

# QTempo Cardiomyocyte Assay for Drug discovery

May 2009

# Cell Types used in Drug Discovery

- Tumor cells or Immortalized cells
  - Unlimited supply of uniform cell population
  - Loss of characters of original tissues
  - Abnormal karyotype
- Primary cells derived from tissues
  - Close to normal cells
  - Lot-to-lot variation
  - Difficult to get sufficient quantity of cells
- **Human ES / iPS cells**
  - Differentiated into various cell types
  - Unlimited supply of uniform cell population
  - Genetic manipulation

# Cardiotoxicity Problem (1)

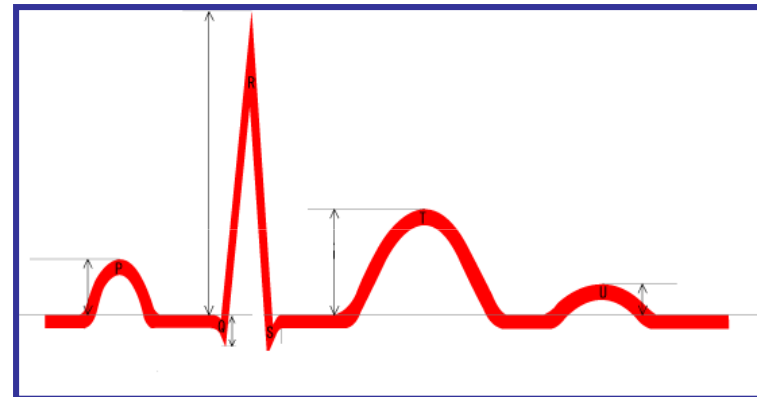
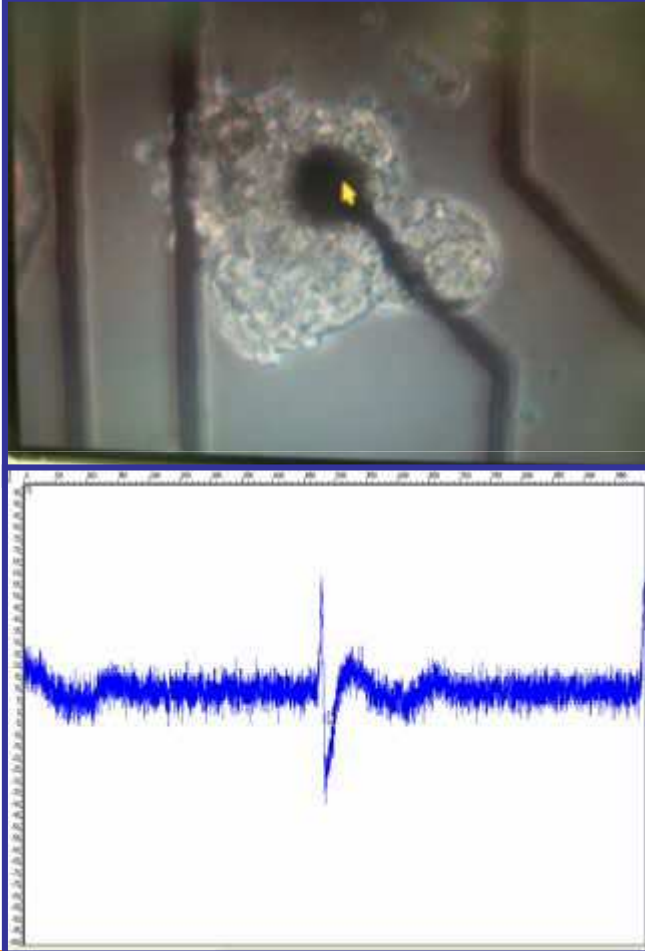
- Significant cause of pre- and post- approval drug failure
  - Vioxx (80m patients, \$2.5bn annual sales, \$4.5bn compensation)
- hERG channel assay dominates pre-clinical market
  - About 80% detection rate
  - False positives as well as false negatives
  - Not a functional assay
- Animal experiments costly, time consuming and unreliable



# Cardiotoxicity Problem (2)

- The goal of cardiotoxicity testing is to prevent arrest of beating human heart cells.
- At present, the first beating human heart cells a drug will interact with are in phase I patients. Higher drug doses which might reveal cardiotoxic potential can not be safely tested in clinical trials.

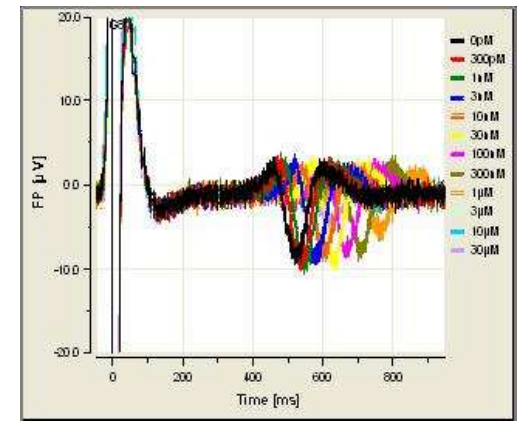
QTempo is a functional assay based on human or monkey beating Cardiomyocytes that detects functional waveform responses in response to therapeutic challenge



Ex. Human ECG

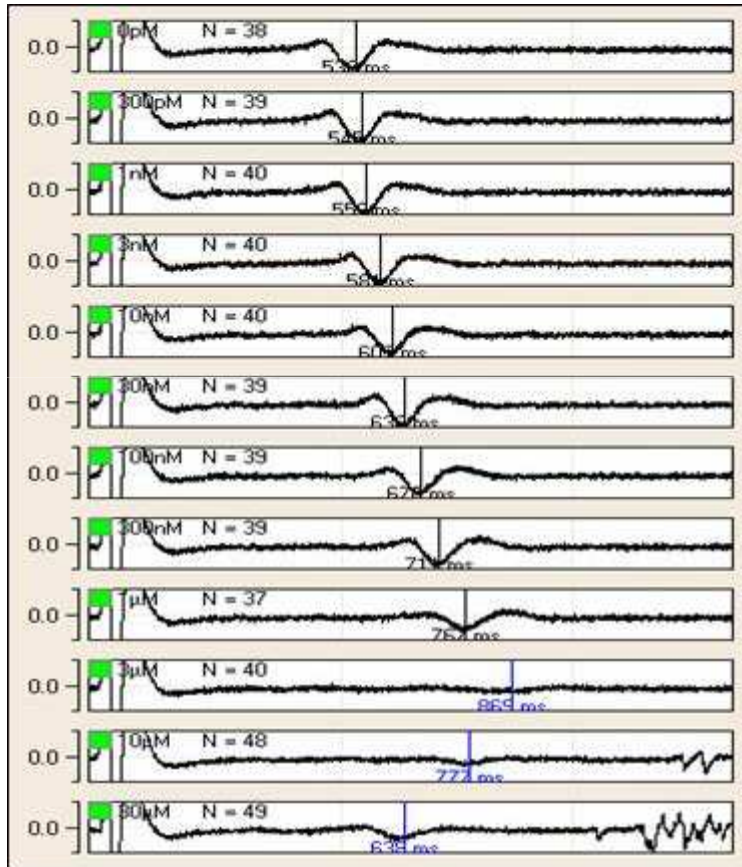
# Standard QTempo Assay service

- Cell:
  - ◆ Human iPS cell derived cardiomyocyte
  - ◆ Human ES cell derived cardiomyocyte
  - ◆ Monkey ES cell derived cardiomyocyte
- Standard Assay conditions :
  - ◆ Number of test concentration: 12 (in maximum);  
Treatment with 11 concentrations and no treatment (accumulative method)
  - ◆ Number of test on each concentration: 1
  - ◆ Lead time: 1-2 week
- Standard Observation points :
  - ◆ Na-K interval ( corresponding to QT interval )
  - ◆ Beating rate
  - ◆ Other observation points

