

Cardiac Safety Assessment Service

**Cardiovascular Research Group
Lankenau Institute for Medical Research
LIMR Suite R230
100 East Lancaster Avenue
Wynnewood, PA 19096
484-476-2688**

About Us

The Cardiovascular Research Group at Lankenau Institute for Medical Research provides drug development companies worldwide with the complete range of cardiac safety assessment services. Led by Professors Gan-Xin Yan, MD, PhD and Peter R. Kowey, MD, renowned cardiologists and electrophysiologists, our cardiac safety team consists of experienced cardiologists, research scientists, and technicians. Our Cardiovascular Research Group has become the international leader in cardiovascular safety pharmacology as well as academic research. We are committed to providing pharmaceutical and biotech companies with drug discovery and development screening services to speed the drug-development process.

Our Cardiovascular Research Group offers an integrated portfolio of cardiac safety assays from cell to organ levels. These assays include **the wedge preparation assay, a hERG potassium current analysis, I_{Na} and I_{Ca} screening, a human cardiomyocyte ion channel assay, and an action potential assay**. The unique ventricular wedge preparation first developed by Dr. Yan has been adopted by many pharmaceutical and biotech companies worldwide for cardiac safety assessment. At the QT conference sponsored by the British Cardiovascular Society in 2007, the rabbit LV wedge preparation was ranked as a better validated preclinical model (compared with other preclinical models including HERG assay and Langendorff model) by the participants from different pharmaceutical companies (Pugsley Hancox and Curtis: Perception of validity of clinical and preclinical methods for assessment of torsades de pointes liability. Pharmacol Ther 2008;119:115-117). Using the unique left ventricular wedge preparation technique, we have screened thousands of compounds for major pharmaceutical companies.

- Wedge Preparation Assay
- hERG Current Assay
- NaV 1.5 and Cav 1.2 Assay
- Human Cardiomyocyte Ion Channel Assay
- Human Cardiomyocyte Action Potential Assay

Price quotes: Please contact Professor Gan-Xin Yan, MD, PhD
(484) 476-2687 (Office); (484) 476-2688 (Lab); 610-256-0999 (Cell); YanGanxin@comcast.net
or YanG@mlhs.org

1. Wedge Preparation Assay

We have developed a new research method, an arterially perfused left ventricular wedge preparation, to access the EKG, AP, and/or muscle contractibility in intact left ventricular wall (see Figure 1). Using this unique ventricular wedge preparation, we are able to record the transmembrane action potentials, EKG and/or contractibility simultaneously in the hearts. The wedge preparation exhibits a number of advantages over other experimental models in accessing the cardiac rhythm and function, such as transmural dispersion of repolarization and cardiac arrhythmias. It has drawn attentions from cardiac safety experts worldwide and has become the powerful tool in the practice of cardiac safety evaluation.

