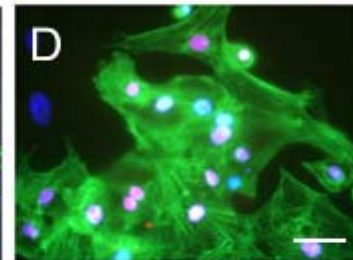
NKX2.5
cTn I



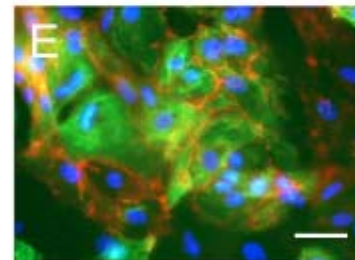
NKX2.5
cTn T



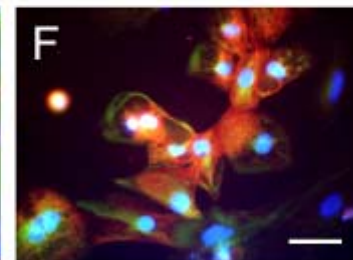
NKX2.5
a-actinin



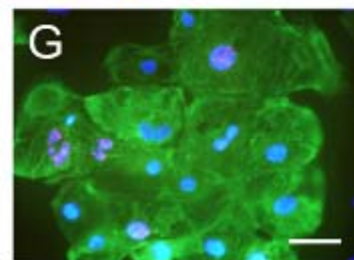
N-cadherin
MF20



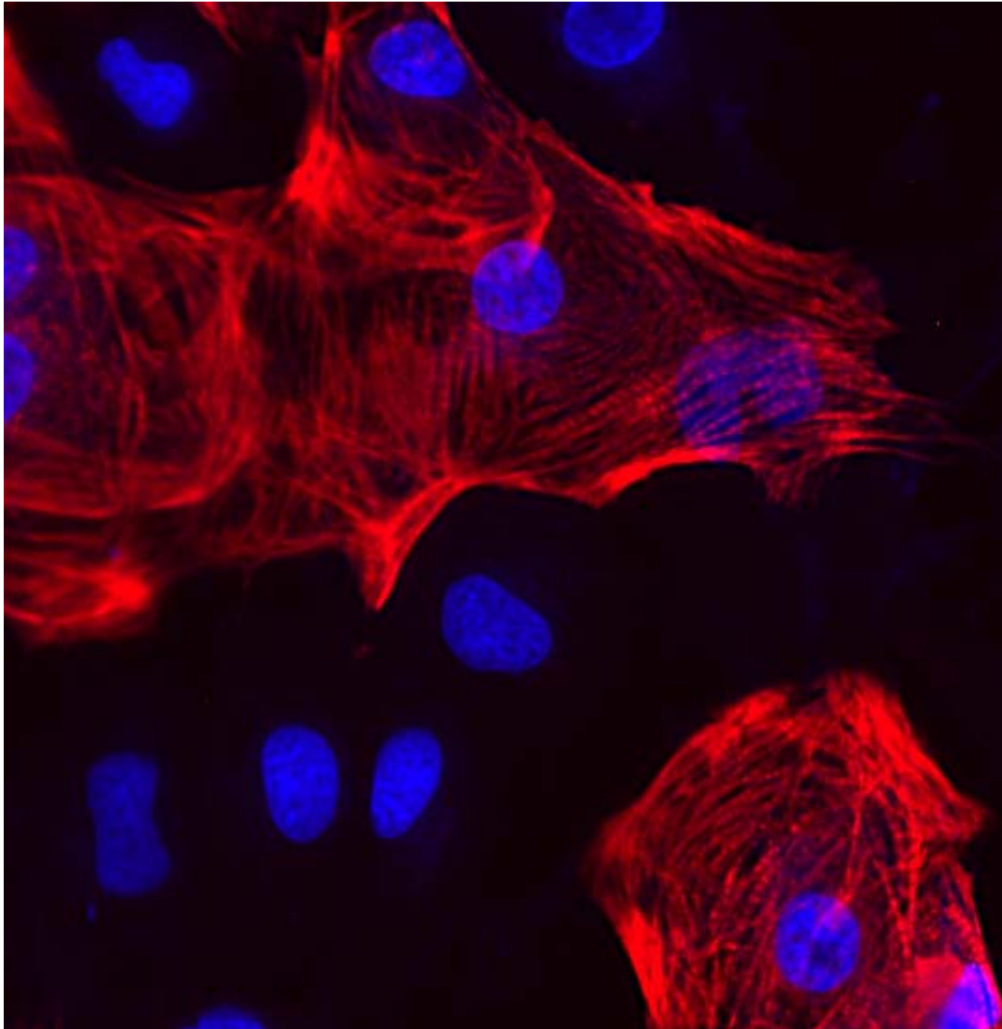
c TnI
CX43



Ki67
a-actinin

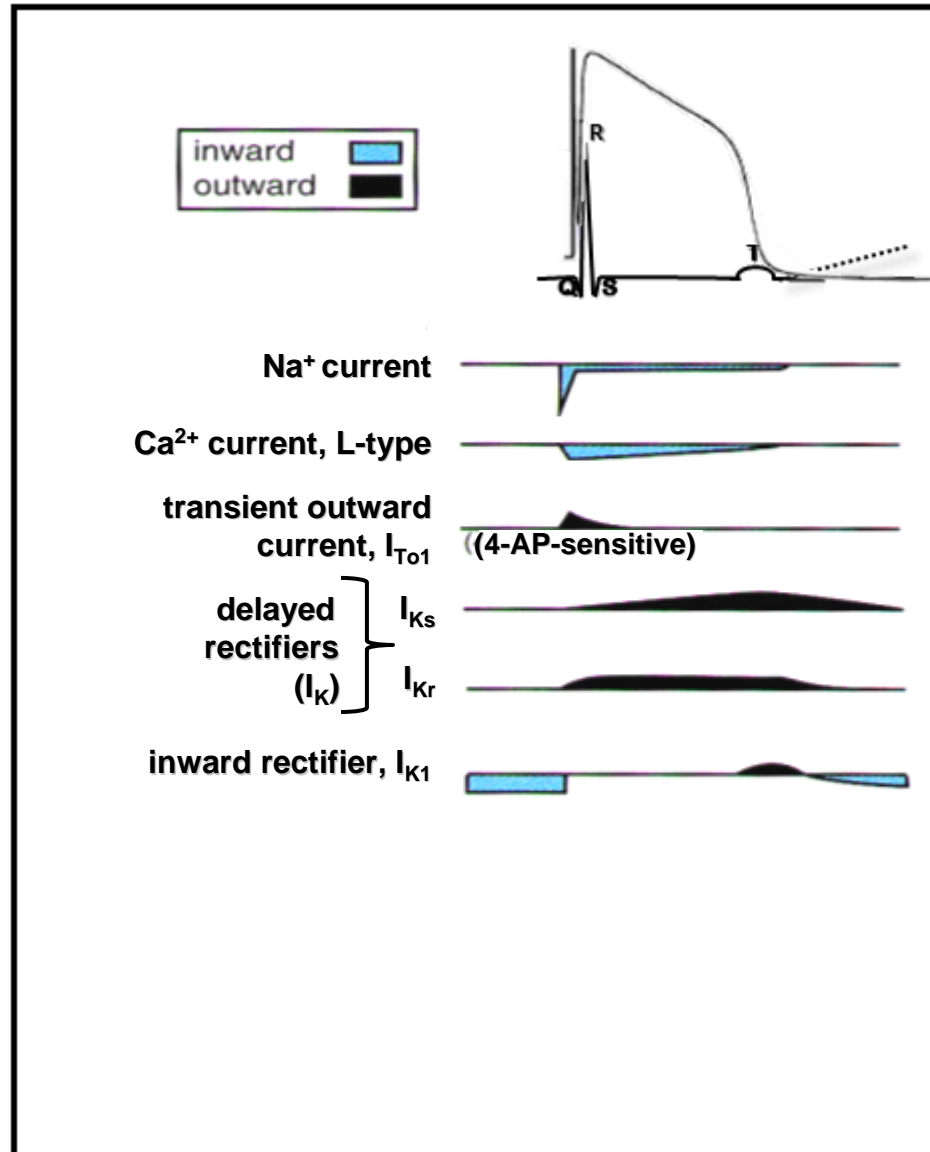


*Images Courtesy of
Geron Corporation*



- CMs are plated at low density to 35 mm dishes for EP experiments
- Single beating cells visually identified under microscope
- Whole cell voltage clamp (currents) or perforated-patch current-clamp recordings (AP) from isolated CMs