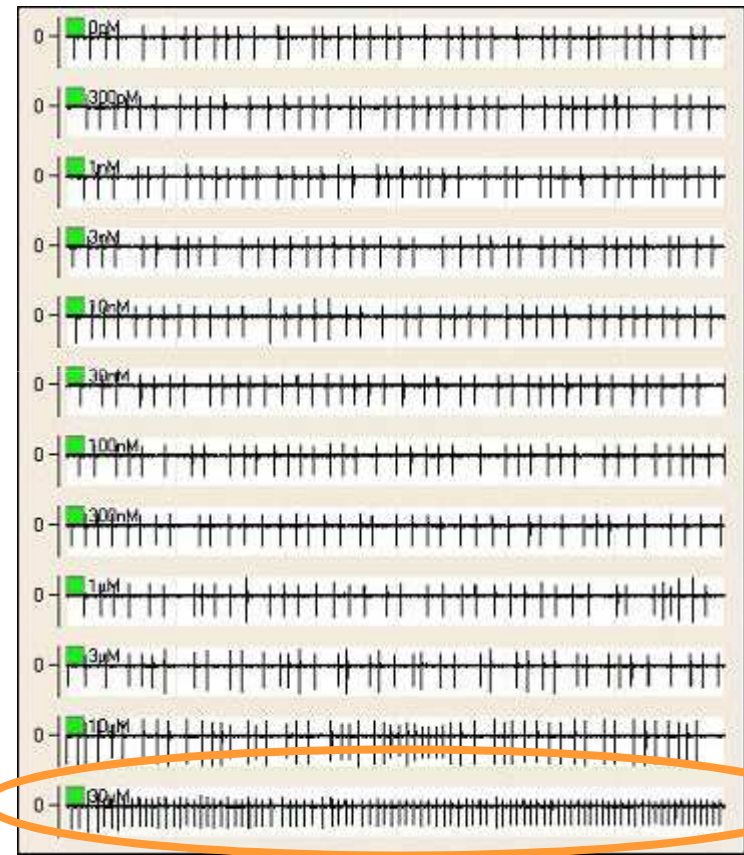


# QT interval prolongation

ECMG



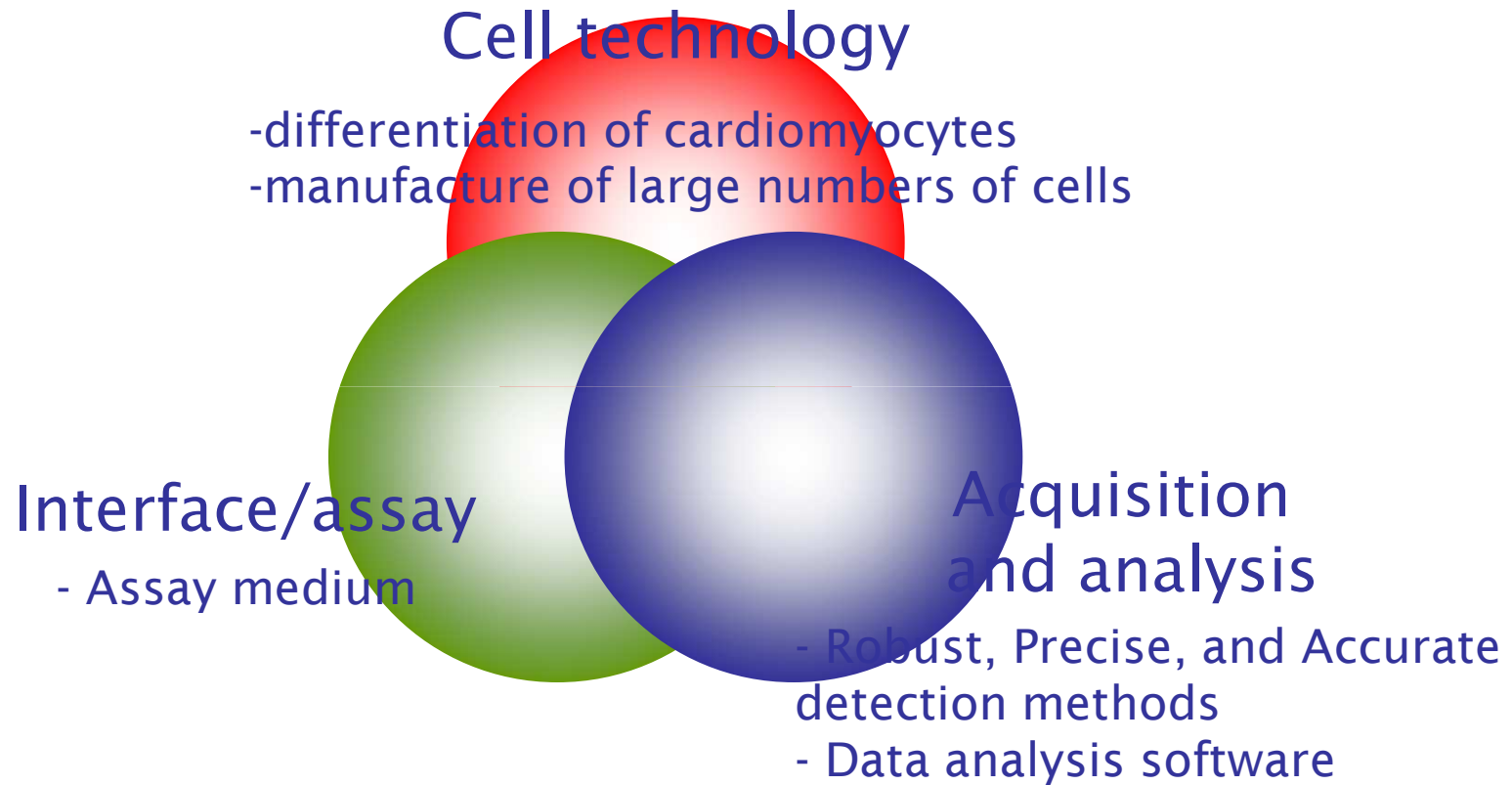
Beat Interval



0  
300pM  
1nM  
3nM  
10nM  
30nM  
100nM  
300nM  
1uM  
3uM  
10uM  
30uM

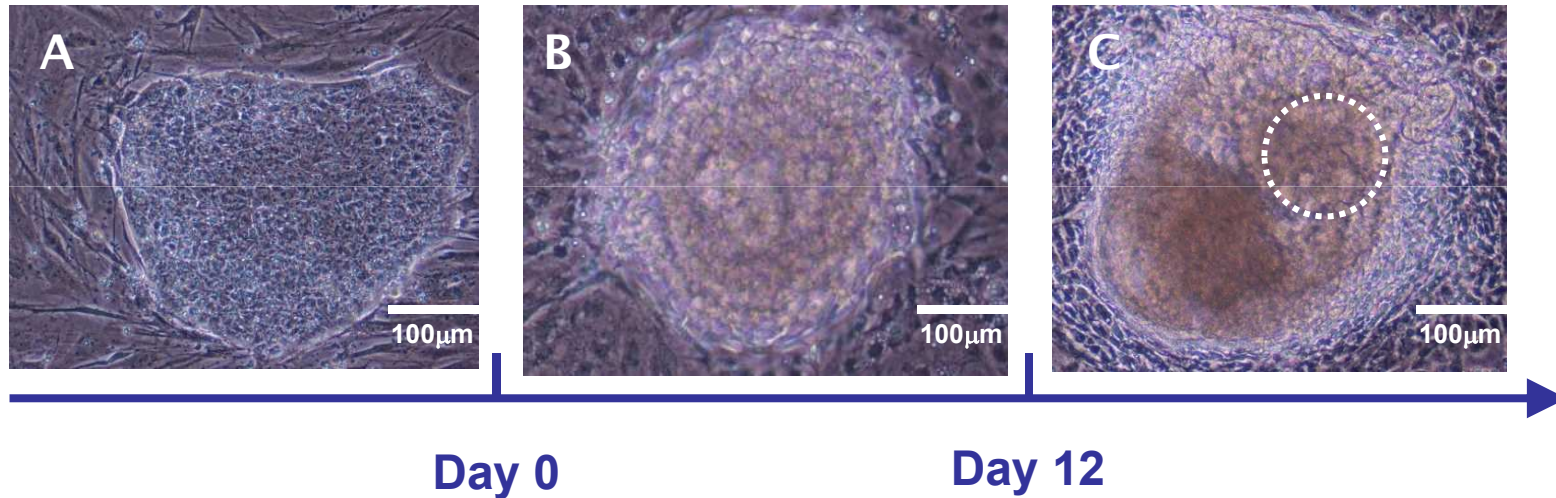


# Technology Elements of assay system

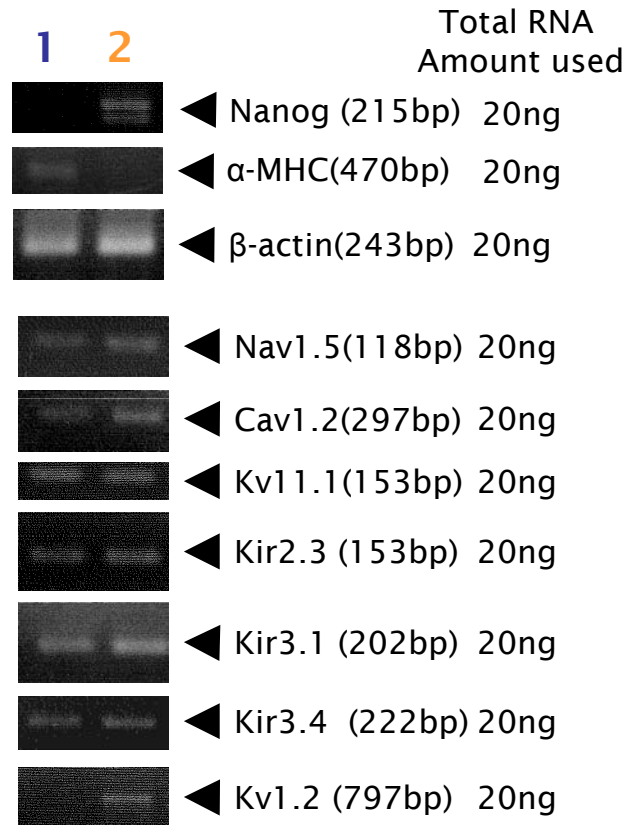




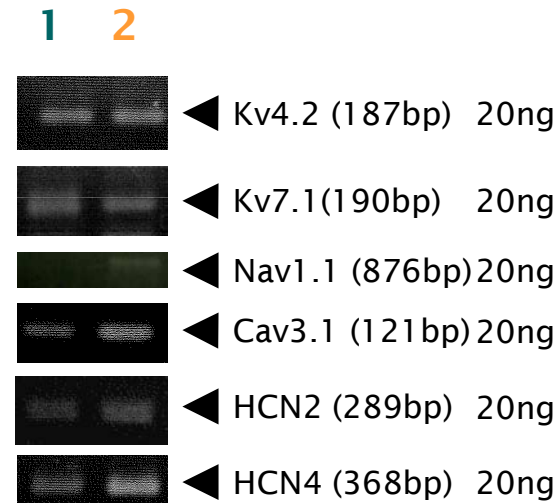
# Cardiomyocyte Differentiation



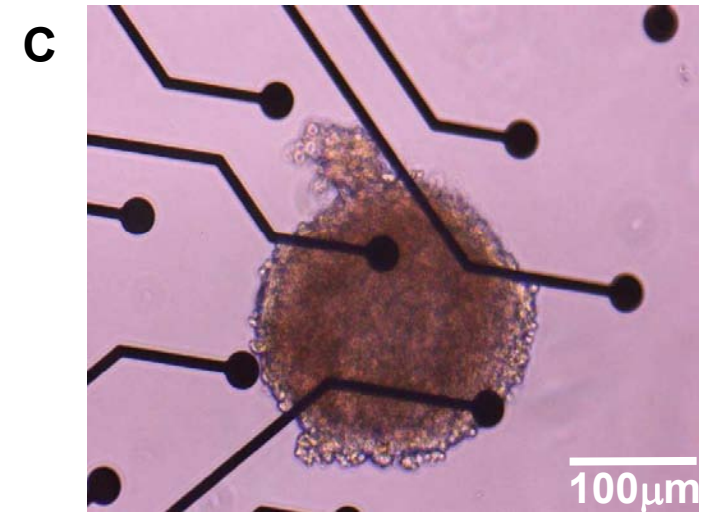
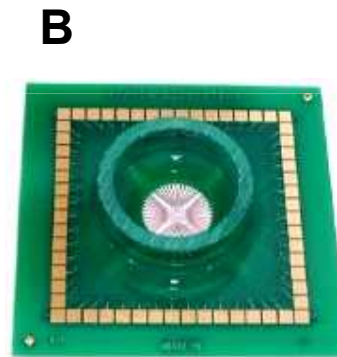
# Gene Expression in Monkey ES cell and Monkey ES-derived beating cells