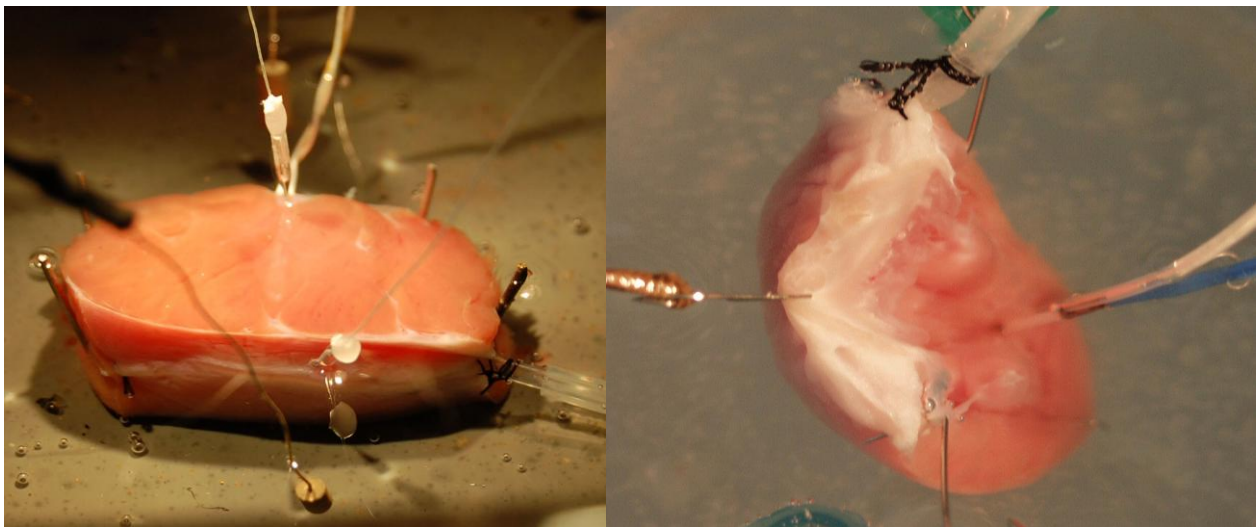


Figure 1. Arterially perfused canine (left) and rabbit (right) left ventricular wedge preparations. The wedges were perfused with Tyrode's solution via a small native branch of left descending coronary artery and stimulated from the endocardial surface. Transmembrane action potentials can be simultaneously recorded from epicardial (Epi), M cells and endocardial (Endo) sites. A transmural ECG is recorded concurrently.

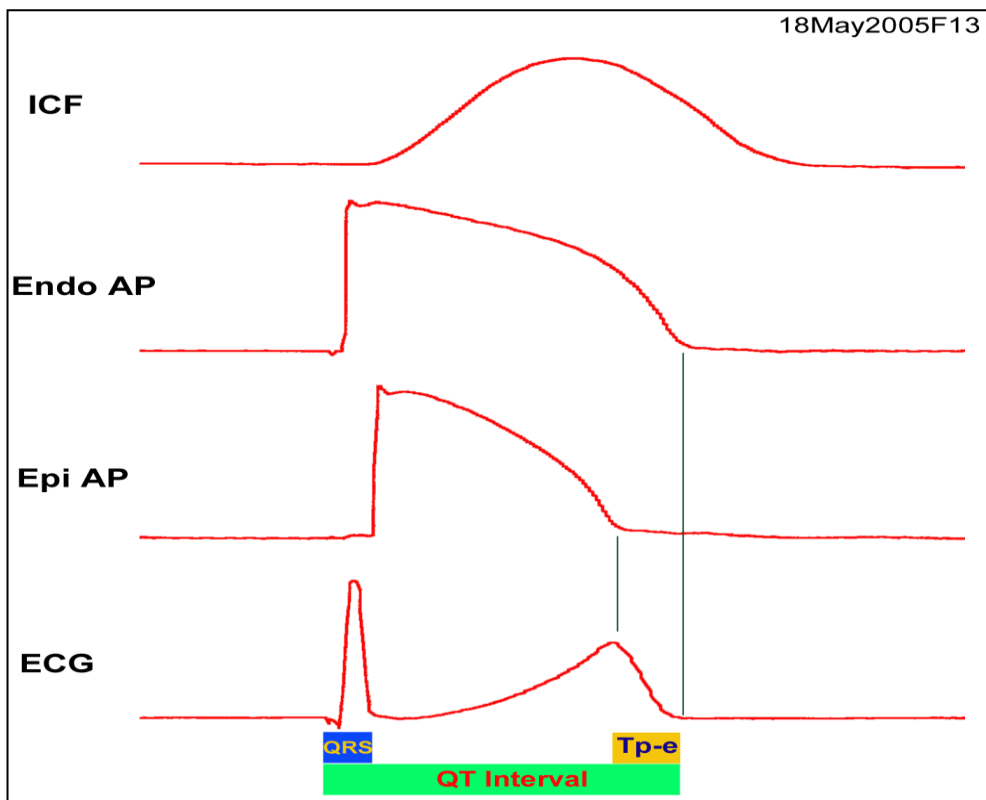


Figure 2. Sample of wedge preparation recording from a canine left ventricular preparation. Panels from top to bottom represent isotropic contraction force (ICF), membrane action potentials from the endocardium (Endo-AP) and epicardium (Epi-AP), and an ECG, respectively. QT Interval, QRS, T_{p-e} intervals were labeled. The stimulating cycle length was 2000 ms and the temperature was maintained at 35.7 ± 0.3 °C.

