Electrophysiological profiling of Axiogenesis CorV.4U iPSC-derived cardiomyocytes

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Introduction

Metrion offers high quality cardiac ion channel services to predict human arrhythmia risk, as required by the FDA's Comprehensive *in vitro* Proarrhythmia Assay (**CiPA**) initiative. For CiPA, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) expressing a ventricular phenotype are needed.

Here, we have assessed the suitability of Axiogenesis CorV.4U 2nd generation iPSC-CM for cardiotoxicity screening by evaluating their biophysical and pharmacological characteristics using three different methodologies:

1. Whole-cell voltage clamp recordings to quantify inward Na^+ and Ca^{2+} currents (I_{Na} and I_{Ca}), as well as outward and inward K^+ currents (I_K).
2. Current clamp measurements of action potential (AP) parameters and pharmacology. Representative compounds from the CiPA validation toolbox in addition to compounds discriminating between atrial and ventricular phenotypes, were utilised in these experiments. These data confirmed the functional expression and pharmacology of typical cardiac currents, including I_{Na}, I_{Ca}, and I_{Kr}.
3. Phenotypic measurements of impedance (contraction) and extracellular field potential (excitability) were made on the xCELLigence RTCA CardioECR platform.

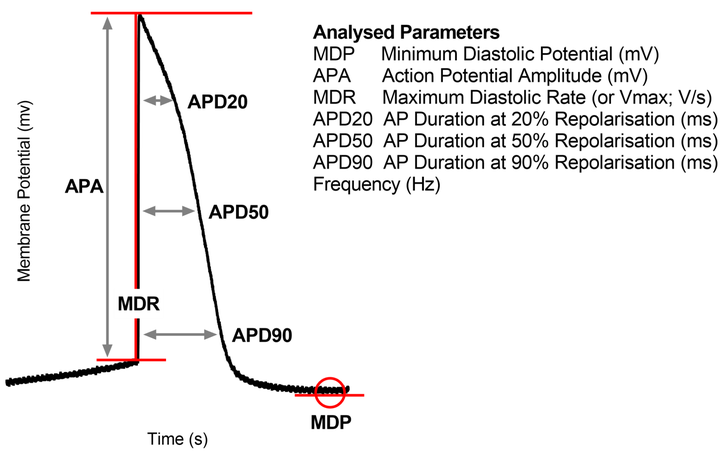
Materials and Methods

Manual patch clamp: Axiogenesis CorV.4U cells were seeded onto fibronectin-coated coverslips and cultured at 37 °C (5 % CO_2). AP were recorded 7-10 days after cell seeding at RT in current clamp mode using perforated patch (100 µg/ml gramicidin). For evoked AP cells were paced at 1 Hz with a field stimulator. Voltage clamp recordings were obtained from single cells using the conventional whole-cell patch clamp configuration with protocols and solutions designed to isolate the ionic current of interest (Table 1).

	External				Internal			
	I_{Na}	I_{Ca}	I_K	CC	I_{Na}	I_{Ca}	I_K	CC
NaCl	50	-	140	140	5	5	5	5
TEA-Cl	1	140	-	-	-	-	-	-
KCl	5.4	5.4	5.4	5.4	-	-	20	125
K-Asp	-	-	-	-	-	-	110	-
$CaCl_2$	1.8	1.8	1.8	1.8	-	-	-	-
$MgCl_2$	1	1	1	1	-	-	1	5
CsCl	90	-	-	-	130	130	-	-
Glucose	10	10	10	10	-	-	-	-
HEPES	10	10	10	10	10	10	10	10
MgATP	-	-	-	-	5	5	5	-
$CdCl_2$	0.3	-	0.3	-	-	-	-	-
EGTA	-	-	-	-	10	10	10	5
4-AP	-	2	-	-	-	-	-	-
pH	7.4 CsOH	7.4 CsOH	7.4 NaOH	7.4 NaOH	7.2 CsOH	7.2 CsOH	7.2 KOH	7.2 KOH

Table 1: Manual patch clamp solutions.
Composition (in mM) of external and internal solutions used for voltage and current (CC) manual patch clamp experiments.

Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitization at 20 kHz. Spontaneous AP were analysed with CAPA software (SSCE UG, Germany) and evoked AP data in FitMaster. The analysed AP parameters are shown in Figure 1. Data are reported as mean ± SEM.



Analysed Parameters
MDP Minimum Diastolic Potential (mV)
APA Action Potential Amplitude (mV)
MDR Maximum Diastolic Rate (or Vmax; V/s)
APD20 AP Duration at 20% Repolarisation (ms)
APD50 AP Duration at 50% Repolarisation (ms)
APD90 AP Duration at 90% Repolarisation (ms)
Frequency (Hz)

Figure 1: Action potential parameters analysed.
Example action potential indicating the parameters which are quantified using HEKA FitMaster (evoked AP) and CAPA software (spontaneous AP) in this study.

xCELLigence RTCA CardioECR:

Day 0; Axiogenesis CorV.4U iPSC-CM were seeded at 30 K cells/well onto fibronectin-coated E-plates (Cardio ECR 48) according to the manufacturers instructions.

Days 1-4; cells were cultured at 37°C (5% CO_2) for 4 days prior to drug application; media exchanges were performed daily.

Day 5; 3-4 hours prior to compound application a complete media exchange was performed and baseline data recorded. Compounds were prepared at 2x concentration in complete culture media. Data were recorded at regular intervals for 24 h and acquired at a sampling rate of 12 msec in Cardio mode (impedance) and 0.1 msec for ExtraCellular Recordings (ECR; field potential). Beat rate and contraction amplitude were measured in Cardio mode, whereas firing rate and field potential amplitude were measured in ECR mode. All data were analysed using xCELLigence software and are presented as the mean ± SEM.

1. Ionic Current "Snapshot"

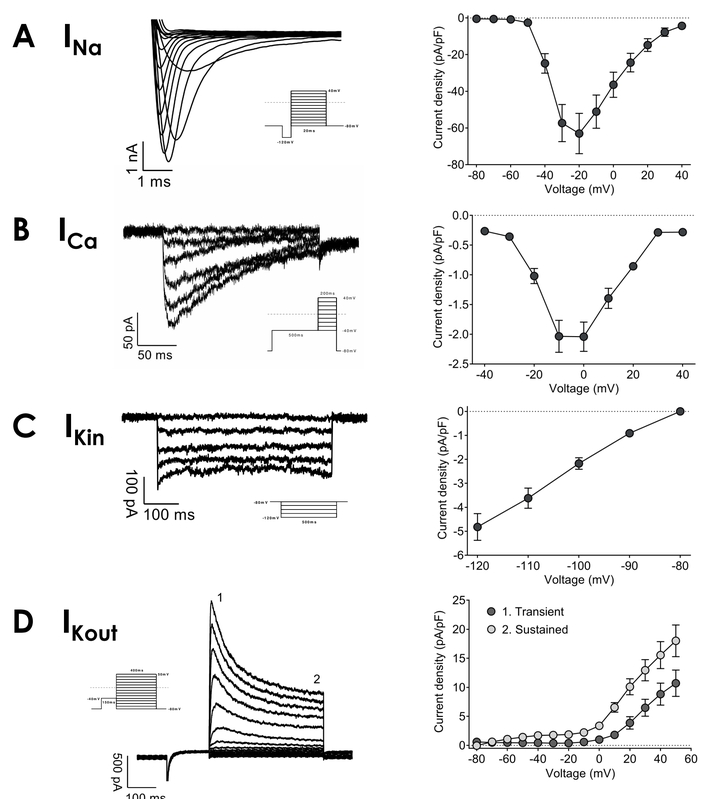
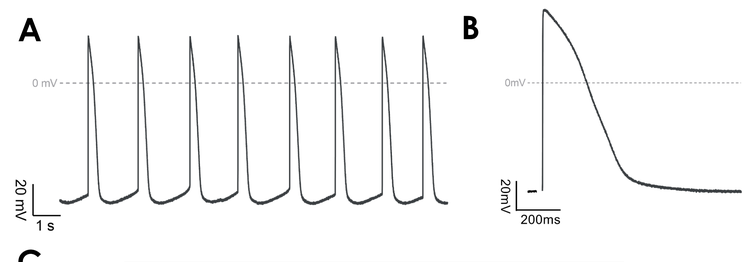


Figure 2: Voltage clamp "snapshot" of cardiac ionic currents in CorV.4U.
Left; representative traces of sodium (I_{Na}; **A**), L-type calcium ($I_{Ca,L}$; **B**), inward (I_{Kin}; **C**) and outward (I_{Kout}; **D**) potassium currents elicited by the voltage protocols shown. *Right*; *I-V* relationships shown for I_{Na} (peak; **A**), $I_{Ca,L}$ (peak; **B**), I_{Kin} (end of the pulse; **C**) transient I_{Kout} (peak; **D**) and sustained I_{Kout} (end of the pulse; **D**).