## 1. Monkey ESC-derived beating cell 2. Monkey ESC

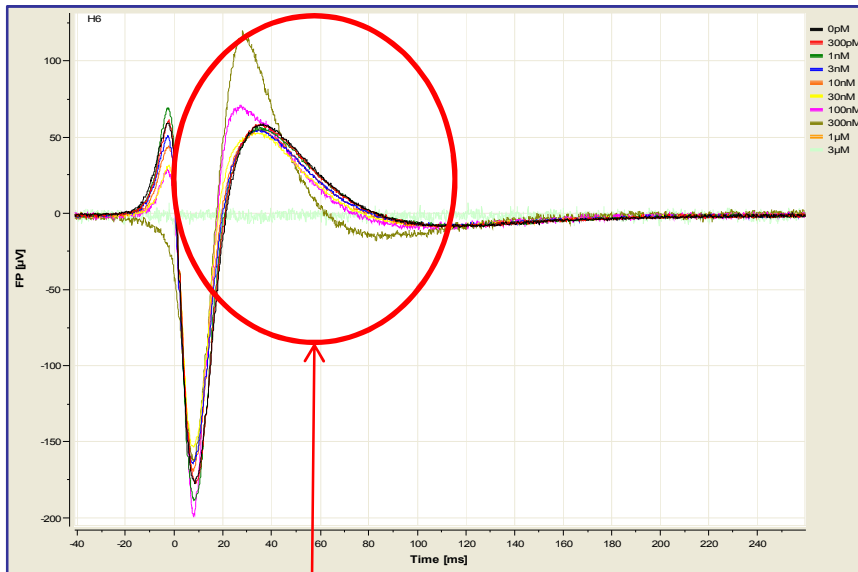


# Data Acquisition Platform MEA Instruments

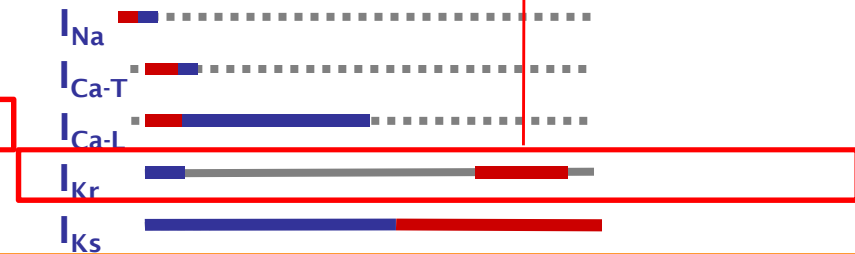
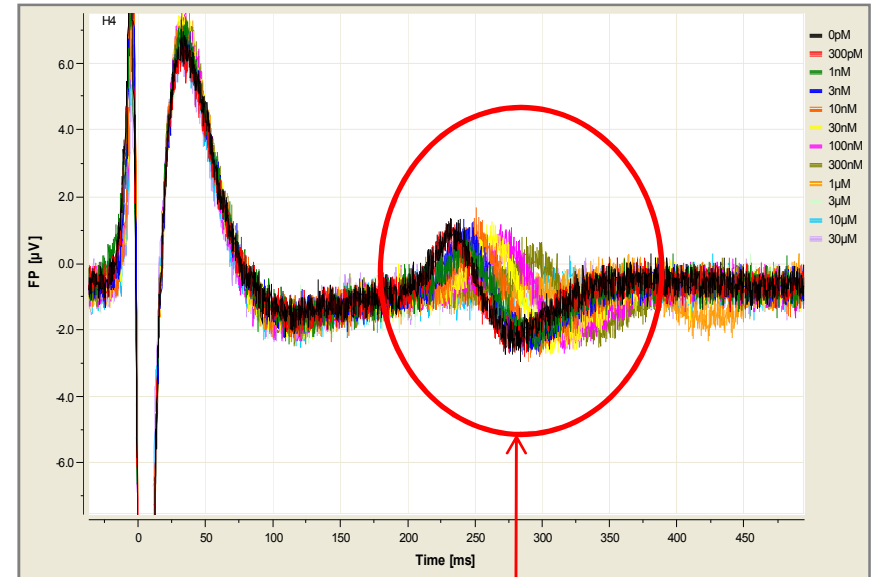


# Covers multi ion channels

## L-type Ca blocker



## K blocker



# Cell Characteristics/Quality control for QTempo (1/3)

## 1-a. Cardiomyocyte cluster diameter

between 100-300 um ( about 10,000 cells, thickness not defined ) .

## 1-b. Magnitude/amplitude of voltage from beating clumps:

Na amplitude: 40 uV or larger (between first positive and negative peak)

K amplitude: 2 uV or larger (between first positive and negative peak) and opposite polarity of Na wave. Prior to treatment, the start of the K-wave is within 600 msec. from start of the Na-wave.

## 1-c. Beat rate:

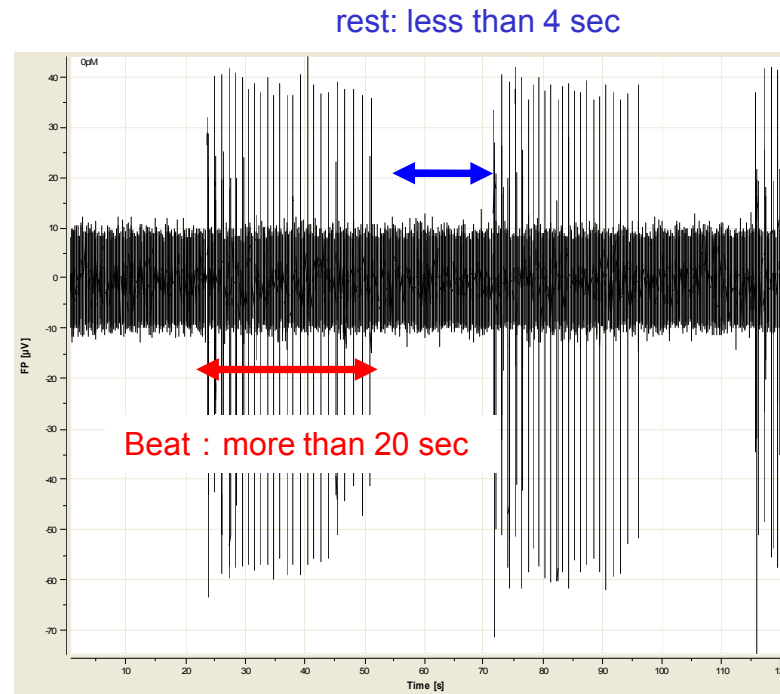
30~120 beats/minute (average from 3-minute measurement of untreated cells)

Allowable variation in beat rate < 10bpm form single cluster

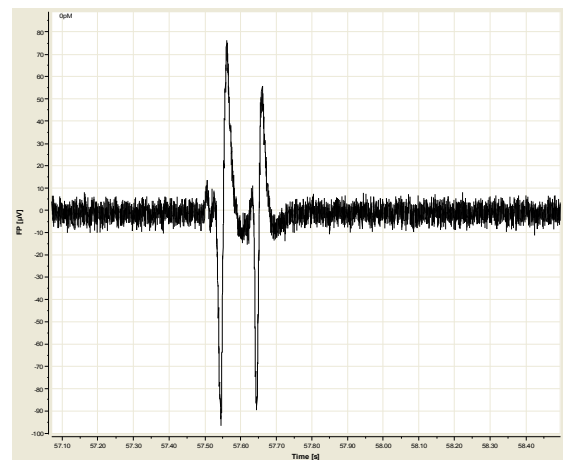


# Cell Characteristics/Quality control for QTempo (2/3)

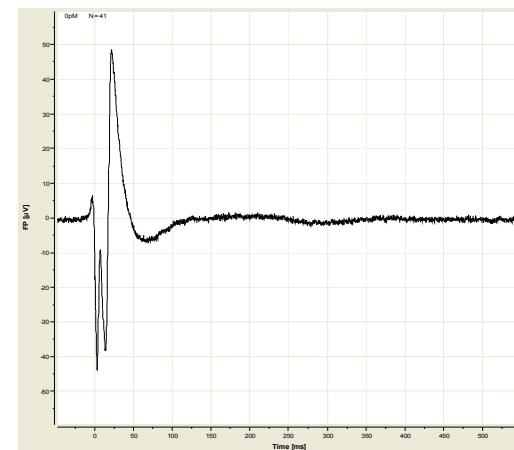
1-d. Cells must beat continuously for more than 20 sec. Any resting interval between beating states must be  $< 4$  sec.