Parameters Assessed in the Wedge Preparation and Their Clinical Safety Relevancies

Parameters Assessed	Clinical Safety Relevancies
QRS	An index of I_{Na} : An increase in QRS indicates I_{Na} inhibition. Strong use-dependent QRS increase, like Class 1c drugs, is associated with ventricular tachycardia.{} The wedge preparation has higher sensitivity and specificity than other assays to detect I_{Na} blockade.
QT	The rabbit LV wedge is sensitive to QT prolonging agent. The specificity is also very high.{}
APD	Similar to QT; action potential recording can be used to detect EAD.
T_{p-e}	T_{p-e} is an index of transmural dispersion of repolarization. It is amplified by pure I_{Kr} blockers and reduced by I_{Na} or I_{Ca} blockers
QT-BCL Slope	The QT-BCL slope is amplified by the QT prolonging agents particularly I_{Kr} blockers and blunted by I_{Na} blockers. Amplified QT-BCL slope is associated with a higher risk of TdP.
EAD/TdP	EAD/TdP can occur in the wedge in presence of strong QT prolonging agents.
VT/VF	VT/VF can be produced by I_{Na} blockers with strong use-dependence, QT shortening

	agents, drugs like digitalis and sympathetic stimulants.
TdP Score	The relative TdP risk of a compound can be hemi-quantitatively estimated based on its effect on QT, T _{p-e} and incidence of EAD/TdP.{}.
Contractility (optional)	Marked reduction and loss of positive staircase phenomenon by a compound indicates I _{Ca} blocker. The wedge is very sensitive and specific in detecting I _{Ca} inhibition.

Summary of left ventricular wedge preparation assay

Animal Species	Guinea Pig, <u>Rabbit (most common)</u> , dog
Technology	Arterially-pefused Wedge Preparation
Compound(s) Info.	Molecular weight, solubility. Minimal 2 ml of up to 100 mM stock in DMSO.
Concentrations	4 concentrations, n=4.
Positive Control	Yes, per client's request.
Testing Conditions	500,1000,2000 ms cycle lengths in 35.7 °C
Reporting	Protocol summary, concentration response curves and safety analysis.

2. Ion Channel Cell Expression Assay

- **hERG Potassium Channel Assay (Screening)**

hERG (the **h**uman **E**ther-à-go-go-**R**elated **G**ene) is a gene (KCNH2) that codes for a protein known as **K_v11.1** potassium ion channel. The hERG channel mediates the repolarizing I_{Kr} current in the cardiac action potential and contributes to the electrical activity of the heart that coordinates the heart's beating. KCNH2 mutations cause inherited forms of cardiac disorders including both long QT (loss-of-function) and short QT (gain-of-function) syndromes. Inhibition of HERG channels by compounds/agents is the primary cause of acquired long QT syndrome and drug-induced torsade de pointes. It is estimated that 20-40 % of all lead compounds show level of hERG cardiac toxicity. Therefore, hERG channel is an important target in cardiac safety assessment.

Our laboratory provides manual patch clamp hERG assay. In this assay, IC₅₀ of the testing compound on hERG channel will be determined.

Ion Channel	hERG
Cell Line	HEK 293/CHO
Technology	Manual patch clamp
Sample size	Minimal 100 µl of 100 mM stock in DMSO.
Compounds info	Molecular weight, solubility
Concentration	5 concentrations, n=4.
Positive control	Yes, dofetilide or terfenadine
Testing condition	2-5 minutes of exposure in room temperature or 36 °C
Reporting	Protocol summary, concentration response curve and IC50 value.