2. Action Potential Properties



C

AP parameter	Spontaneous	Evoked
MDP (mV)	-72.0 ± 0.4	-73.3 ± 0.6
MDR (V/s)	57.4 ± 3.9	45.2 ± 7.7
APA (mV)	105.6 ± 0.9	119.9 ± 1.8
APD20 (ms)	125.7 ± 3.5	99.3 ± 2.9
APD50 (ms)	241.5 ± 4.8	211.7 ± 3.6
APD90 (ms)	511.0 ± 18	426.8 ± 11.7
Frequency (Hz)	0.4 ± 0.0	1

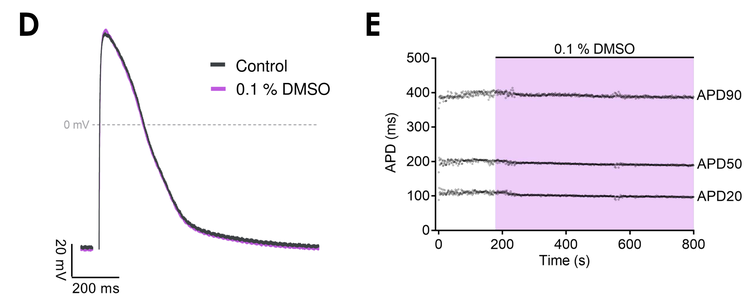


Figure 3: Characteristics of spontaneous and evoked action potentials.
Representative traces of spontaneous (**A**) and evoked (1 Hz; **B**) AP recorded from CorV.4U iPSC-CM under control conditions. ***C***; Average AP parameters for spontaneous (n = 79) and evoked (n = 70) AP in control conditions. ***D***; Representative trace showing stability of evoked AP following 0.1 % DMSO application (10 min). ***E***; Stability of evoked AP over time (APD *vs* time) during the control period and following 0.1 % DMSO application (10 min).