Ephys Phenotype of hESC-Derived Ventricular Myocyte

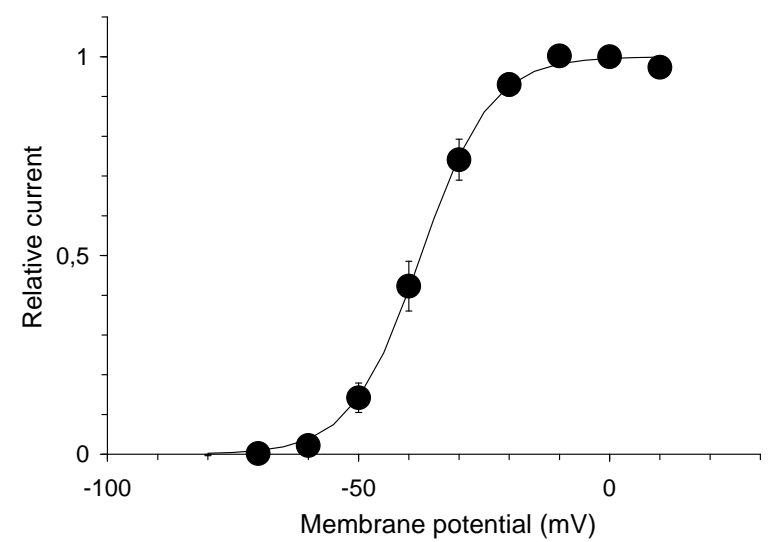
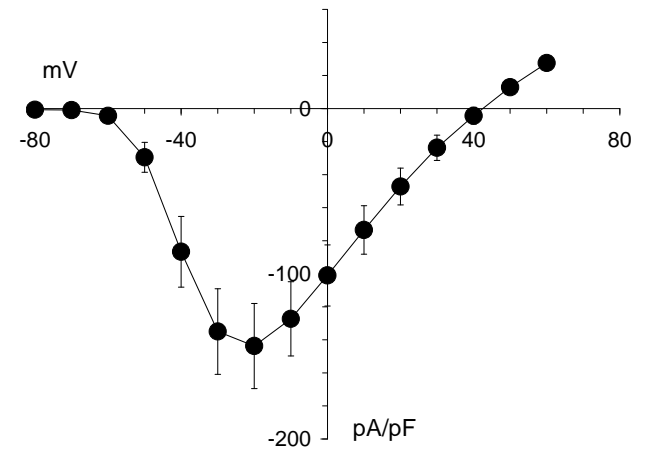
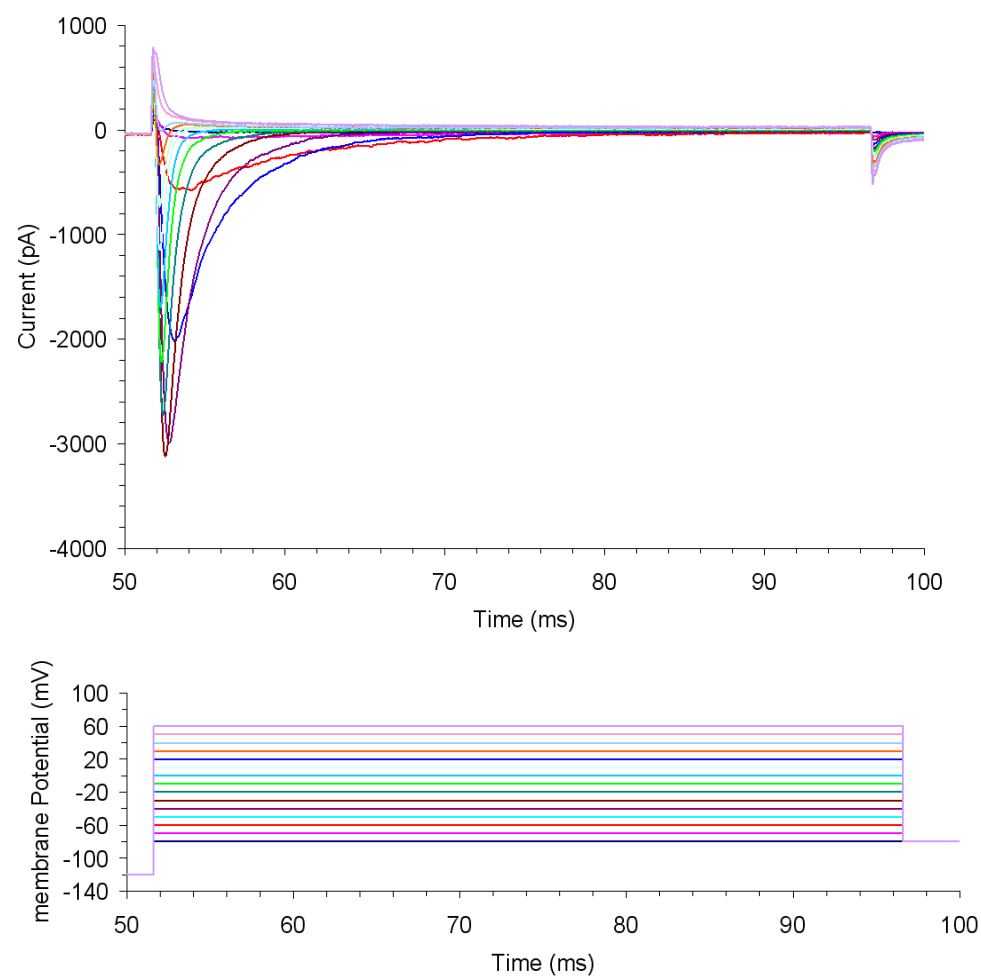


Fetal Action Potential

Major adult Currents?

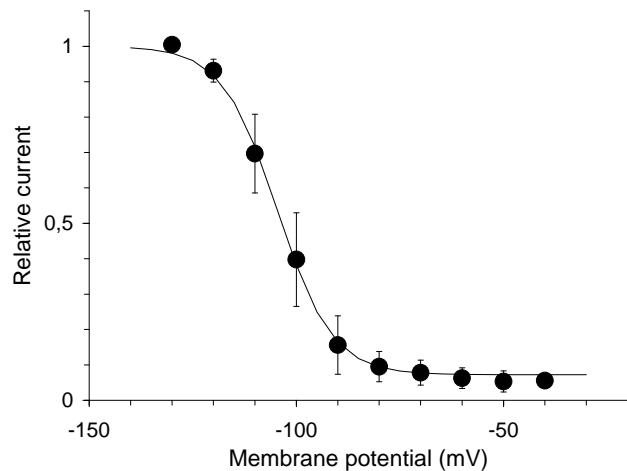
SC-hCM Sodium Channel (I_{Na})

Voltage-Dependent Activation

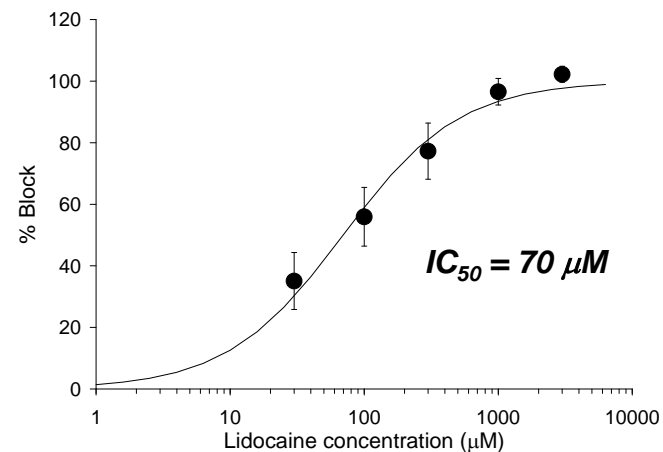


SC-hCM Sodium Channel (I_{Na})