- **Sample --- Effects of Dofetilide on hERG currents**

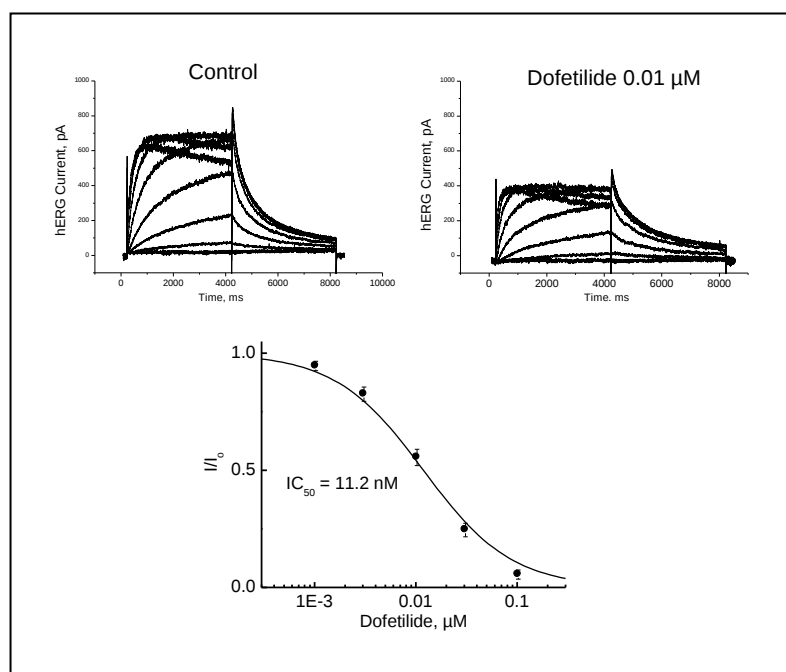


Figure 3. Effect of dofetilide on hERG currents. A&B: The superimposed currents response to step depolarizations ranging from -20 mV to $+60$ mV from a holding potential of -50 mV were obtained under control condition (A) and after 4 min of superfusion with 10 nM of dofetilide (B). C: Concentration-response relationship of dofetilide on hERG current. Data were fitted with an equation $I/I_0 = 1/(1+[C]/[IC_{50}])$. All data are expressed as Mean $\pm$ SEM, $n=4$ cells.

- ### Other Expressed Ion Channel Assay

We can also provide service for other expressed ion channels assay, such as NaV1.5 (subunit of fast voltage-dependent sodium channel) and Cav1.2 (subunit of L-type voltage-dependent calcium channel).

Ion Channel	NaV1.5, CaV 1.2
Cell Line	HEK 293/CHO
Technology	Manual patch clamp
Sample size	Minimal 100 μ l of 100 mM stock in DMSO.
Compounds info	Molecular weight, solubility
Concentration	5 concentrations, $n=4$.
Positive control	Yes, dofetilide or terfenadine
Testing condition	2-5 minutes of exposure in room temperature or 36 $^{\circ}$ C
Reporting	Protocol summary, concentration response curve and IC50 value

3. Native Cardiomyocyte Ion Channel Assay

- Available Cardiac Myocytes

Cardiomyocyte	source
Human (atrial or ventricular tissue)	From patients during open-heart surgery or heart transplant.
Canine (atrial or ventricular tissue)	From commercial vendors
Rabbit (atrial or ventricular tissue)	From commercial vendors
Guinea Pig (atrial or ventricular tissue)	From commercial vendors
Rat (atrial or ventricular tissue)	From commercial vendors
Mouse (atrial or ventricular tissue)	From commercial vendor

- Available Cardiac Ion Channel Assays

In the heart, the concerted opening and closing of cardiac ion channel is responsible for the action potential formation and cardiac excitability. Inhibition of these ion channels can lead to antiarrhythmic or proarrhythmic. Therefore, the most direct and promising method of evaluating cardiac safety of a compound is to measure its effects on cardiac ion channel.