3. Core Cardiac Channel Pharmacology

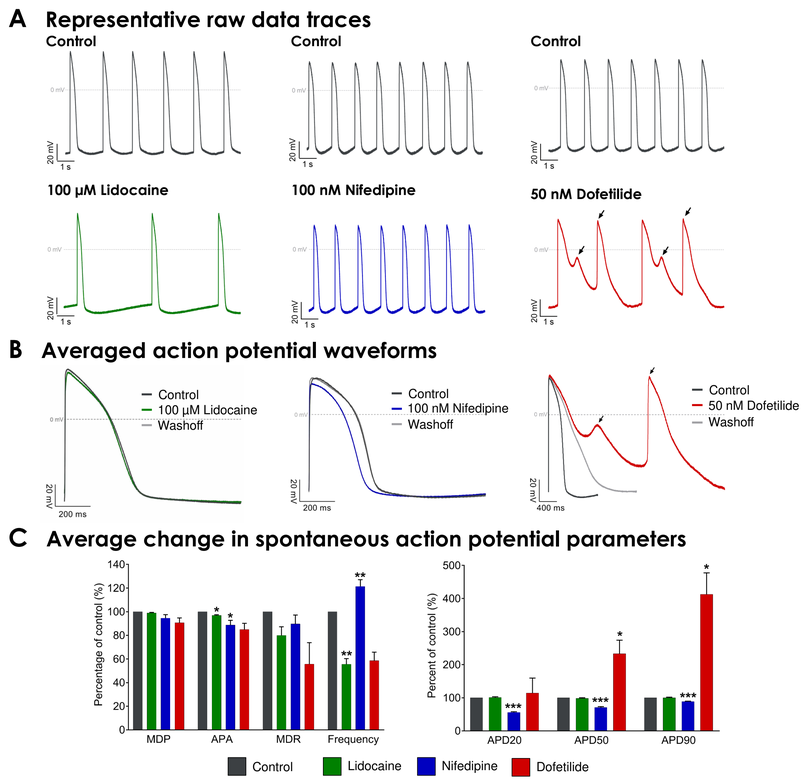


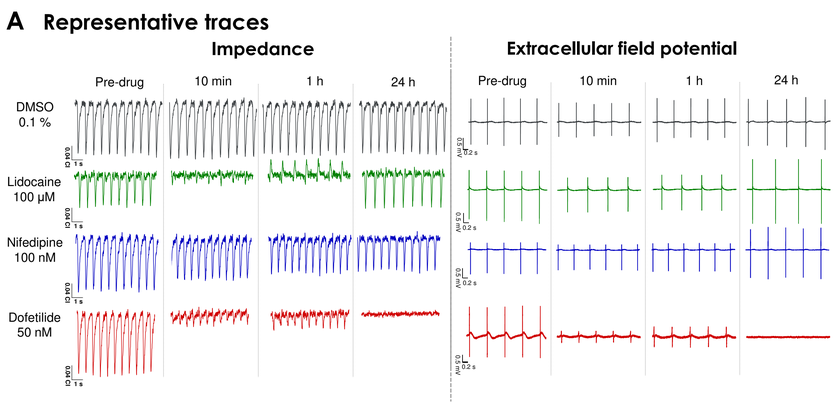
Figure 4: Effect of cardiac channel blockers on CorV.4U spontaneous AP.
A; Typical spontaneous AP recorded under control conditions (grey) and in the presence of 100 µM lidocaine (green), 100 nM nifedipine (blue) or 50 nM dofetilide (red). Early after depolarisations (EADs) were detected following dofetilide addition (arrows). ***B***; Average AP profile for each condition. AP waveform calculated as the mean of N > 3 individual AP. ***C***; Average effect (% of control) for each compound on spontaneous AP parameters. Data presented as mean ± SEM, N ≥ 4. Significance calculated by a paired two-tailed Student's *t*-test, * p<0.05, ** p<0.01, *** p<0.001.

4. Summary of AP Pharmacology

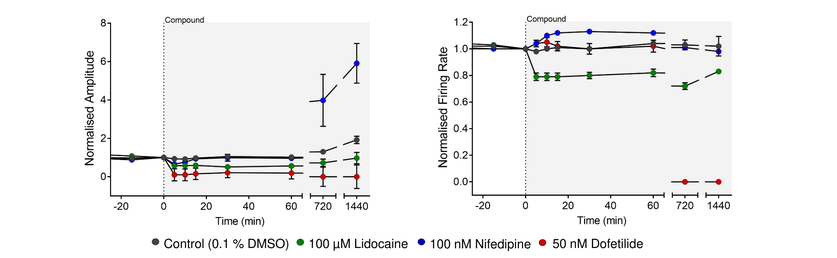
Compound	Conc (µM)	Target	Expected effect	Response	
E-4031	0.1	I_{kr}	↑ APD90 , ↓ BR	+++ (EADs)	
Dofetilide*	0.05-0.5		↑ APD90, ↓ BR	+++ (EADs)	
Lidocaine*	30-100	I_{Na}	↓ Vmax, ↑ TTP, ↓ BR	+++	
TTX	5	I_{Na}	↓ Vmax, ↑ TTP, ↓ BR	+++	
Ranolazine*	10	$I_{Na,L}$	↓ MDP, ↑ APD90	-	
Nifedipine*	0.1	$I_{Ca,\ L}$	↓ APDs, ↑ BR	+++	
Verapamil*	0.3	$I_{Ca,\ L}$	↓ APDs	++	
	1	$I_{Ca,\ L}$ + I_{Kr}	↓ APD20, ↑ APD90	+	
JNJ-303*	1	I_{Ks}	↑ APD90 , ↓ BR	-	
Ivabradine*	10	I_f	↓ MDP, ↓ MDR, ↓ BR	+	
Isoprenaline	0.1	β_2 receptor	↑ BR , ↓ APDs	+	
4-AP	50	I_{Kur}	↓ APD20 & 50	++	Atrial
Carbachol	1	rI_{KACh}	↓ MDP ↓ APDs	-	Atrial
Tertiapin Q	0.1	cI_{KACh}	↑ MDP ↑ APDs	-	Atrial
Apamin	0.1	I_{KCa}	↑ MDP, ↑ APDs	-	Atrial
ML-365	0.1	I_{K2P}	↑ MDP, ↑ APD90	-	Atrial