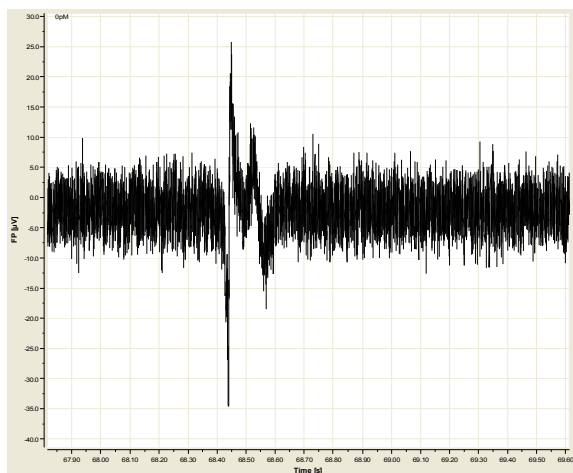
# Cell Characteristics/Quality control for QTempo (3/3): Aberrant waveforms



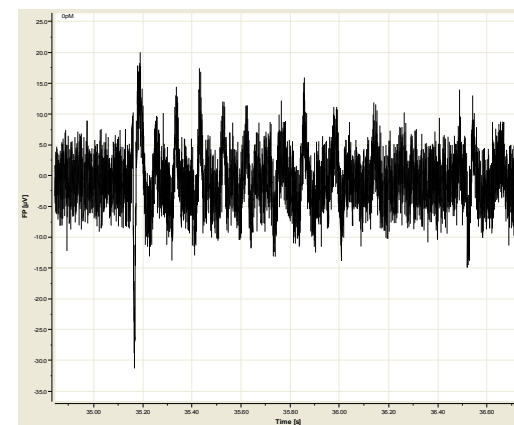
Multiple peak



Split peak



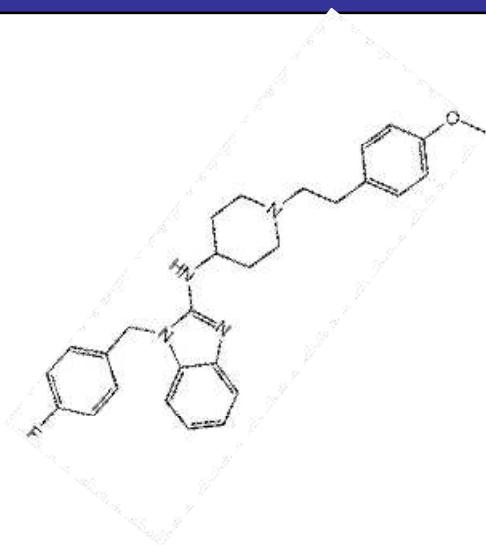
Collapsed



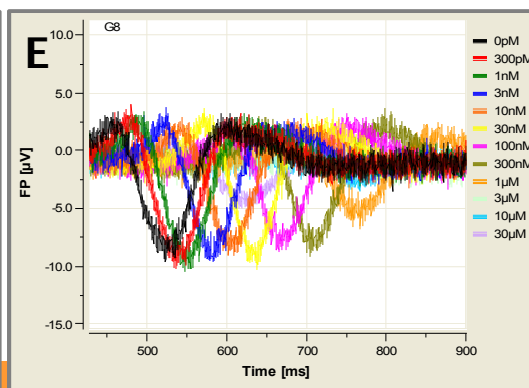
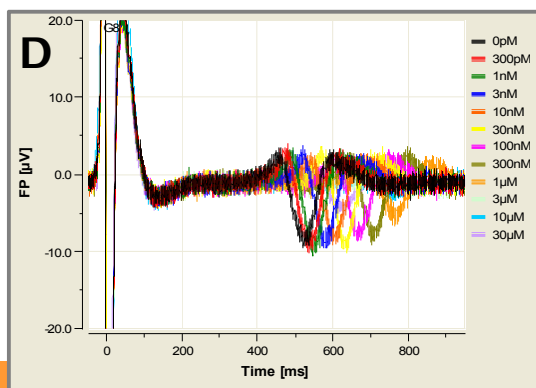
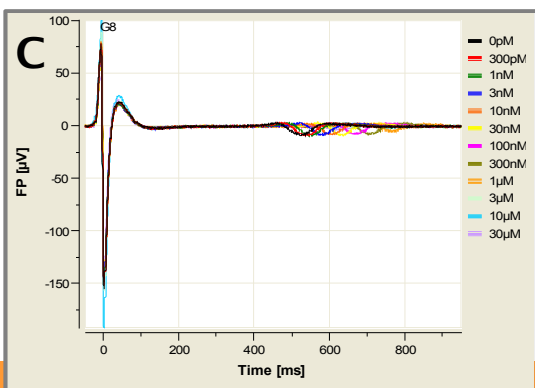
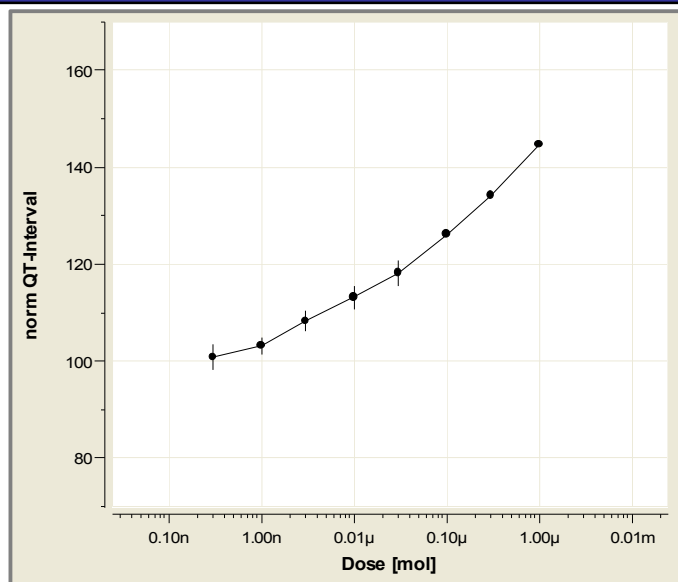
Fibrillating

# Astemizole on Monkey ES-derived: a potent histamine H1-receptor antagonist withdrawn from market due to QT prolongation