Abbreviation	Name	Role
I_{Na}	Fast sodium current	phase 0
I_{to}	Transient outward potassium current	Phase 1
I_{Ca-L}	L-type calcium current	Phase 2 and 3
I_{Kr} and I_{Ks}	Rapid activa	Phase 2 and 3
I_{Na-L}	Slow sodium current	Phase 2 and 3
I_{K1}	Inward rectifier potassium current	Phase 3 and 4

- Available cardiac ion channel assays packages

Whole Package (Cardiac Profiler)	I_{Na} , I_{to} , I_{Kur} , I_{Ca-L} , I_{Na-L} , I_{Ks} , I_{Kr} , I_{K1}
Outward Current Package	I_{to} , I_{Kur} , I_{Ks} , I_{Kr} , I_{K1}
Inward Current Package	I_{Na} , I_{Ca-L}
Individual Ion Channel Evaluation	Channel biophysical properties, such as use-dependence properties studies in I_{Na} and I_{Ca-L} currents.
Technology	Manual patch clamp technique
Protocol	Standard protocols for all current recording
Conditions	Physiological or Room Temperature
Turn-Around Time	1-2 weeks from receipt to draft report for one testing compound (depending on assay type)

- Example 1 --- Effect of Compound X on fast sodium current (I_{Na})
in human atrial myocytes