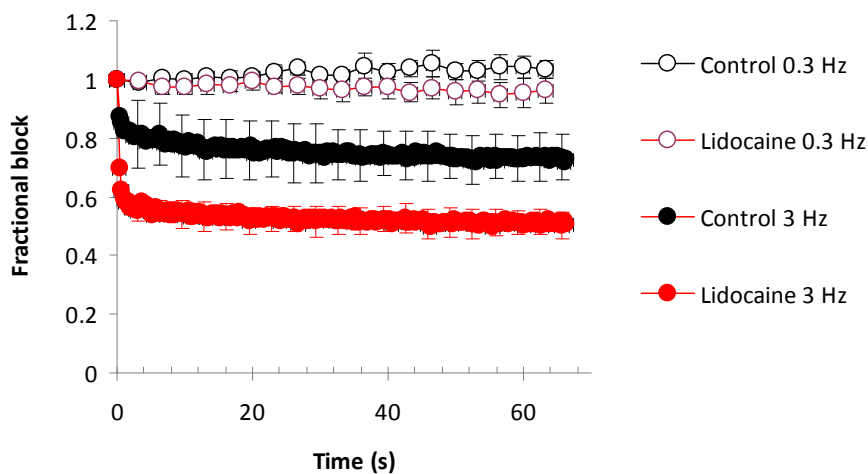
Inactivation



Lidocaine Block

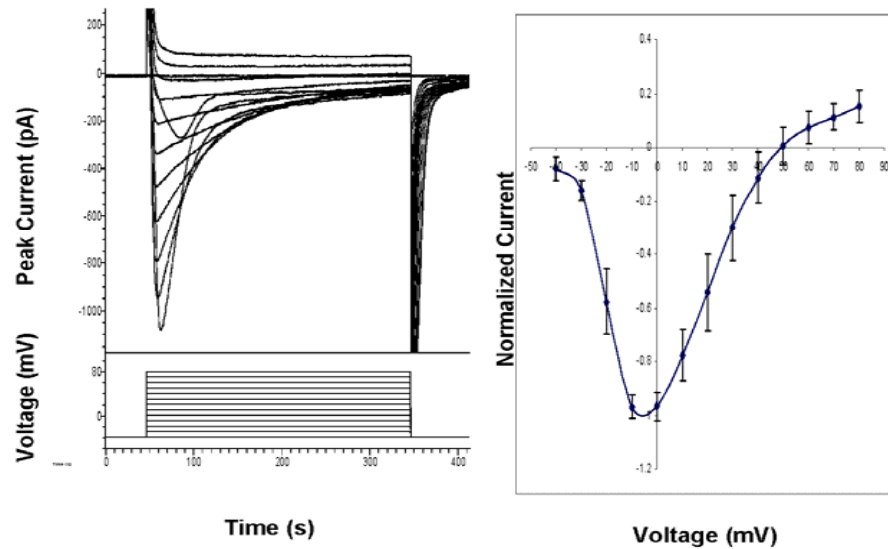


Lidocaine Use-dependence

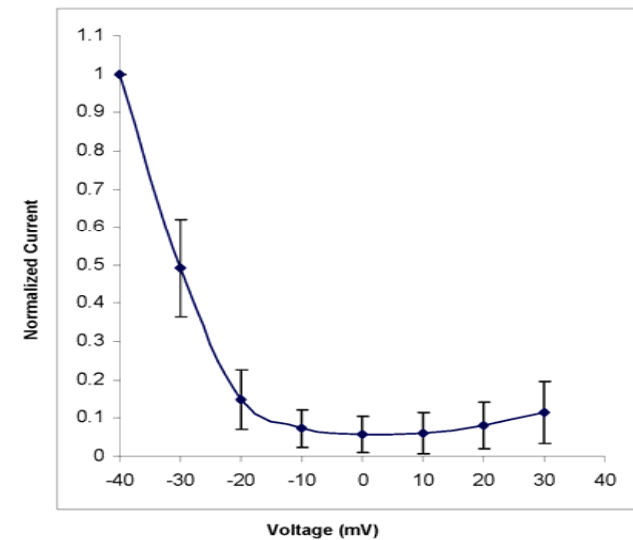


SC-hCM Calcium Channel ($I_{Ca,L}$)

Activation

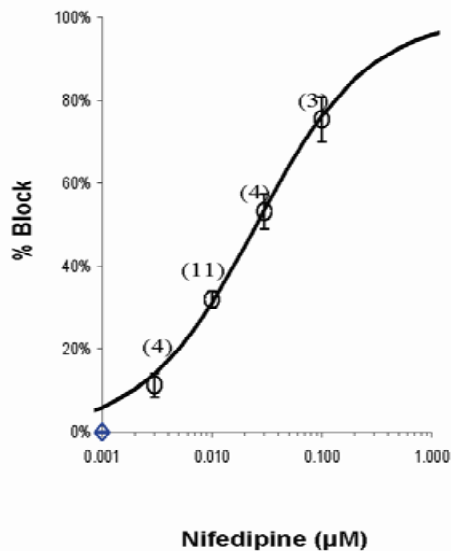
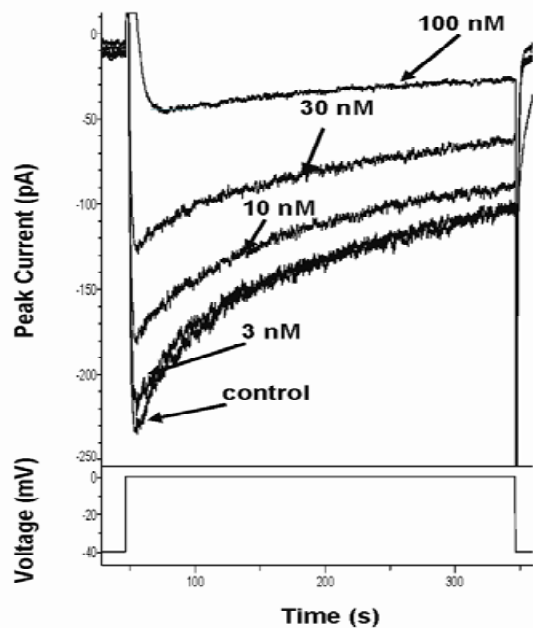


Inactivation

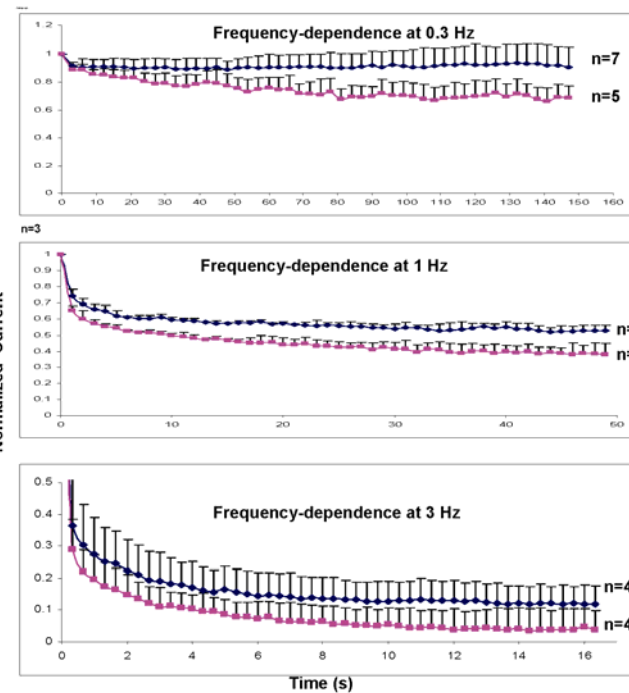


SC-hCM Calcium Channel ($I_{Ca,L}$)