Table 1: Summary of compound effects on CorV.4U action potentials
The change in AP property is shown by the following scale; – no effect, + small (< 10 % change in AP parameter), ++ moderate (10-25 % change in AP parameter), and +++ significant (> 25 % change in AP parameter or statistically significant change). The target channel and expected effects are indicated. Compounds which are used to selectively target atrial currents are highlighted in grey. * indicates a compound from the **CiPA** validation panel.

5. Phenotypic Assay Characterisation



Condition	Amplitude (cell index)				Beat rate (bpm)			
	0 min	60 min	% change	p-value	0 min	60 min	% change	p-value
Control	0.10 ± 0.01	0.10 ± 0.03	0.0	ns	97.2 ± 1.3	99.0 ± 0.6	↓ 1.8	ns
Lidocaine	0.08 ± 0.01	0.07 ± 0.00	↓ 12.8	ns	89.8 ± 1.8	78.1 ± 10.3	↓ 13.0	ns
Nifedipine	0.14 ± 0.01	0.09 ± 0.01	↓ 33.6	*	92.6 ± 1.4	109.0 ± 4.9	↑17.7	*
Dofetilide	0.14 ± 0.02	0.04 ± 0.01	↓ 72.3	*	93.1± 1.4	111.6± 12.7	↑19.9	ns



Condition	Amplitude (mV)				Firing rate (events per min)			
	0 min	60 min	% change	p-value	0 min	60 min	% change	p-value
Control	1.32 ± 0.2	1.27 ± 0.2	↑ 3.3	ns	94.0 ± 0.3	97.5 ± 0.6	↓ 3.6	***
Lidocaine	1.15 ± 0.2	0.61 ± 0.2	↓ 46.6	*	88.8 ± 0.6	72.7 ± 4.9	↓ 18.2	**
Nifedipine	0.39± 0.10	0.38± 0.12	↓ 1.8	ns	91.2 ± 0.8	101.8 ± 2.0	↑ 11.6	ns
Dofetilide	0.64± 0.16	0.11± 0.03	↓ 83.1	*	91.1 ± 1.0	92.6 ± 3.0	↑ 1.6	ns

Figure 5: Spontaneous phenotypic pharmacology of CorV.4U iPSC-CM.
A; Representative traces showing the contractile activity (impedance; left) and electrical activity (extracellular field potential; right) in control conditions (grey) and the presence of 100 µM lidocaine (green), 100 nM nifedipine (blue) or 50 nM dofetilide (red). **B**; Graphs showing the normalised drug effects over time for impedance amplitude (left) and beat rate (right). The average values for control (0 min) and after 60 min drug exposure, % change and significance are summarised in the table. **C**; Normalised drug effects on ECR amplitude (left) and firing rate (right). The average values for control (0 min) and after 60 min drug exposure, the % change and significance are summarised in the table. Data are normalised to the last baseline value before drug application (time = 0). Significance calculated by a paired two-tailed Student's *t*-test, ns not significant, * p<0.05, ** p<0.01, *** p<0.001.

Conclusions

- Extensive profiling of cardiac ion channel expression with a variety of selective pharmacological tools and high fidelity techniques available at Metrion has enabled us to profile the physiology of CorV.4U iPSC-CM:
  - AP were stable over time during both spontaneous and evoked recording conditions
  - The cells exhibit appropriate core cardiac channel pharmacology, including EADs and I_{Kr} inhibition
  - Phenotypic assays confirmed core channel pharmacology and suitability of the cells and the xCELLigence CardioECR platform for acute and chronic drug application studies.
- In the light of the incoming CiPA guidelines, Axiogenesis CorV.4U cells represent a suitable model for *in vitro* pre-clinical cardiac safety evaluation of compounds to identify potential human pro-arrhythmic activity.

Acknowledgements

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