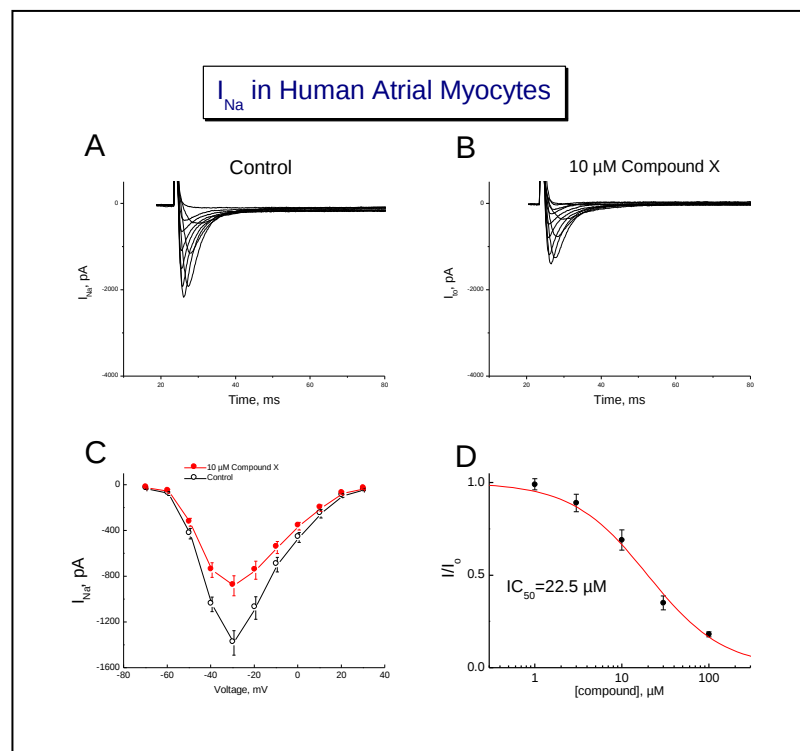
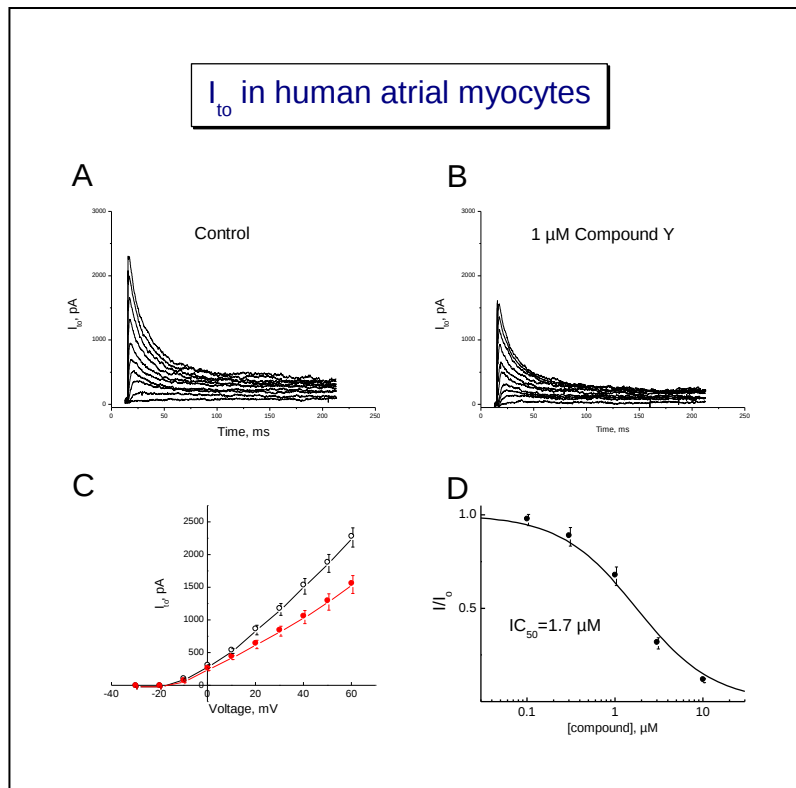
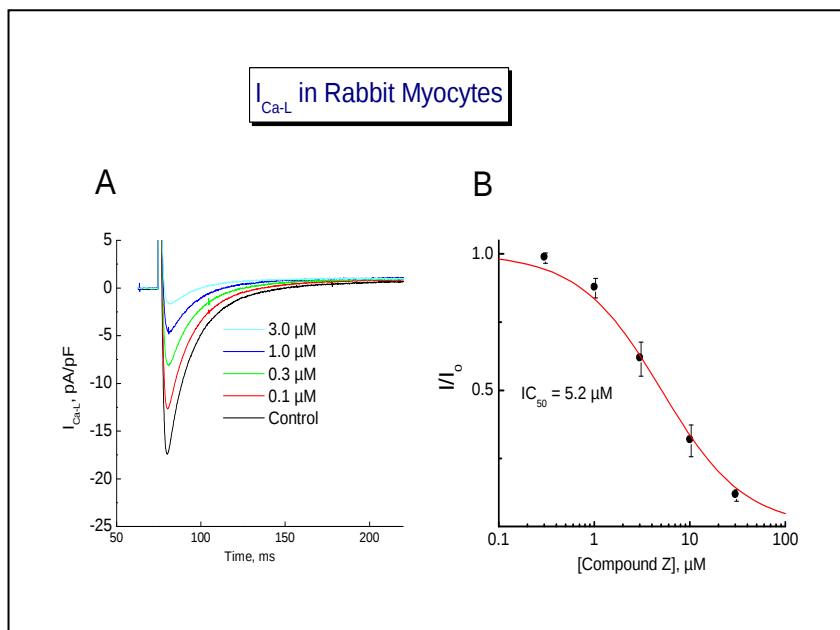


Figure 4. Effect of Compound X on fast sodium current in human atrial myocytes. A&B: The superimposed currents response to step depolarizations ranging from -70 mV to $+40$ mV from a holding potential of -100 mV were obtained under control condition (A) and after 4 min of superfusion with $10 \mu\text{M}$ of Compound X (B). C: Effect of Compound X on the peak current-voltage relationships of I_{Na} $I-V$ curves before (open circles) and after (solid circles) 4 min superfusion with $10 \mu\text{M}$ of Compound X. D: Concentration-response relationship of Compound X on sodium current. Data were fitted with an equation $I/I_0 = 1/(1 + [C]/[IC_{50}])$. All data are expressed as Mean $\pm$ SEM, $n=4$ cells.