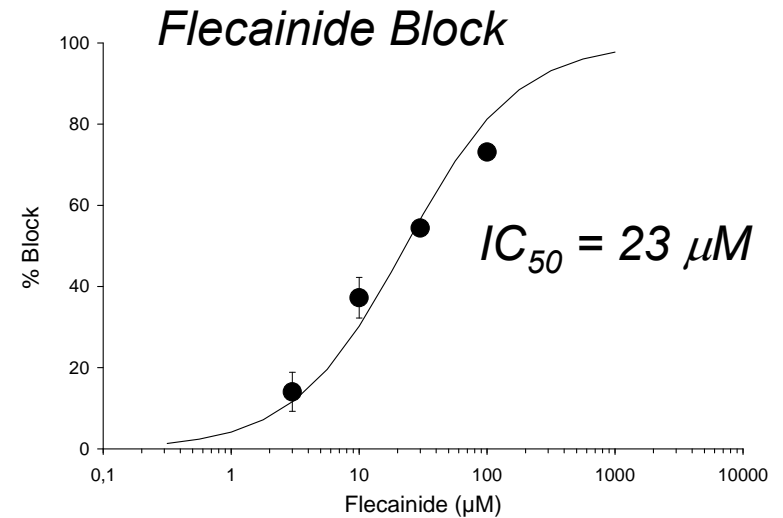
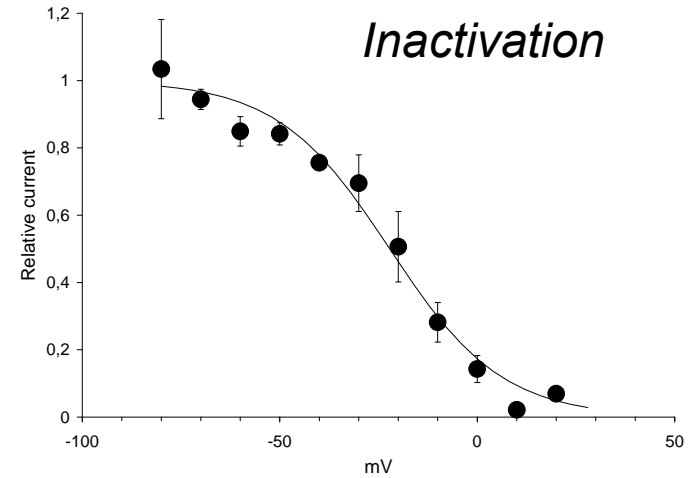
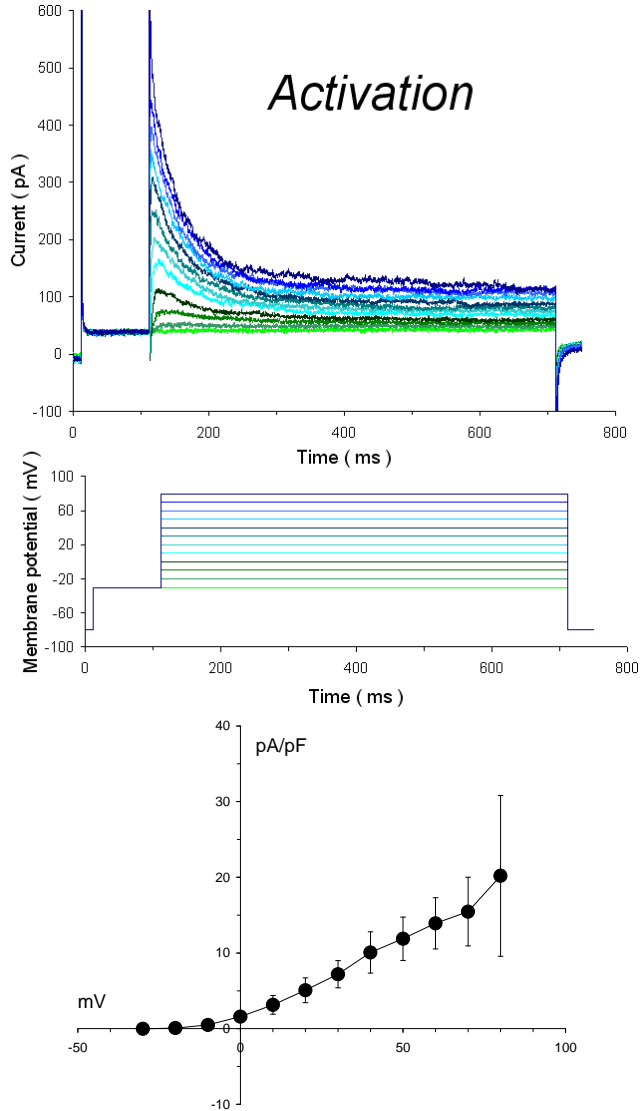
Nifedipine Block



Nifedipine Use-dependence

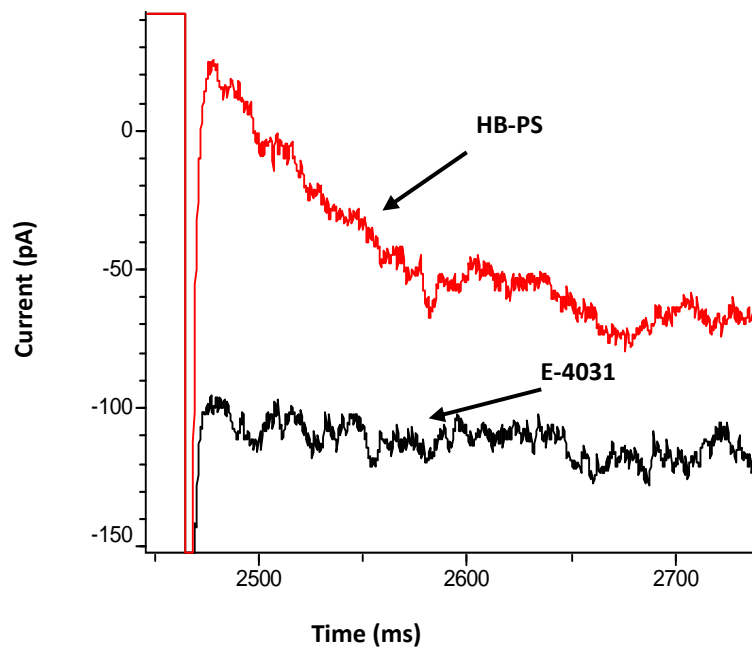


SC-hCM Potassium Channel (I_{T0})

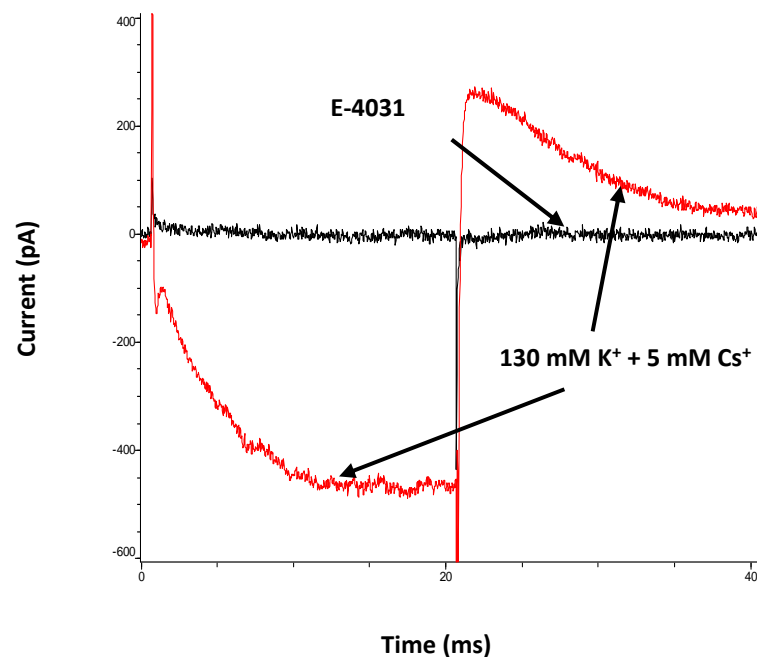


SC-hCM hERG Potassium Channel (I_{Kr})

I_{Kr} (hERG) tail currents
physiological solutions

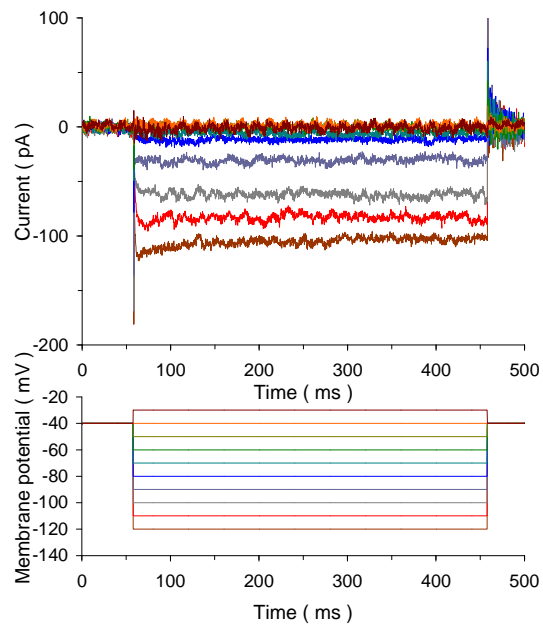


I_{Kr} (hERG) currents
modified solutions

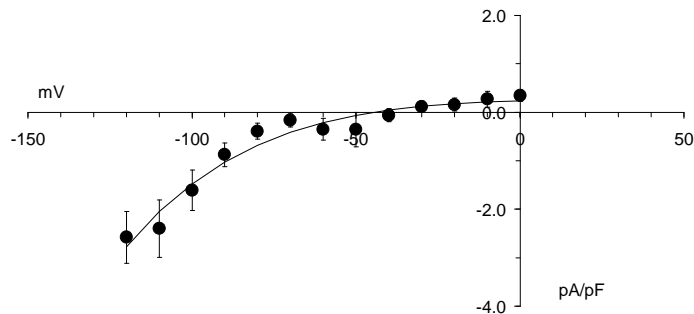
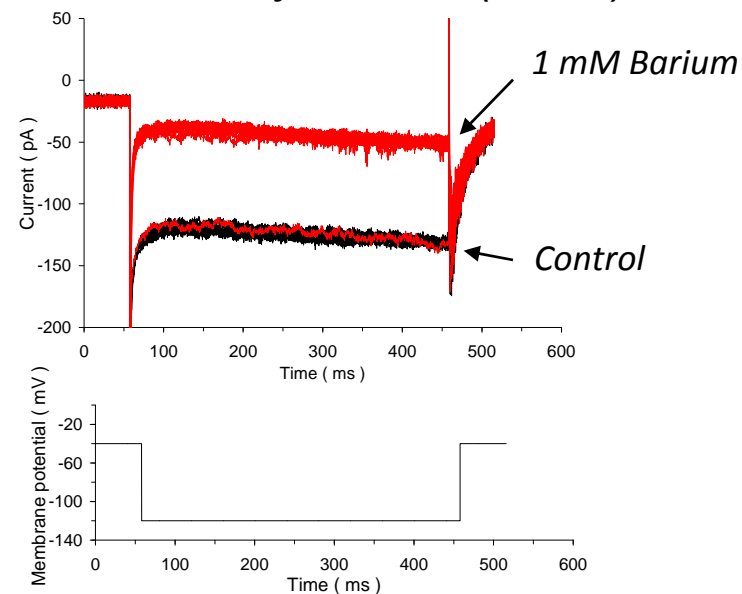


