

Delayed or accelerated cardiac repolarization is potentially proarrhythmic. In this study, we compared a guinea pig papillary muscle APD (GPPM-APD) assay to a canine Purkinje fiber APD (CPF-APD) assay in assessing drug effects on cardiac repolarization. Papillary muscles (right ventricle) and Purkinje fibers were stimulated at 0.5 Hz, action potentials were recorded using microelectrode techniques, repolarization assessed using APD₃₀, APD₅₀, APD₉₀ values, and the calculated triangulation parameter, APD₃₀₋₉₀. Eight compounds (6 positives, 2 negatives) were tested in both preparations. Percent changes in APD₉₀ values obtained with the GPPM-APD assay were less than those obtained in the CPF-APD assay (table). Repolarization parameters in the GPPM-APD assay exhibited a rank sensitivity order of APD₃₀ < APD₅₀ ≈ APD₉₀ < APD₃₀₋₉₀ in detecting effects of the five hERG blockers on repolarization. APD₉₀ was the most sensitive parameter in detecting effects of the hERG activator (A-935142.0). These results suggest the GPPM-APD assay and the CPF-APD assay are valuable in assessing drug effects on cardiac repolarization with comparable effects in both assays.

| Compounds | APD ₉₀ | | |
|---------------------|-------------------|-------------------|-------------------|
| | CPF [†] | GPPM [‡] | GPPM [‡] |
| DMSO 0.1% | 2 ± 1 | 1 ± 2 | 3 ± 3 |
| Tobifenacin 700 nM | 55 ± 3 | 15 ± 5* | 39 ± 10* |
| Moxifloxacin 450 μM | 178 ± 15 | 81 ± 12* | 140 ± 20* |
| E-4031 0.2 μM | 146 ± 11 | 36 ± 5* | 53 ± 6* |
| Cisapride 10 μM | 34 ± 4 | 22 ± 4* | 40 ± 11* |
| Haloperidol 2.66 μM | 3 ± 4 | 12 ± 3* | 32 ± 5* |
| A-935142.0 60 μM | -24 ± 2 | -18 ± 2* | -13 ± 5* |

Values are expressed as a mean percent change from baseline ± SEM. n = 4-6
[†]p < 0.05 vs. vehicle (unpaired t-test); [‡]p < 0.05 vs. vehicle (Mann-Whitney)

1759-Pos

Increased Cardiac Risk in Concomitant Methadone and Diazepam Treatment: Pharmacodynamic Interactions in Cardiac Ion Channels

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Methadone, a synthetic opioid used in the treatment of chronic pain and in maintenance of withdrawal from opioid dependence, has been linked to QT prolongation, potentially fatal torsades de pointes, and sudden cardiac death. Concomitant use of benzodiazepines, such as diazepam, in methadone maintenance treatment appears to increase the risk of sudden death. Our objective was to determine the effects of methadone and diazepam singly and in combination on the major cardiac ion channels, responsible for the cardiac repolarization, stably expressed in mammalian cells. Using automated patch-clamp technique (PatchXpress[®]) for ion channel current recording, we found that methadone produced concentration-dependent block of hERG (IC₅₀ = 1.7 μM), hNa_v1.5 (11.2 μM tonic block; 5.5 μM phasic block), hCa_v1.2 (26.7 μM tonic; 7.7 μM phasic) and hK_vLQT1/hminK (53.3 μM). Diazepam demonstrated much less potent block to block of these ion channels: the IC₅₀ values were 53.1, >100 tonic and 47.7 phasic, 89.0 tonic and 82.1 phasic, and 86.4 μM for hERG, hNa_v1.5, hCa_v1.2 and hK_vLQT1/hminK, respectively. Co-administration of 1 μM diazepam with methadone had no significant effects on methadone-induced block of hERG, hCa_v1.2 and hK_vLQT1/hminK channels, but caused a 4-fold attenuation of hNa_v1.5 block (44.2 μM tonic and 26.6 μM phasic). Thus, although diazepam alone does not prolong the QT interval, the relief of the methadone-induced Na⁺ channel block may leave hERG K⁺ channel block uncompensated, thereby creating a potentially greater cardiac risk.