A

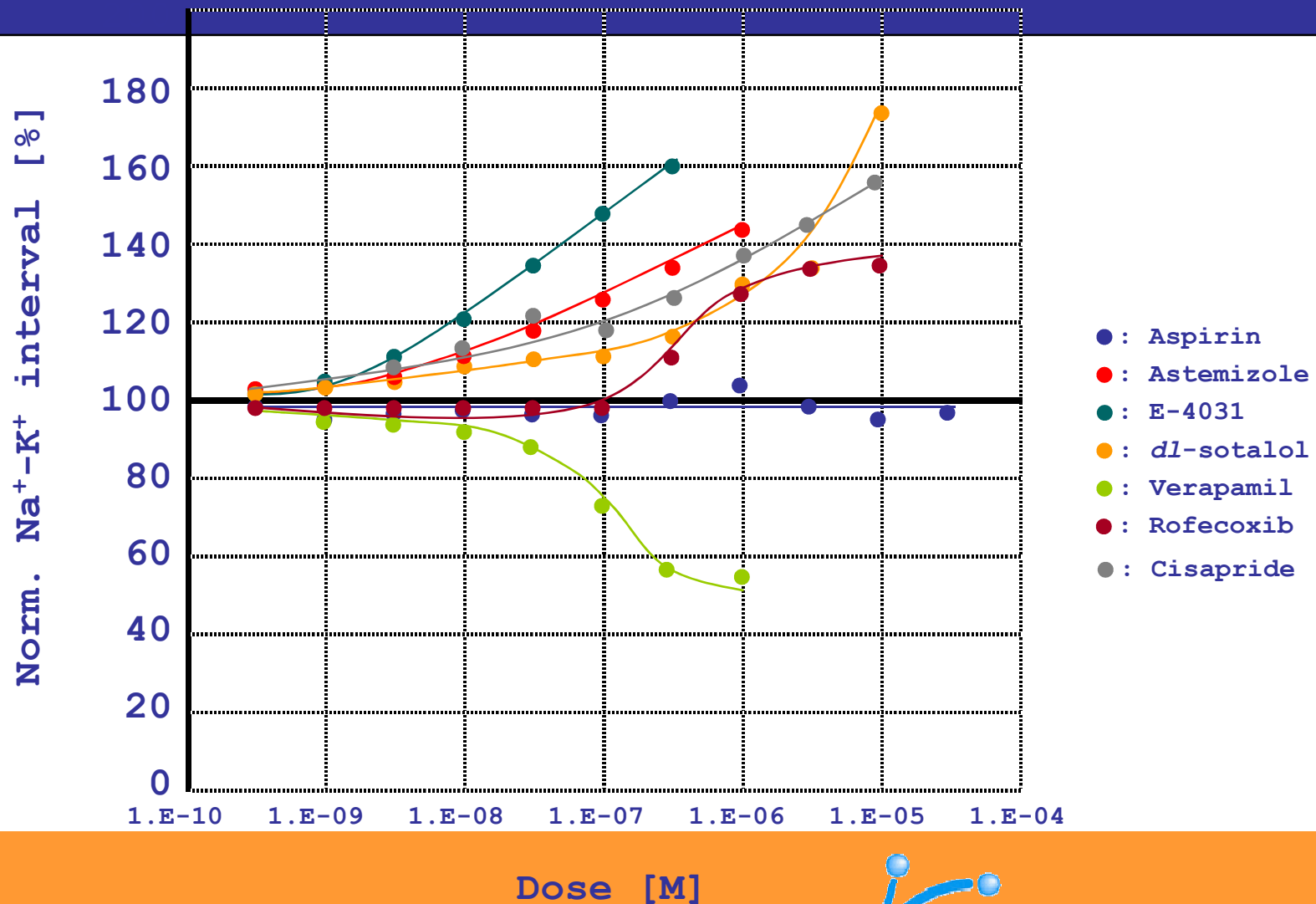


B

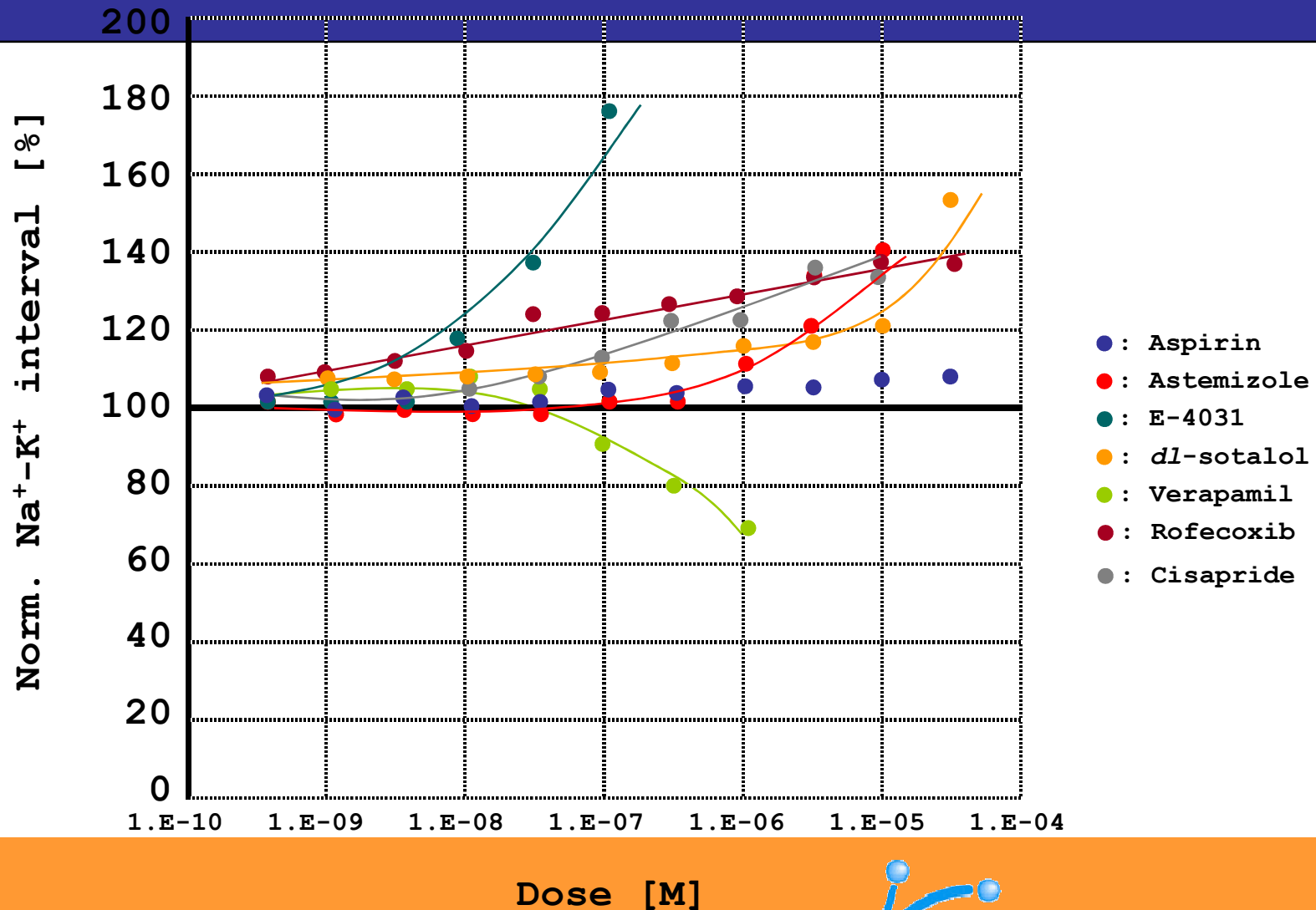




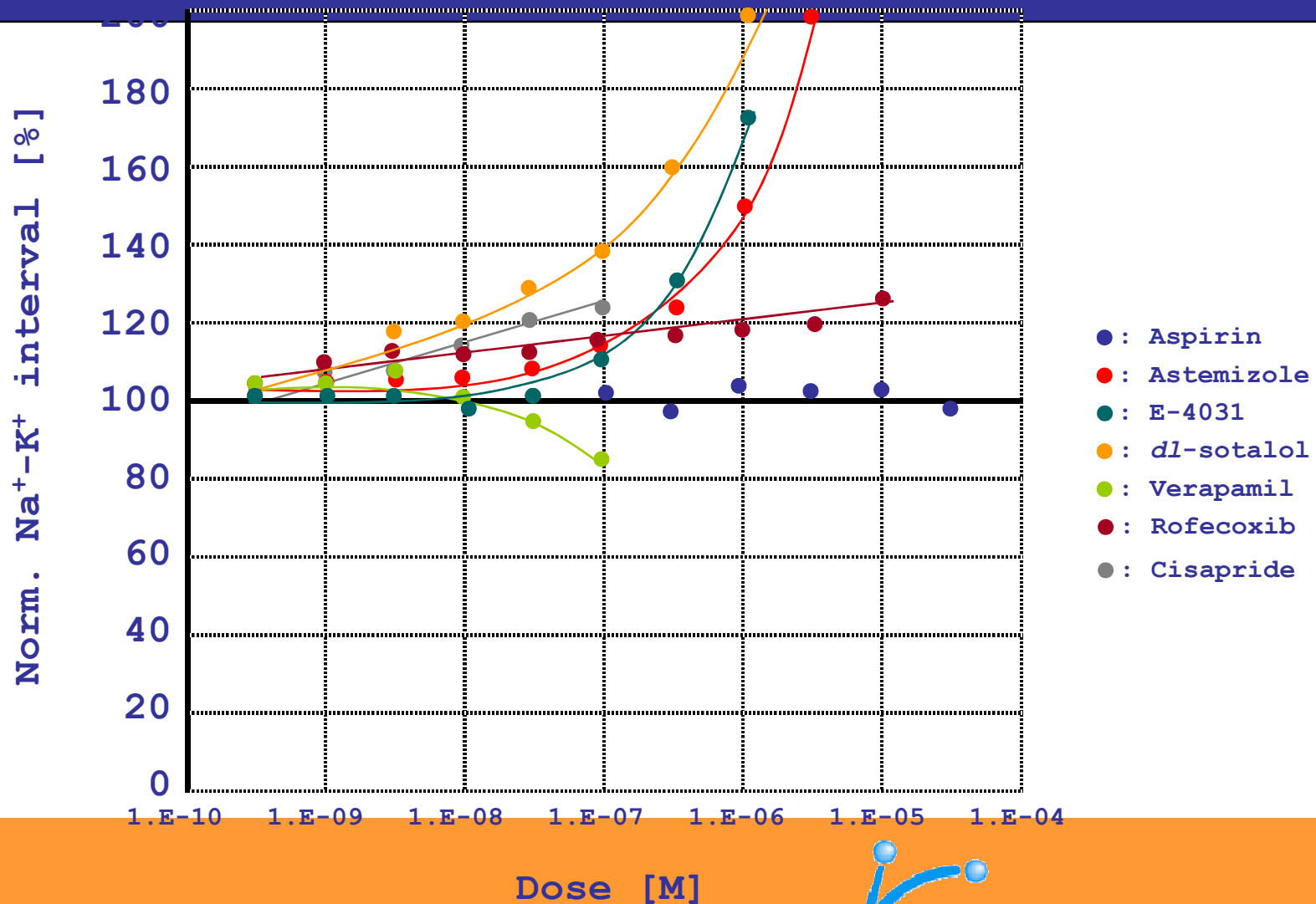
# Na<sup>+</sup>-K<sup>+</sup> interval prolongation on **monkey ES-derived** cardiomyocytes treated with various compounds



# Na<sup>+</sup>-K<sup>+</sup> interval prolongation on **human ES-derived** cardiomyocytes treated with various compounds