SC-hCM Potassium Channel (I_{K1})

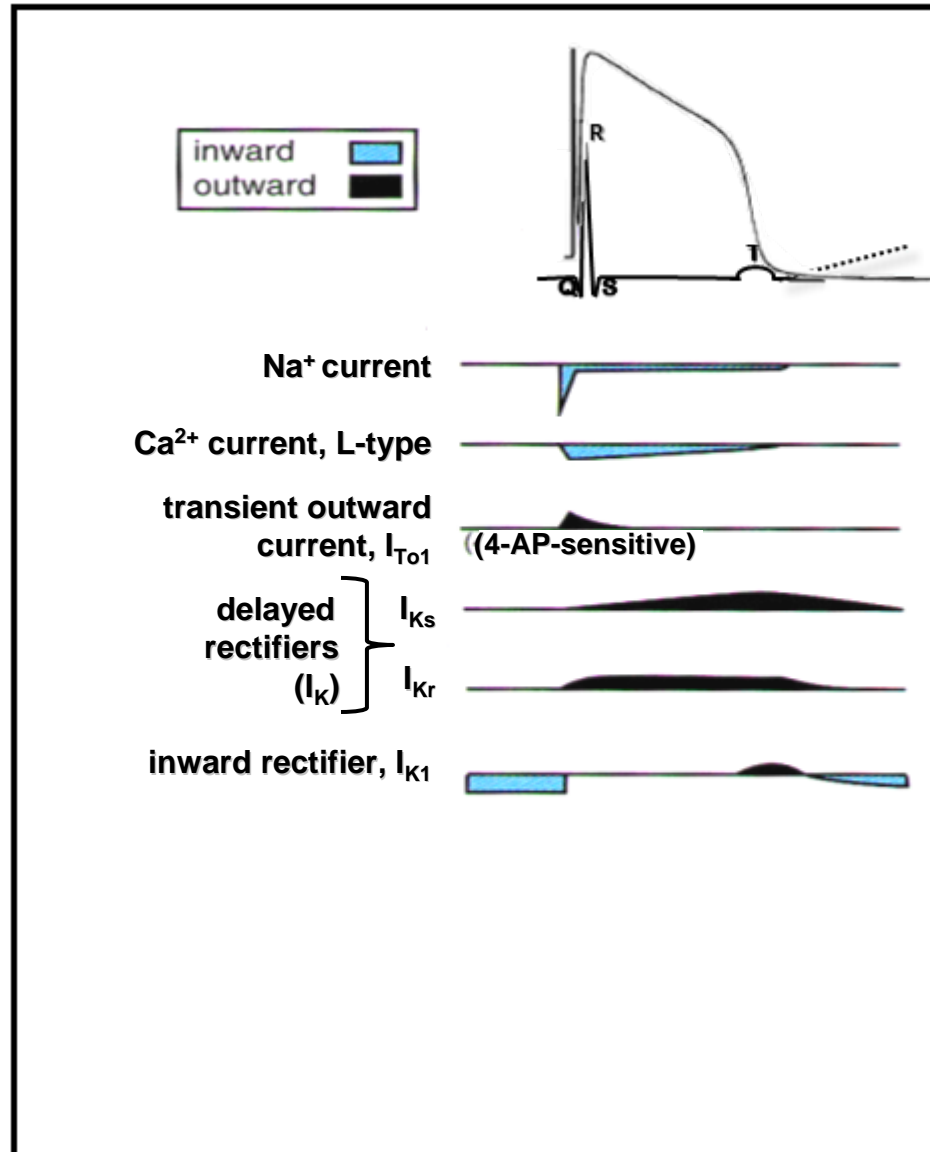
Voltage-Dependence



Block by Barium (1 mM)



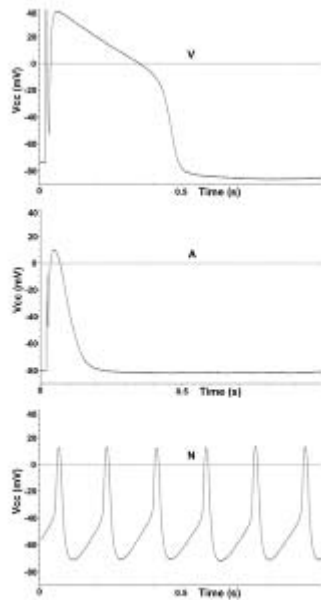
Ephys Phenotype of hESC-Derived Ventricular Myocyte



Fetal Action Potential

Major adult Currents

SC-hCM Action Potentials



Ventricular-like (82%)

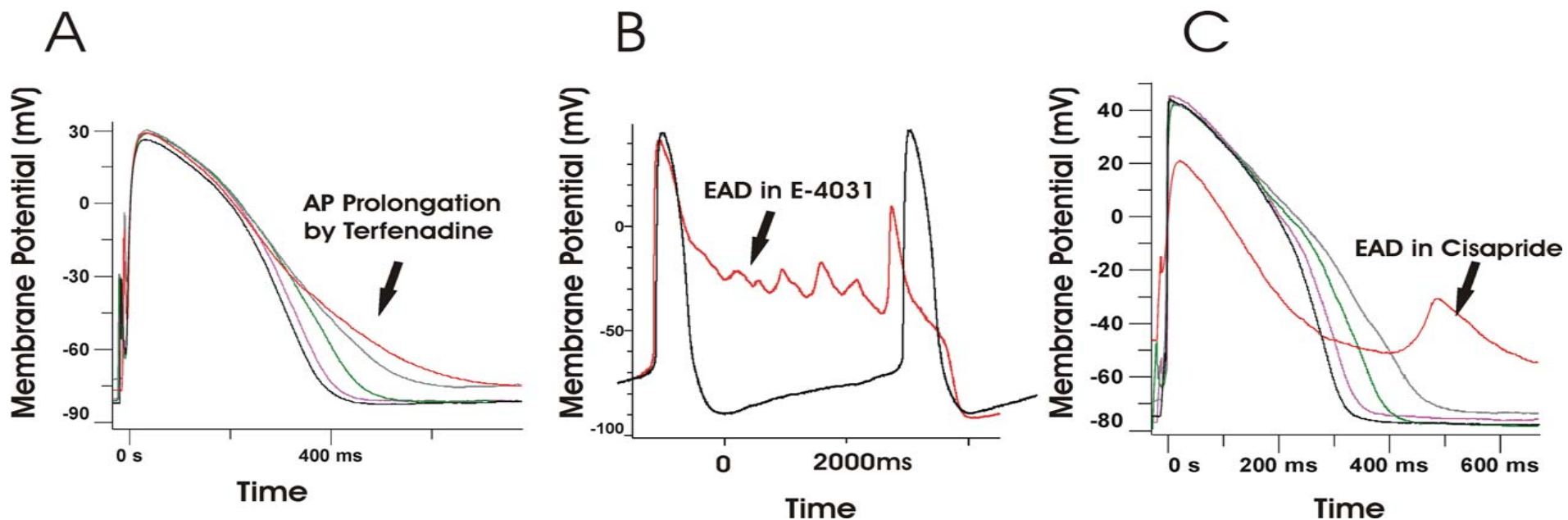
Atrial-like (18%)

Nodal-like (<1%)

Peng et al., J Pharmacol Toxicol Methods. 2010 May-Jun;61(3):277-86

Cell Type	Number of Cells	% Total	RMP (Mean ± SEM)	APA (Mean ± SEM)	V _{max} (Mean ± SEM)	APD ₉₀ (Mean ± SEM)
Ventricular	102	82	-72.2 ± 1.9	102.1 ± 2.7	11.6 ± 1.4	350.5 ± 16.8
Atrial	22	18	-64.7 ± 6.6	86.5 ± 4.5	25.7 ± 14.1	102.0 ± 6.9
Nodal	1	<1	N/A	N/A	N/A	N/A
Total Number of Cells (n)	125	100	125	125	125	125

hERG channel blockers prolong and de-stabilize the ventricular-type SC-hCM action potential