1760-Pos

Optimization of a Cav1.2 Cell Line for Use on QPatch and PatchXpress

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Ion channel currents comprise the cardiac action potential and are important in cardiac safety liability assessment of potential drug candidates. The gold standard for assessing ion channel activity is the voltage clamp technique, but this technique is a very low throughput process. Planar patch technology (QPatch and PatchXpress) allows for moderate throughput by providing automated, simultaneous whole cell voltage clamp recordings from cells heterologously expressing the channel of interest. Ion channels routinely screened for cardiovascular safety are hERG (Kv11.1), Nav1.5, Kir2.1 and KvLQT/minK using either PatchXpress or QPatch instruments. In this study, we highlight the validation of Cav1.2 (L-type calcium channel) on our automated electrophysiology systems for cardiovascular safety screening. The L-type calcium channel is expressed in the cardiovascular system both in smooth and cardiac muscle. Potent L-type calcium channel antagonists can lower blood pressure, reduce cardiac contractility, and potentially increase the P-R interval on the electrocardiogram (ECG). Cav1.2 was expressed in CHO cells using a tetracycline inducible vector. Because of this we needed to optimize expression level by varying the induction variables. Addition of an L-type antagonist (verapamil) also provided benefit by keeping well-expressing cells viable after induction. In order to optimize flexibility in performing experiments, we also prepared the cells as a cryo-pre-

served substrate. Cav1.2 channel kinetics for both activation and inactivation were investigated, and potencies of 8 reference compounds (weak and strong antagonists) were assessed on both platforms. In conclusion, we have optimized tissue culture conditions, cell preparation and voltage clamp protocols on two automated electrophysiology platforms to provide cardiac safety evaluation of drug candidates using an inducible Cav1.2 cell line.

1761-Pos

Pacemaker Cells of the Atrioventricular Node are Ca_v1.3 Dependent Oscillators

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The atrioventricular node (AVN) can generate pacemaker activity in case of failure of the sino-atrial node (SAN). However, the mechanisms underlying pacemaking in AVN cells (AVNCs) are poorly understood. Voltage-dependent ion channels such as hyperpolarization-activated HCN channels, L-type Ca_v1.3 and T-type Ca_v3.1 channels are known to play a role in pacemaking of sino-atrial node cells (SANCs). Here, we investigate the role of these channels in AVNCs pacemaker activity using genetically modified mouse strains and show that they differentially impact pacemaking of AVNCs than of SANCs. Indeed, contrary to SANCs, Ca_v1.3 channels are necessary for pacemaking of AVNCs and accounted for the predominant fraction of I_{Ca,L}. Inactivation of Ca_v1.3 channels impaired automaticity in AVNCs by promoting sporadic block of automaticity and spontaneous cellular arrhythmia.

Abolition of the cAMP sensitivity of HCN channels shifted the I_f activation to voltages negative to that spanning the diastolic depolarization and prevented AVNCs automaticity in basal conditions. However pacemaker activity could be restored to control levels by adrenergic receptor stimulation.

Inactivation of both Ca_v1.3 and Ca_v3.1 results in abolishment of pacemaking. Inhibition of TTX-resistant (I_{NaT}) Na⁺ current showed that this is a key contributor of the action potential (AP) threshold and upstroke velocity.

Conclusion: 1. Spontaneous firing rate in AVNCs is strongly dependent from Ca_v1.3-mediated L type calcium current and from TTX resistant sodium current (I_{NaT}). 2. In AVNCs the Ca_v1.3 isoform seems to be predominant compared to Ca_v1.2 isoform. 3. The fact that hyperpolarization of Ca_v1.3^{-/-}AVNCs pacemaking can be observed suggests that the absence of pacemaker activity is not due to the impossibility to generate the upstroke phase of the AP but probably due to an imbalance between outward and inward currents during the diastolic depolarization.

1762-Pos

Development of Novel System for the Functional Analysis of the Cardiomyocytes Network Model Using On-Chip Cellomics Technology

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Spatial and temporal regulation of cellular orientation is one of the key to resolve the mechanism of organs and tissues that are complexly intertwined with epigenetic factors, such as cellular network size and orientation of cellular-type. To study the dynamics of synchronous beating rhythm in the cardiac myocytes, we tried to develop the agarose micro-chamber (AMC) system on the multi-electrode array (MEA) chip, and extra-cellular signals of cardiomyocytes in geometrically patterning chambers were recorded with On-Chip MEA system. The chip set consists with the type of MEA chip, pattern of AMC and cellular-type, for example, primary mouse embryonic, ES and iPS derived cardiomyocytes. By using the cell handling by micropipette and additional fabrication to the AMC during the cultivation, we are able to construct the normal and disordered model. For example, we made the loop structure as reentry model having length of the circuit above the millimeter. Under this system, it is possible to obtain a multiple of information about individual cell (as constitutional unit) and entire network (as organ model) by field potential recordings (FPs) and optical imaging. Pseudo-ECG, which sum the FPs obtained from each electrode, means whole network signal and duration time of pseudo-ECG corresponds to QT interval. From analysis of individual FPs, direction of the excitement propagation and conduction velocity is resolved. Waveform analysis of FPs give us the relative intensity of Na⁺, Ca²⁺ and K⁺ currents and field potential duration (FPD) corresponding to action potential duration (APD). On-Chip geometric re-constitutive approaches are powerful tools for stepwise cell network construction and long-term measurement of it, and also will make possible the development of the novel system for the toxicity studies of drugs.