# Na<sup>+</sup>-K<sup>+</sup> interval prolongation on human iPS-derived cardiomyocytes treated with various compounds



# QTempo comparison with hERG for eight reference compounds

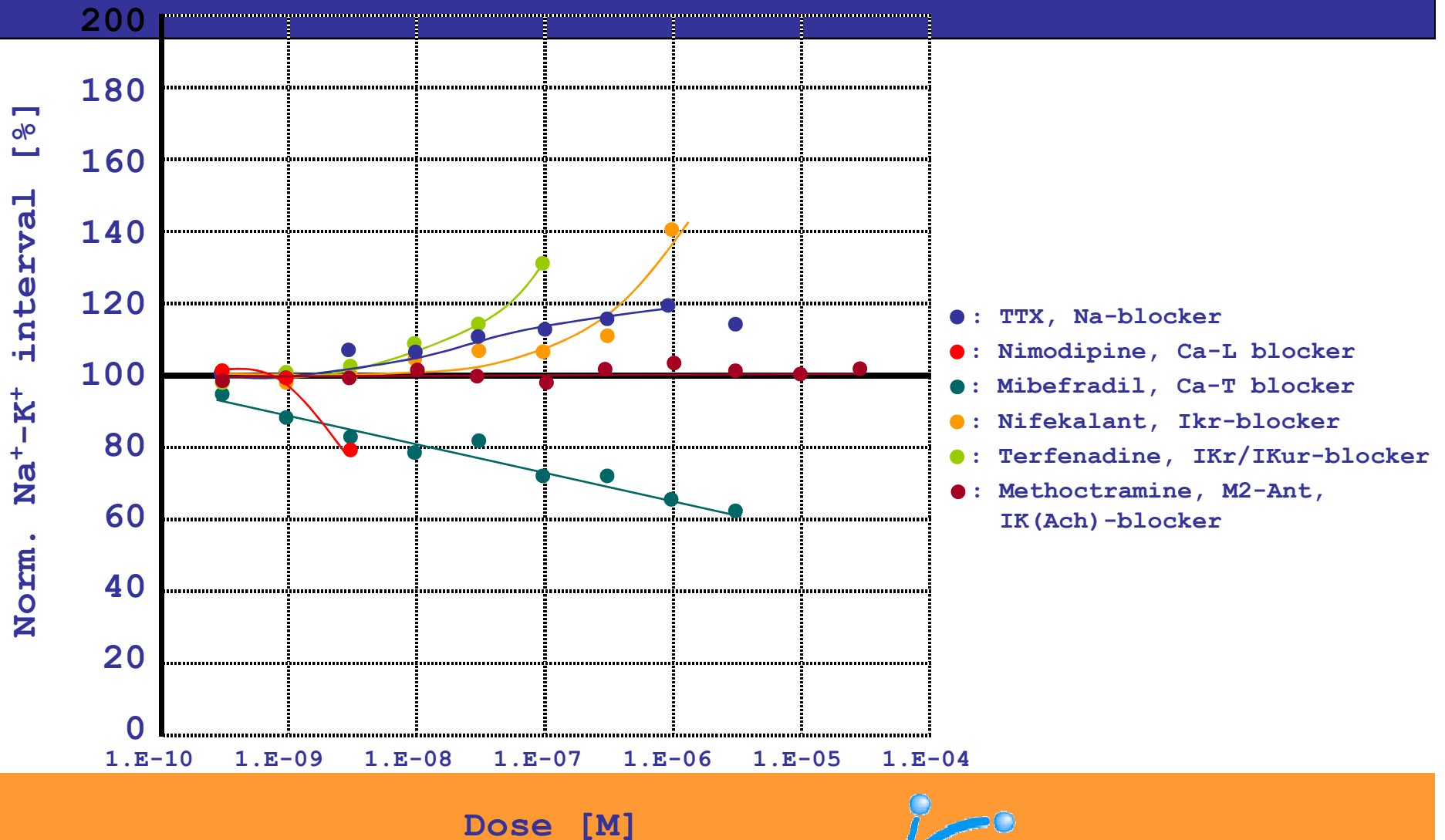
## QTempo

Compound	Repro CELL's ES/iPS-derived cells with MEA 10% prolongation, nM			HERG with patch clamp (conventional or automated)*1	in vivo or ex vivo: dog or human
	From Monkey ES	From Human ES	From Human iPS	HERG block, IC <sub>50</sub> , nM	Indication
Astemizole	30 to 100	100 to 300	30 to 100	1 to 30	prolongation
Cisapride	3 to 30	30 to 100	3 to 30	30 to 100	prolongation
E-4031	1 to 10	3 to 10	30 to 100	10 to 100	prolongation
Nimodipine	shortening	shortening	shortening	no reported	no prolongation
Rofecoxib	300 to 1000	10 to 100	30 to 100	no reported	no reported
<i>dl</i> -Sotalol	10 to 100	10 to 100	3 to 30	>30000/no effect	prolongation
Verapamil	shortening	shortening	shortening	140 to 800	no prolongation
Aspirin	no prolongation	no prolongation	no prolongation	no effect	no prolongation

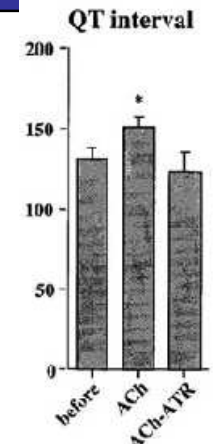
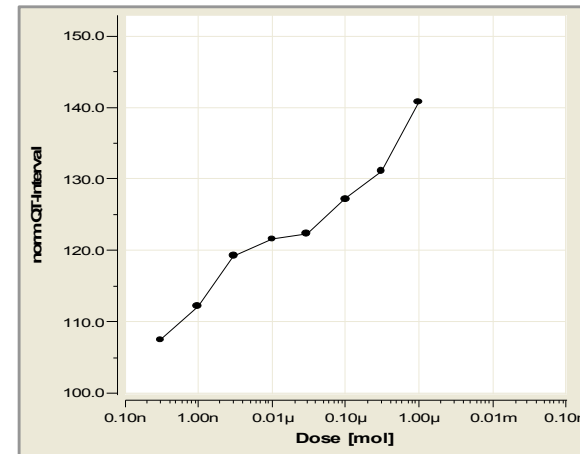
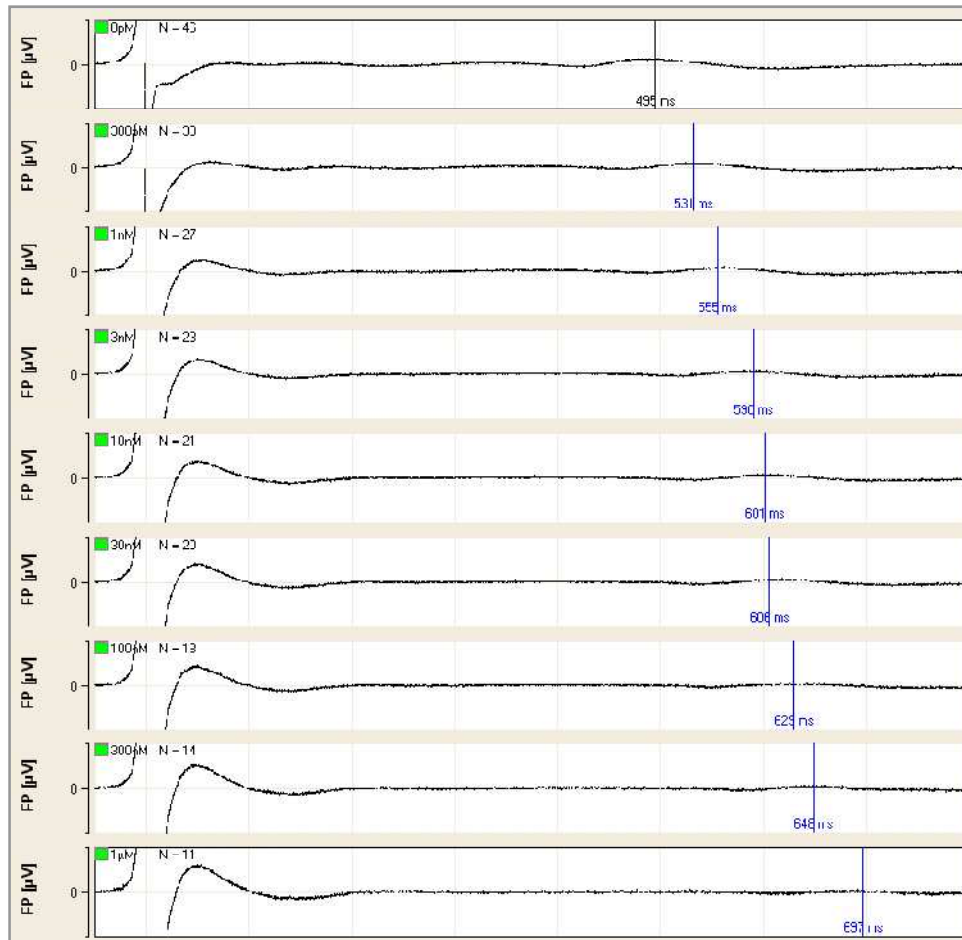
\*1 Redfern WS, et al. Cardiovasc Res 2003; 32-45.  
 Ducroq J, et al. J Pharmacol Toxicol Methods. 2007; 159-70  
 Kiss L, et al. Assay and Drug Development Technologies 2003; 127-135.  
 Schroeder, K. et al Journal of Biomolecular Screening 2003; 50-64.



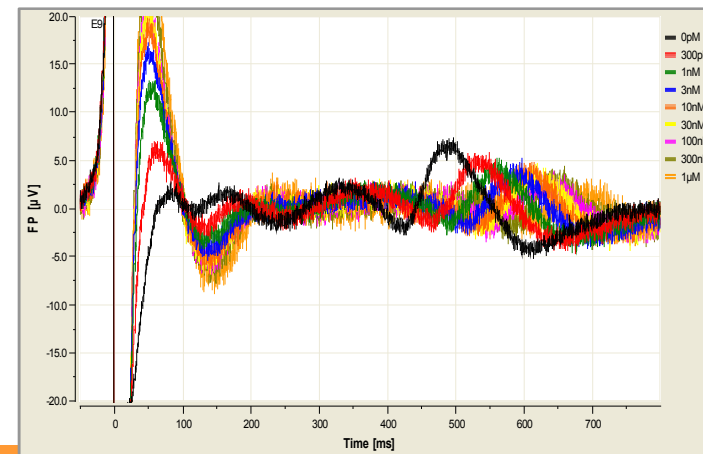
# Na<sup>+</sup>-K<sup>+</sup> interval prolongation on Monkey ES-derived cardiomyocytes treated with various specific blockers