- **Example 2 --- Effect of Compound Y on transient outward potassium current (I_{to}) current in human atrial myocytes**



- **Example 3 --- Effect of Compound Z on L-type calcium current (I_{Ca-L}) in rabbit ventricular myocytes**



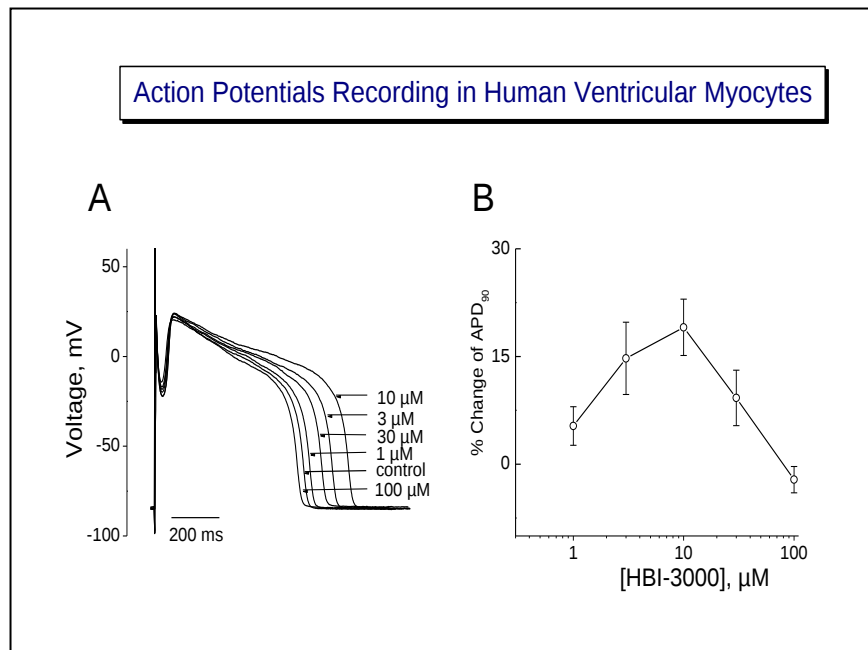
4. Native Cardiomyocyte Action Potential Assay

- Available cardiac myocyte types and species

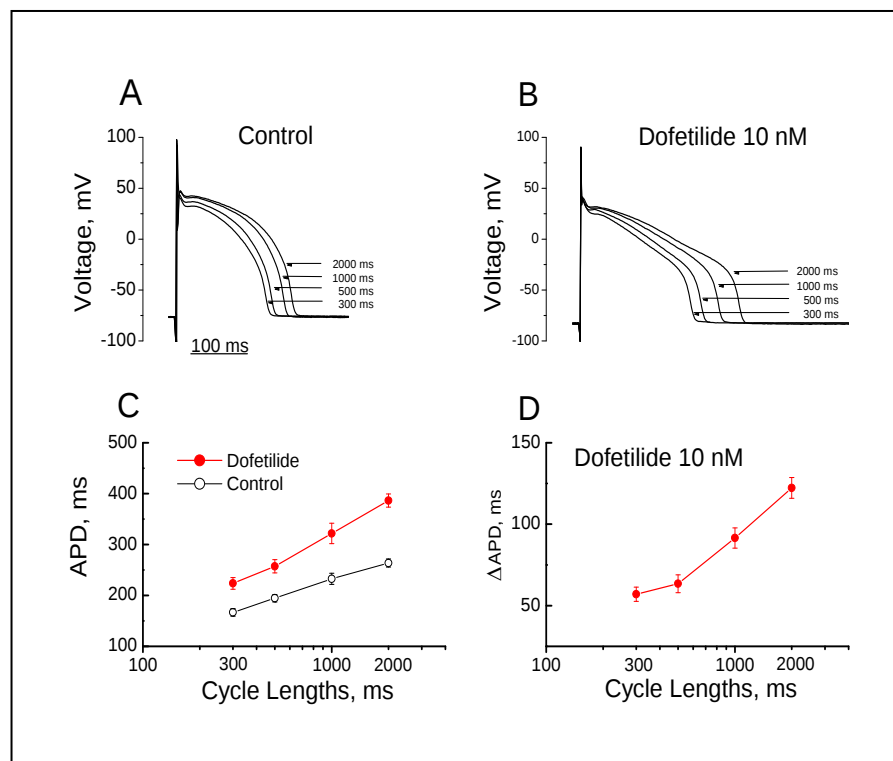
Action potentials are generated by the movement of ions through the transmembrane ion channels in the cardiac cells. Action potentials are dramatically different in morphology and duration on myocyte cell type and species.