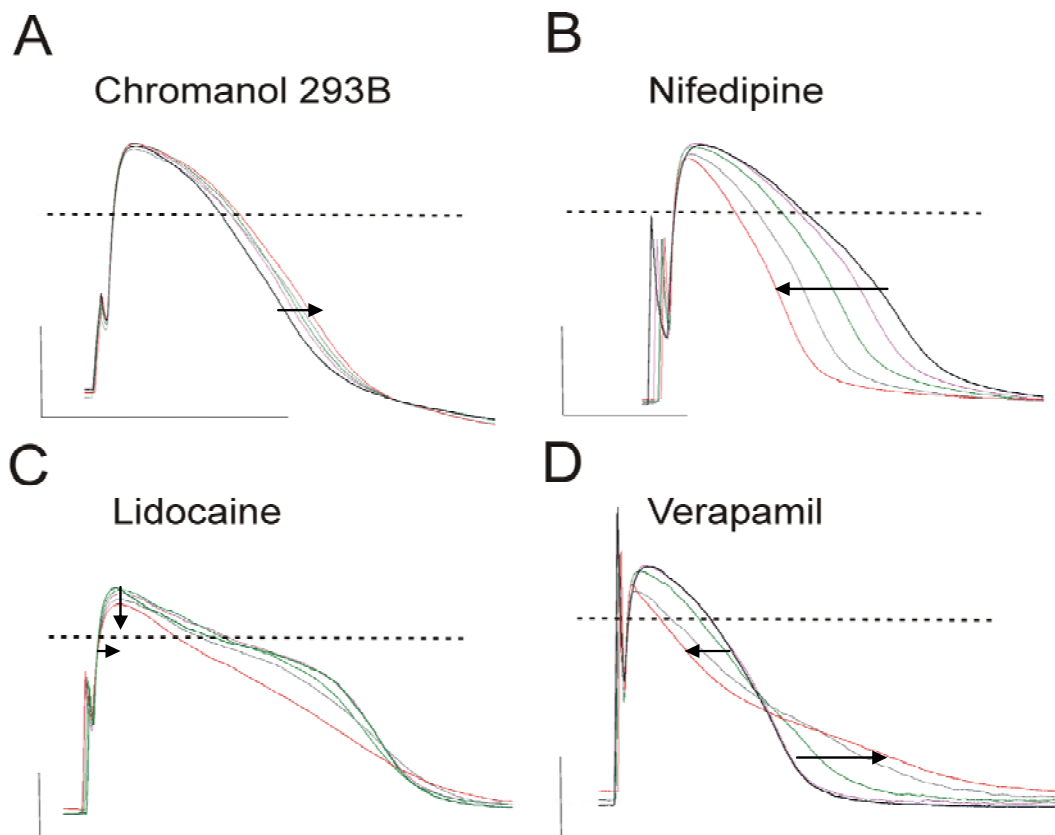
AP Prolongation and torsadogenicity induced by hERG channel blockers terfenadine, E-4031, and cisapride.

A, The selective hERG channel blocker terfenadine, which does not induce AP prolongation in canine PFs, was applied to ventricular cardiomyocytes at concentrations of **3, 10, 30 and 100 nM** (purple, green, gray and red traces, respectively), and the changes in AP parameters were measured. Concentration-dependent AP prolongation was observed.

B, The selective hERG channel blockers E-4031 and cisapride were applied to ventricular CMs. **100 nM E-4031** induced early afterdepolarizations (EADs), a trigger for Torsade de Pointes.

C, Cisapride at 3, 10 and 30 nM (purple, green and gray traces, respectively) prolonged the APD, while **100 nM cisapride** (red traces) induced EADs.

The SC-hCM AP assay detects I_{Ks} , $I_{Ca,L}$, I_{Na} blockers and mixed ion channel effects (MICE)



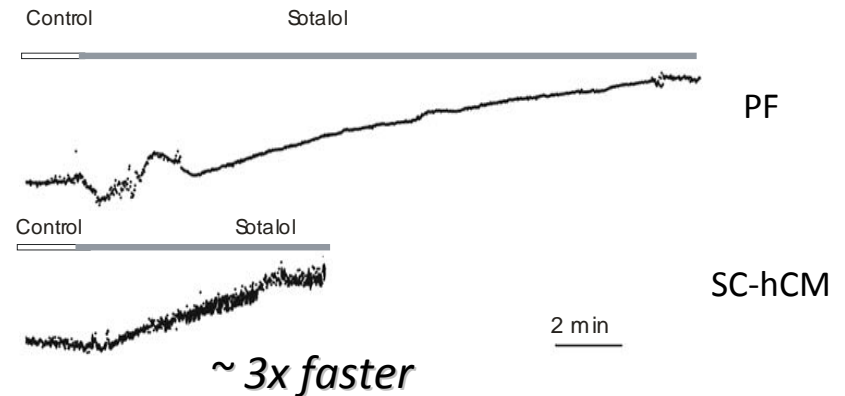
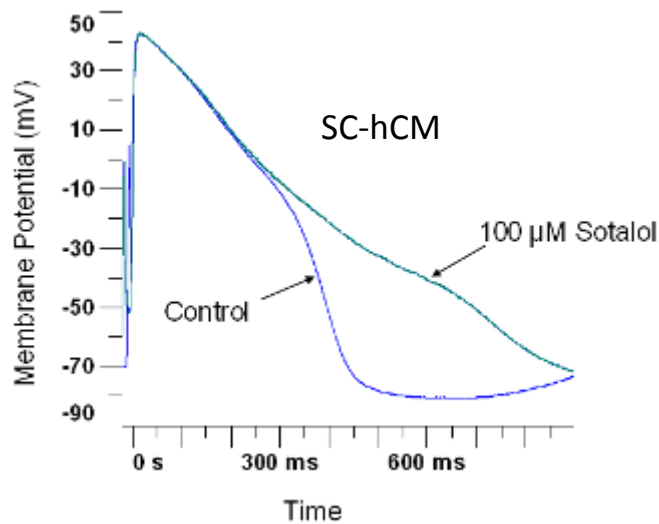
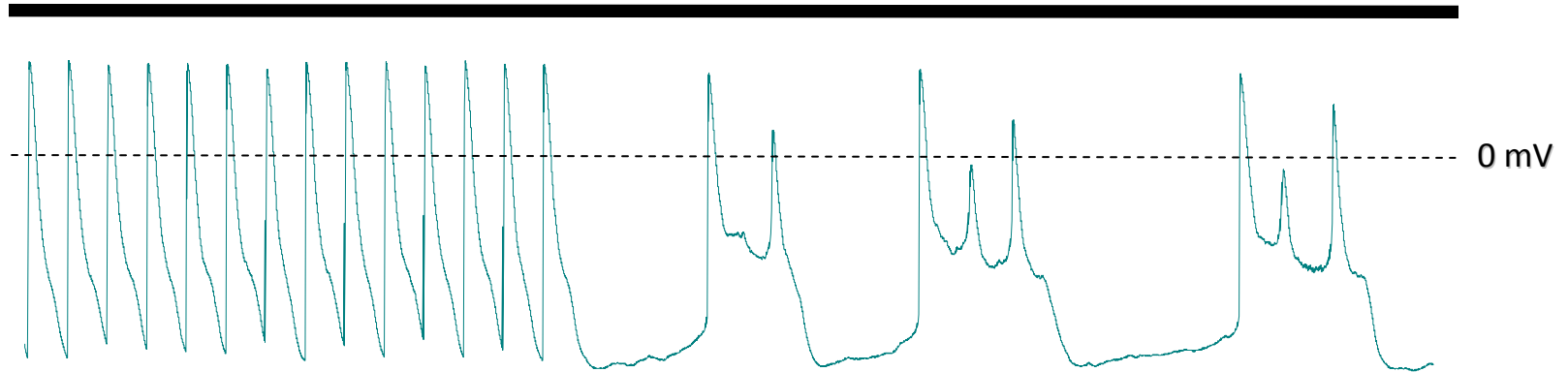
- (A) I_{Ks} : Chromanol 293B conc.
0 μ M, 10 μ M, 30 μ M, 100 μ M, 300 μ M
- (B) $I_{Ca,L}$: nifedipine conc.
0 μ M, 0.03 μ M, 0.1 μ M, 0.3 μ M, 1 μ M
- (C) I_{Na} : lidocaine conc.
0 μ M, 10 μ M, 30 μ M, 100 μ M, 300 μ M
- (D) APD: verapamil conc.
0 μ M, 0.1 μ M, 1 μ M, 3 μ M, 10 μ M