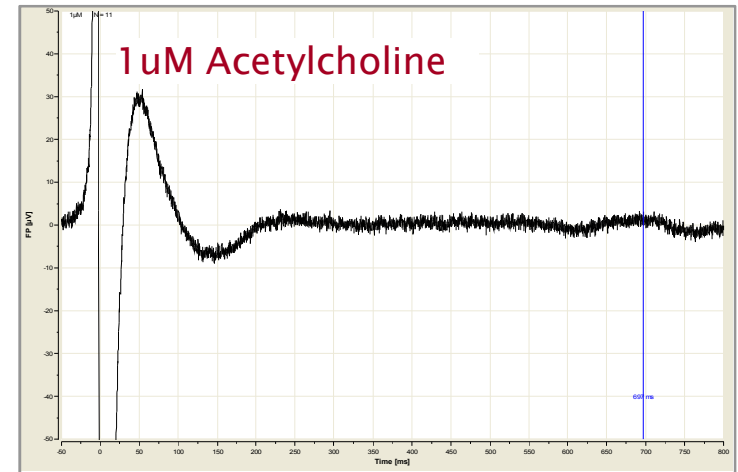
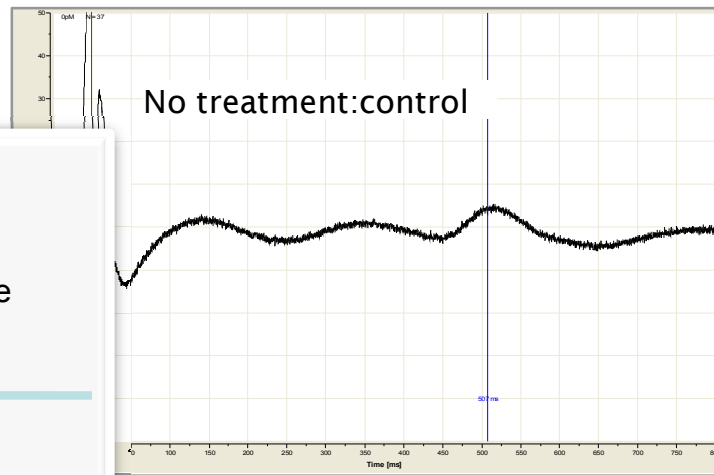
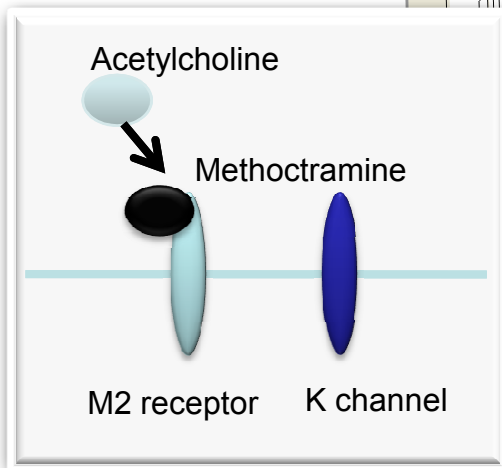
# Na<sup>+</sup>-K<sup>+</sup> interval prolongation observed on Human ES-derived cardiomyocytes treated with Acetylcholine



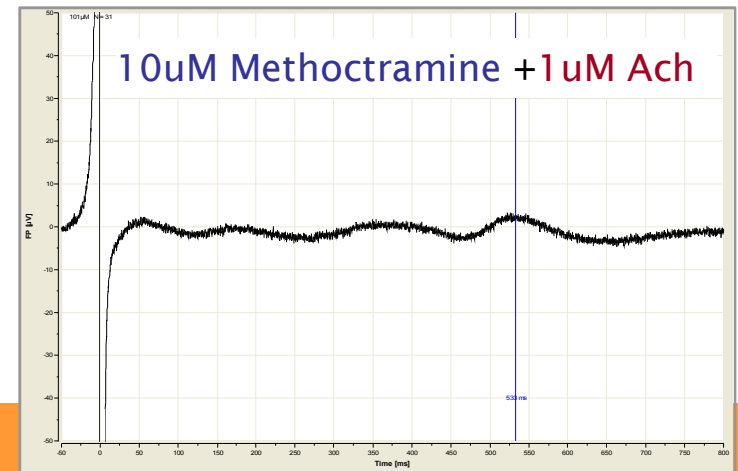
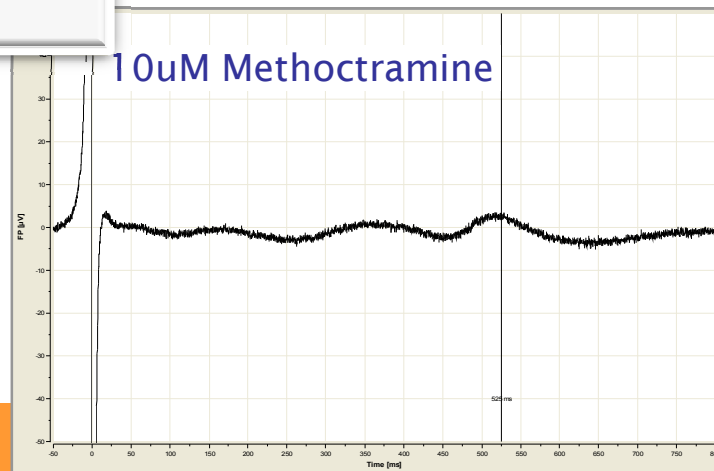
*Folia Pharmacol. Jpn.* **125**, 358 ~ 364 (2005)



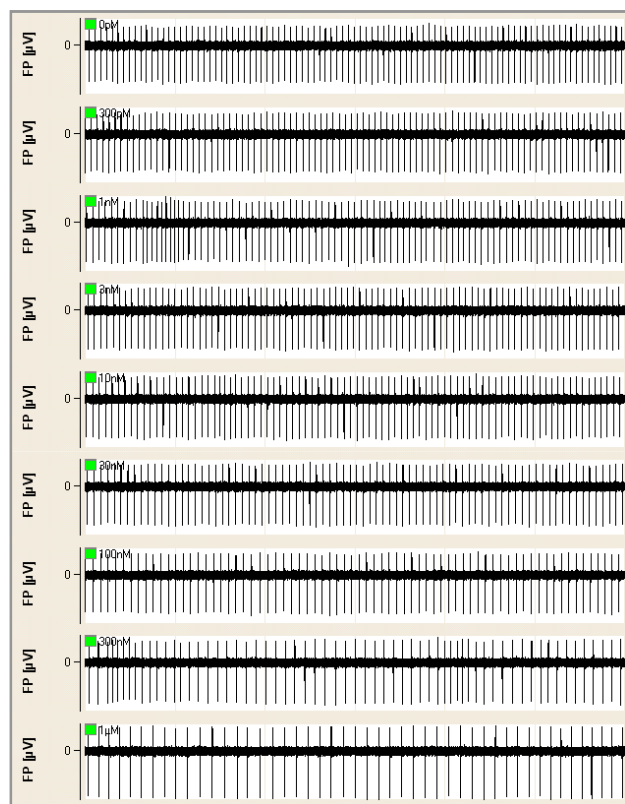
# Effect to $\text{Na}^+\text{-K}^+$ interval in human ES-derived cardiomyocytes treated with Acetylcholine and/or Methoctramine



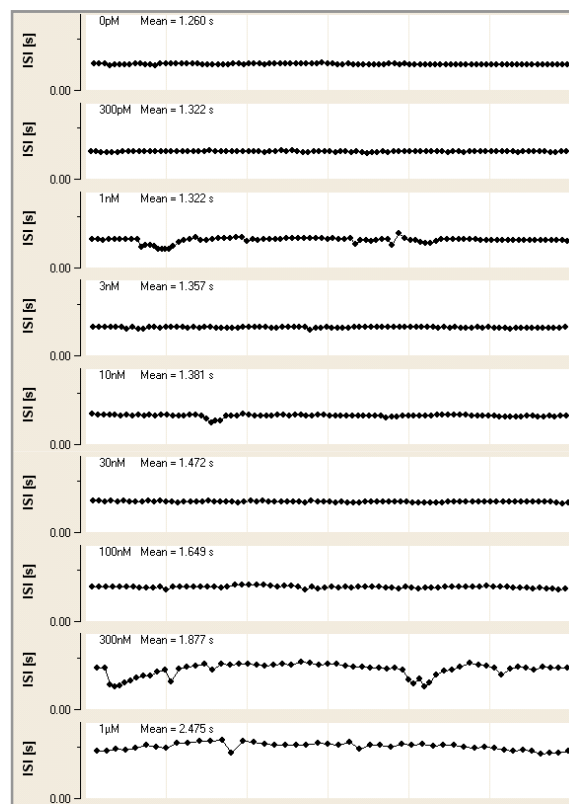
Acetylcholine: cardiac M2 muscarinic acetylcholine receptor agonist



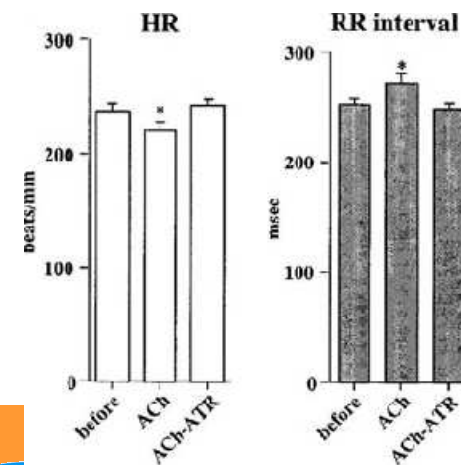
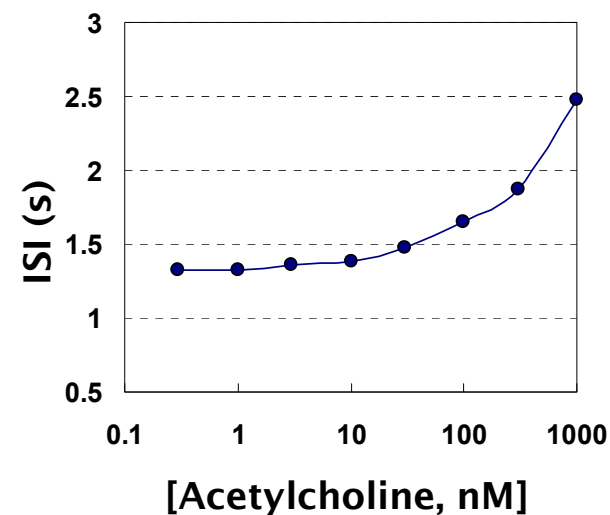
# Acetylcholine treatment causes bradycardia of Human ES-derived cardiomyocytes