Animal Species	Mouse, Guinea Pig, Rat, Rabbit, Dog
Human Tissue	Human atrial and ventricular tissues (freshly isolated from open chest surgery or heart transplantation).
Myocyte Cell Type	Atrial and ventricular myocytes
Technology	Standard Microelectrode Technique (Not using current clamping method)
Conditions	Physiological Temperature ($36.5 \pm 0.5^{\circ}\text{C}$).
Protocol	Standard protocols
Parameters Measured	APD ₂₀ , APD ₉₀ , V _{max} , Resting Potential. Use-dependence property.
Concentrations	4-5 concentrations, n=4.
Turn-Around Time	1-2 weeks from receipt to draft report for one testing compound (depending on assay type)
Note	Ask for discount if more than 5 testing compounds.

- **Example 1 --- Effect of Compound HBI-3000 on the action potentials in human ventricular myocytes**



- **Example 2 --- Frequency-dependent effects of dofetilide on the action potentials in rabbit ventricular myocytes**



- **Example 3 --- Effects of Compound A on the action potentials in rabbit atrial myocytes**

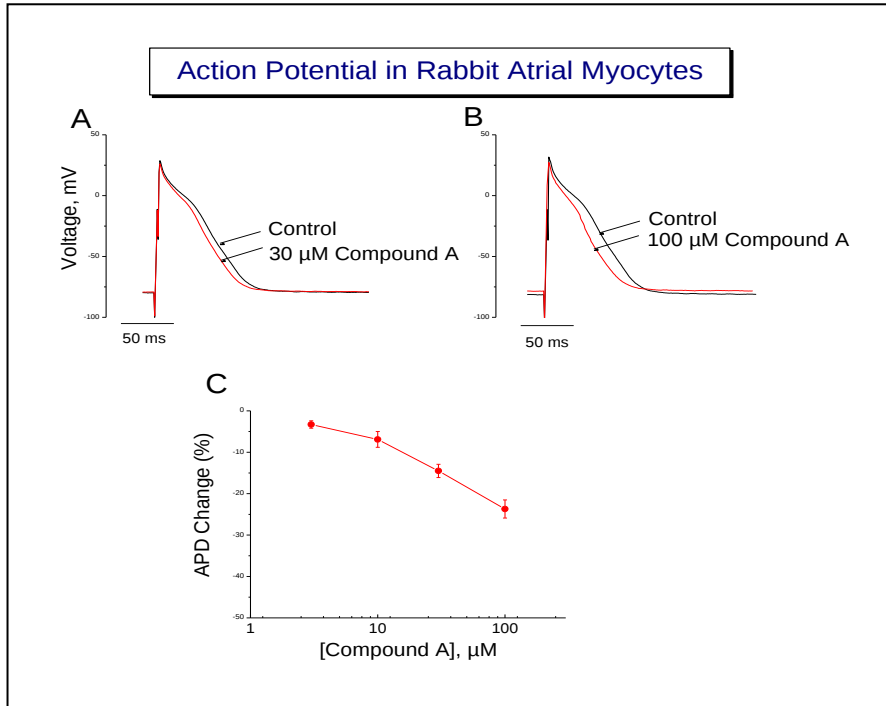


Figure 9. Effects of Compound A on action potentials in rabbit atrial myocytes. A&B: Action potential traces in the presence and presence of 30 or 100 μM Compound A. C: Compound A concentration-dependently shortened the action potential in rabbit atrial myocytes.