Scale bars: 200 ms (x-axis), 40 mV (y-axis)

Black arrows: compound induced direction of change

Sensitivity and Speed of SC-hCM Assay

Sotalol (100 μ M)



SC-hCMs Show High Sensitivity to Modulators of the Cardiac AP

Compound	Isolated Rabbit Heart	Rabbit PF	Canine PF	SC-hCM	hERG IC ₅₀ ³
	Positive ²	Positive ²	Positive ²	Positive ²	
Terfenadine ¹	0.5 μM ⁵	1 μM	False Negative	0.03 μM¹	0.004
Quinidine ¹	1 μM	1 μM	1 μM	0.3 μM¹	0.83
Cisapride ¹	N/A	0.1 μM	0.1 μM	0.01 μM¹	0.026
Sotalol	30 μM	10 μM	100 μM	10 μM	268
Verapamil	NE ⁶	10 μM	1 μM	1 μM	0.125
Chromanol 293B ¹	N/A	False Negative	False Negative	300 μM¹	10.7 ⁴
E- 4031	N/A	N/A	0.01 μM	0.01 μM	0.011
Nifedipine ¹	N/A	N/A	> 10 μM	0.03 μM¹	N/A

1. Red indicates increased sensitivity of SC-hCMs compared to PF
2. Positive indicates APD/QT increase ≥10%
3. From manual patch clamp experiments of hERG channels expressed in HEK cells
4. KvLQT/minK (IKs) IC₅₀
5. Applied via side port injection
6. QT not prolonged

Part 3: Utility for understanding complex mechanisms of action



THE ION CHANNEL EXPERT

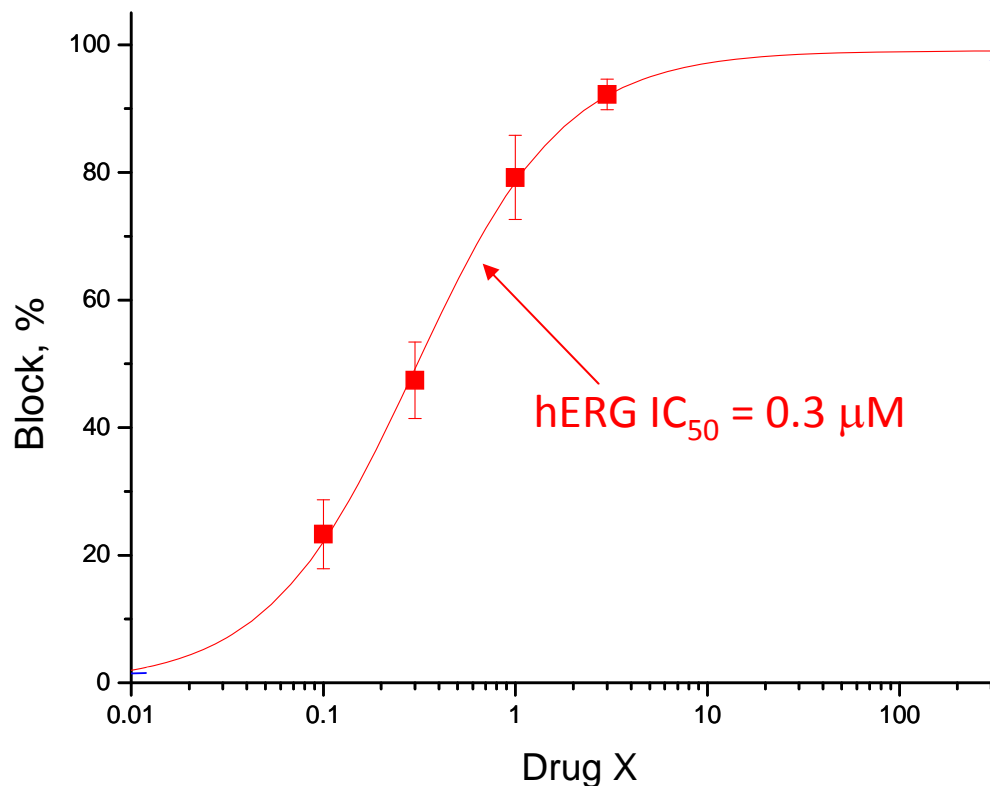
www.chantest.com

Case 1: (+)hERG, (?)QT Risk

Case 2: Vardenafil

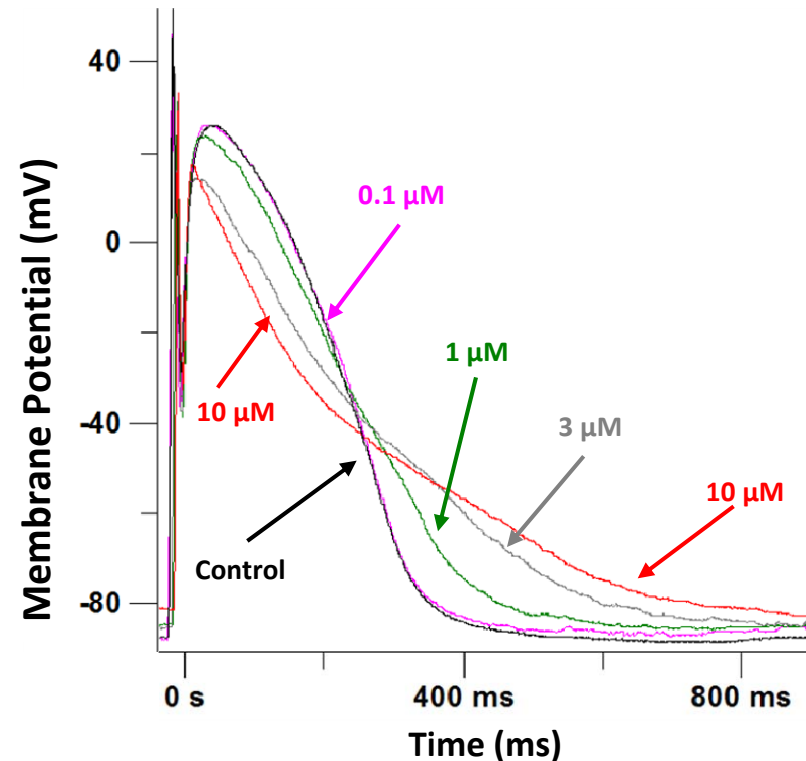
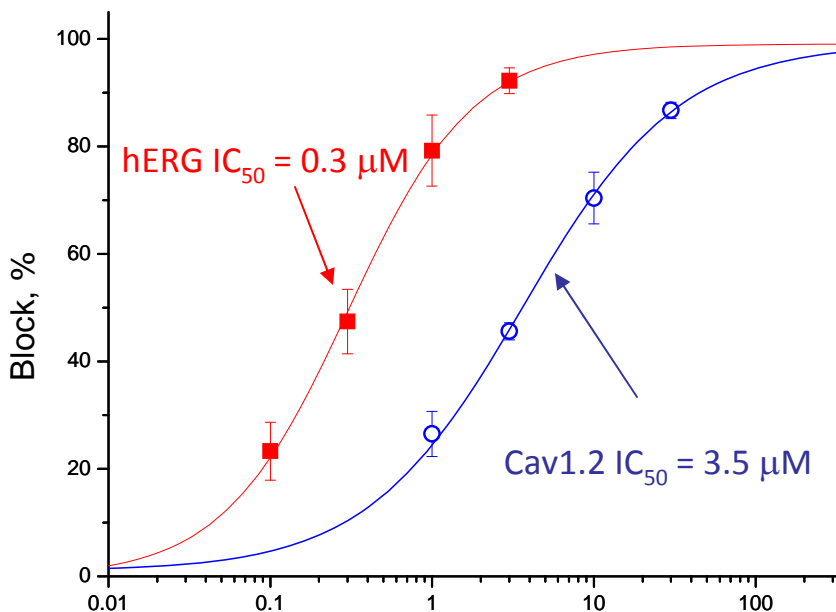
Case 1: (+)hERG, (?)QT Risk

- Drug X is a very potent hERG Blocker
 - IC_{50} in the nanomolar range
- Is there a QT risk?



Drug X: (+)hERG, (?) QT Risk

- **Channel Panel:**
 - Drug X is a very potent hERG blocker but also potently inhibits calcium current.
- **SC-hCM Action Potential Duration:**
 - Blocks hERG Channels (\uparrow APD₉₀) and Calcium Channels (\downarrow APD₃₀) in SC-hCM.
 - No EADs in SC-hCM
- ***in vivo* Dog:**
 - No corrected QT prolongation in the dog.