FP: field potential



ISI: interspike interval

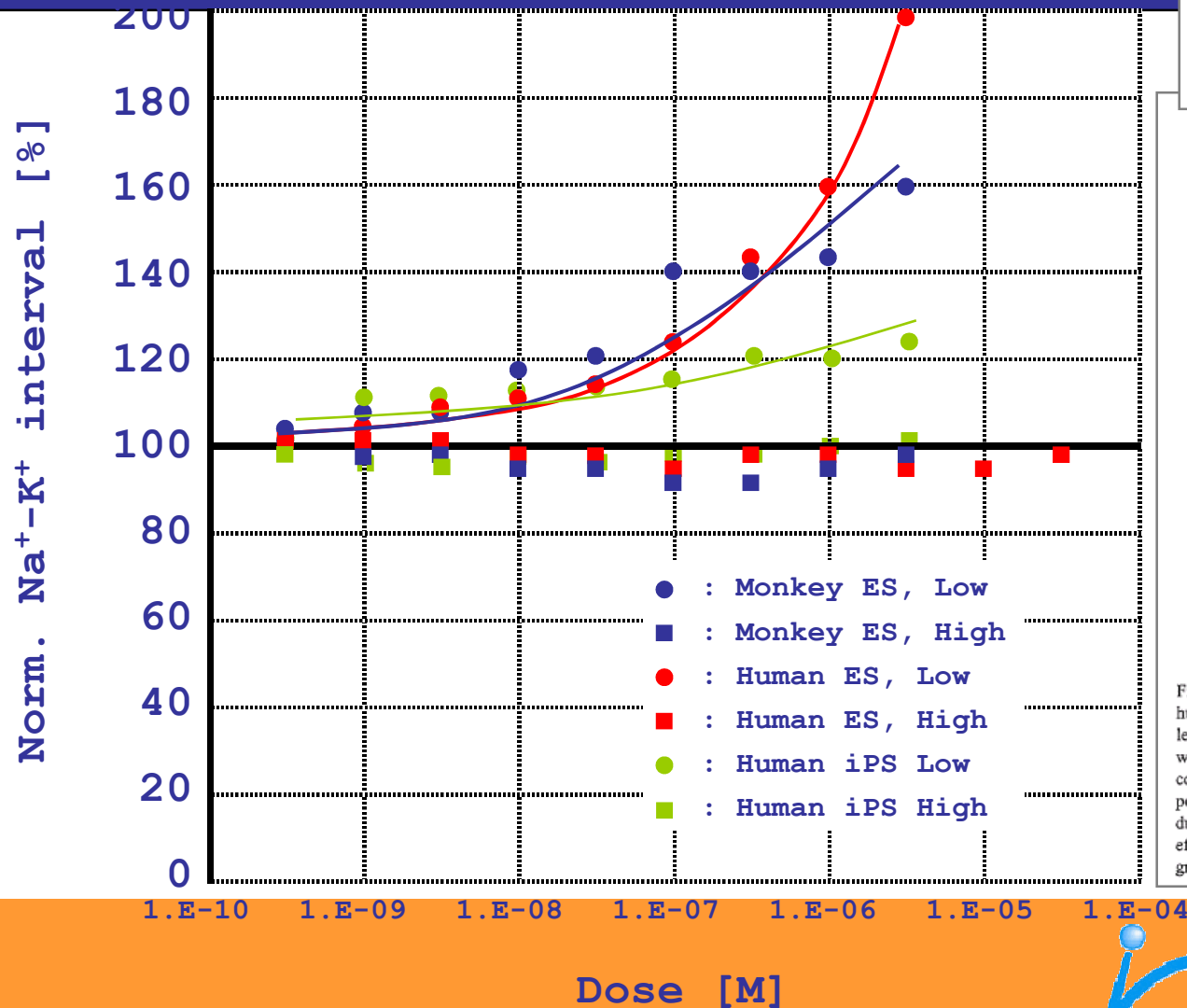


Folia Pharmacol. Jpn. 125, 358 ~ 364 (2005)

ReproCELL



# Na<sup>+</sup>-K<sup>+</sup> interval prolongation on Primate ES/iPS-derived cardiomyocytes treated with Chromanol 293B, IKs blocker



Prolongation observed in low frequency cardiomyocytes, not in high frequency cardiomyocytes

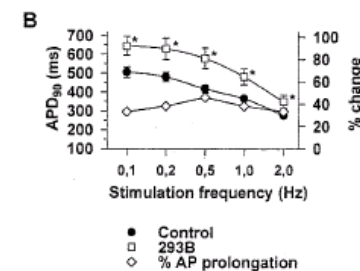
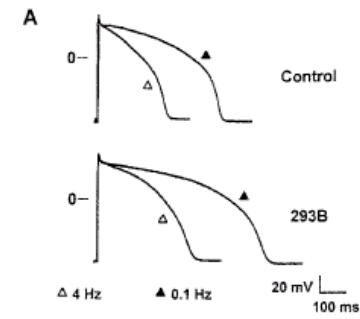


Fig. 8. Effects of 293B (1  $\mu$ mol/L) on action potentials recorded in human ventricular myocytes. (A) Action potentials recorded at 36°C from left ventricular midmyocardial cells from an explanted heart of a patient with dilated cardiomyopathy under control (top) and 293B (middle) conditions. Resting membrane potential is not corrected for the junction potential. (B) Frequency dependence of differences in action potential duration. APD<sub>90</sub> was significantly prolonged at all frequencies and the effect was not rate-dependent. \*  $P < 0.05$  versus control,  $n = 5$  for each group. Raiph f. Bosch, *et al.*, Cardiovascular Research 38 (1998) 441-450

# QTempo assay service & validation study

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Understanding brings Innovation

**Report of QT prolongation by QTTempo**

Service name: QTempo: QT prolongation  
Cat. #: RCESD001

**Customer Information**

Name:  
Division:  
Organization:

**Testing sample**

Sample ID: E-4031  
Dose: 0nM, 0.3nM, 1 nM, 3 nM

**Schedule**

Date of Measurement: October 16th, 2008  
Date of Reporting: October 18th, 2008

**Conducted by**

ReproCELL Inc.  
Shirokane-Utsu Bldg 2F  
Tel: +81-3-5488-7077  
URL: www.reprocell.com

Responsible person: Dr. Yasuyuki Asai  
Chief Technology Officer

10/01/2008 QTempo provided by ReproCELL Inc.

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**Cumulative dose response method**

Drugs have been applied in cumulative dose response. The field potential of beating cardiocytes at 30µM have been monitored in threefold logarithmic concentration. Subsequently recorded in steady state for another 30µM. Parameters recorded and analyzed include amplitude of the field potential, duration of the field potential (measured as the time from the start of the field potential to the end of the field potential), and the duration of the field potential (measured as the time from the start of the field potential to the end of the field potential). The analysis of these parameters on the cardiac field potential recorded on ESC-1000.

**Results**

Summary of Result

10/01/2008 QTempo provided by ReproCELL Inc.

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**Fig. 1 Change in field potential of beating cardiocytes**

**Fig. 2 Change in Inter spike interval of beating cardiocytes**

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**QTempo**

**Fig. 3 Overlay of waveforms recorded under control condition and in the presence of each concentration of E-4031**

**Fig. 4 Dose response curve as a function of E-4031 concentration**

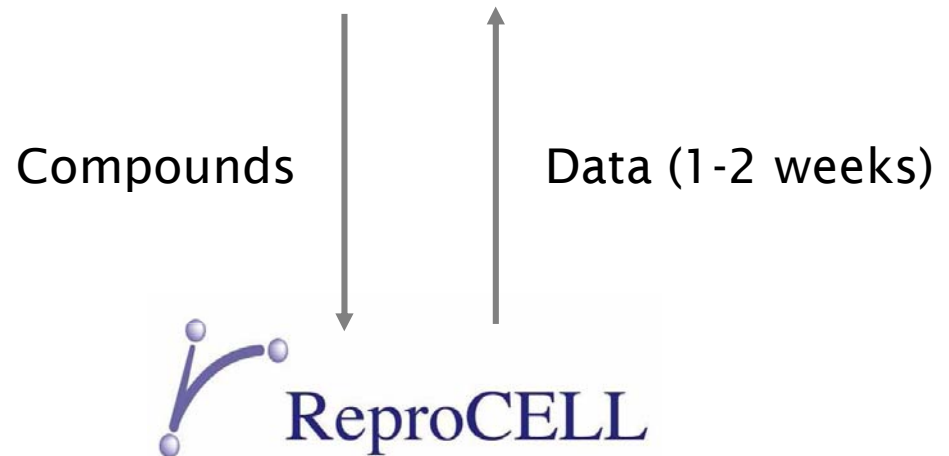
10/01/2008 QTempo provided by ReproCELL Inc.

# QTempo assay service

Typical service is 12 concentrations of each compound against a single cluster of cells.

Modifications of assay are available as required.

Pharma company, CRO, . . .



**3 cell types available**

QTempo monkey ES

QTempo human ES

QTempo human iPS



# QTempo Advantages

- Beating human heart cells
- Multiple data points from ECGM including multi ion channels and beat interval
- More accurate than the hERG assay (QT)
- Cheaper (animal costs and drug synthesis costs), more rapid and possibly more accurate than animal tests
- Screens complex ion channels and receptor interactions
- Scalable



# Contact



People + Science = ∞ Future

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