Case 1: (+)hERG, no QT Risk

A thorough preclinical safety evaluation, including the SC-hCM repolarization assay, predicts that Drug X may not be a QT risk.

Case 1: (+)hERG, no QT Risk

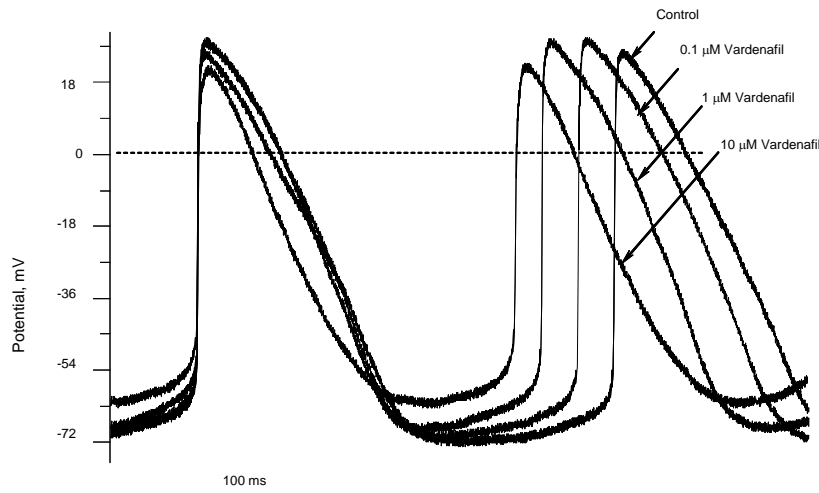
- Drug X is not a QT risk.
- Verapamil is Drug X:
 - Used to treat hypertension and angina.
 - No incidence of QT prolongation at clinical plasma concentrations in humans.
 - No incidence of TdP in humans.

Case 2: Vardenafil

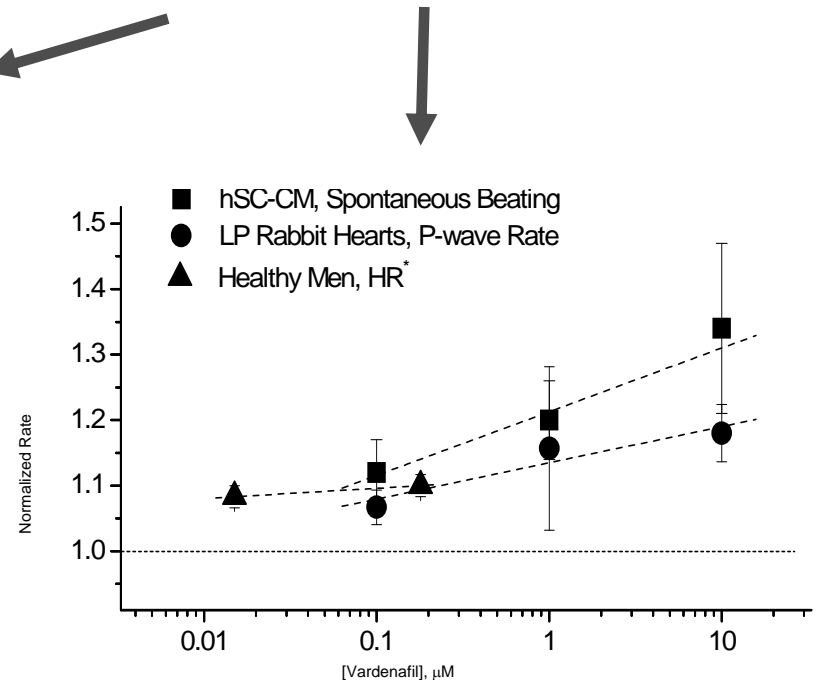
- Erectile dysfunction drug (Levitra™), but also decreases blood pressure and increases heart rate
 - believed to be autonomic events
- Screen ion channels, all IC_{50} values outside of therapeutic range:
 - Cav1.2 $IC_{50} = 16 \mu\text{M}$
 - hERG $IC_{50} = 40 \mu\text{M}$
 - Kv1.5 $IC_{50} = 5 \mu\text{M}$

Case 2: Vardenafil

- But when vardenafil is applied at 0.1 μM to spontaneously beating, isolated SC-hCMs . . .



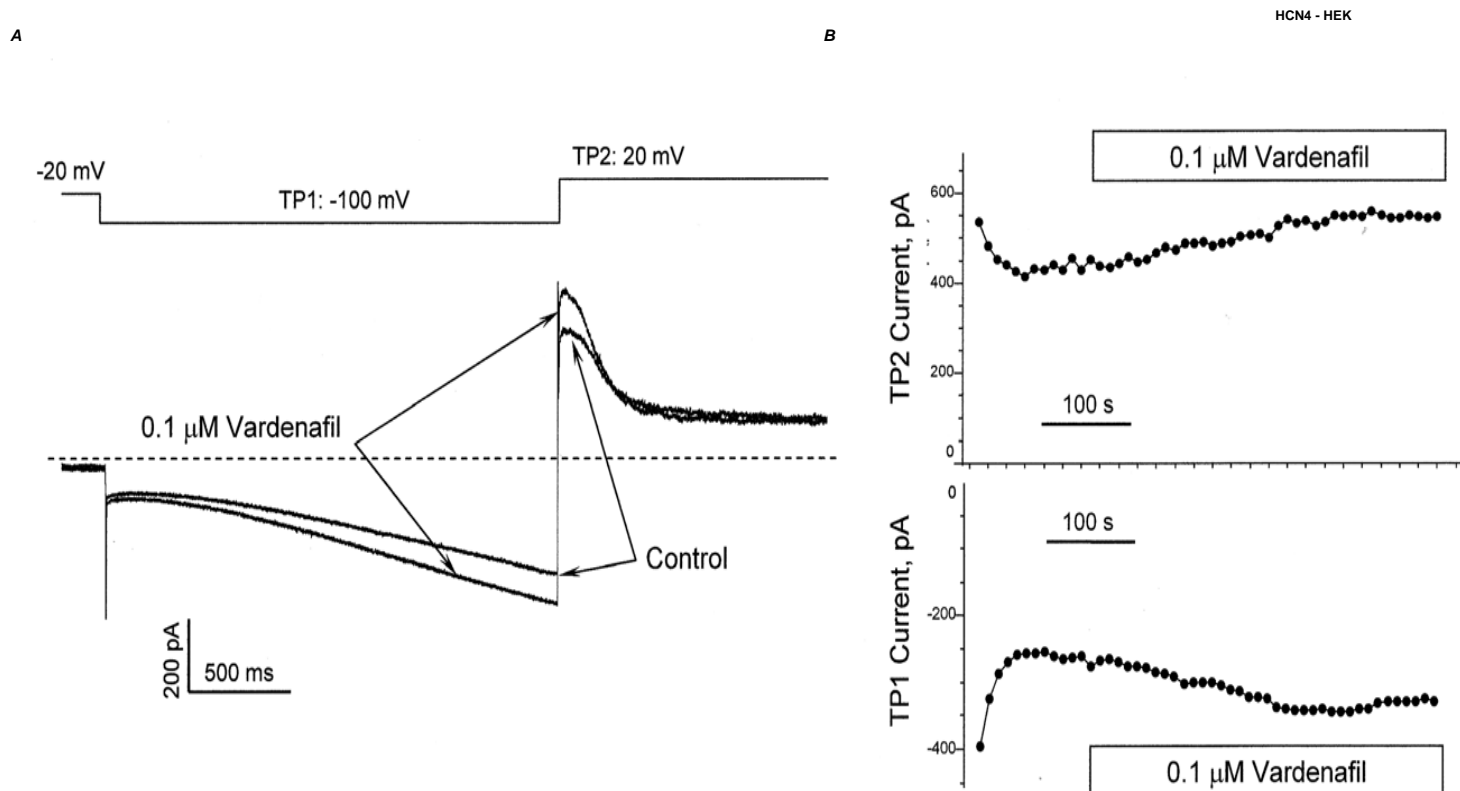
Beat rate increases!



*Data from Morganroth et al, Am J Cardiol, 2004

Vardenafil may act directly on cardiac cells to increase the heart rate

- Mechanism likely via potentiation of HCN4 channels, responsible for I_f



- ChanTest is committed to continually broadening the validation dataset through contract work, collaboration, internal R&D, and grant funding.
- Screened more than 70 proprietary compounds in AP assay (contracted by multiple pharma companies around the world) and 15 commercially available reference or research compounds.
- Leader in CRO field for cardiac risk evaluation using SC-hCMs.

Electrophysiology

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