B-PO01-005

ACUTE PROARRHYTHMIC EFFECT OF ANTI-ACETYLCHOLINEESTERASE DRUGS USED IN THE TREATMENT OF DEMENTIA: BENEFIT OF RIVASTIGMINE

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Background: Acetylcholineesterase inhibitors are used in the treatment of dementia. Recent case reports have suggested torsade de pointes (TdP) with donepezil.
Objective: To evaluate the electrophsiologic effects of donepezil, galantamine and rivastigmine in a sensitive model of proarrhythmia.
Methods: 34 rabbit hearts were retrogradely perfused. Eight catheters were placed epi- and endocardially. Hearts were paced at seven different cycle lengths (300-900ms), thus obtaining cycle-length dependent action potential duration at 90% of repolarization (APD_{90}). Spatial dispersion of repolarization was calculated as the difference of the maximum and minimum of APD_{90}. Hearts were divided into three groups: In the first group (n=10), donepezil (0.5, 1, 2µM) was administered. The second group (n=11) was perfused with galantamine (1, 5, 10µM) and the third (n=13) with rivastigmine (1, 5, 10µM).
Results: Infusion of 0.5 and 1µM donepezil slightly abbreviated APD_{90} (0.5µM: -4ms, 1µM: -4ms). However, 2µM donepezil prolonged APD_{90} (+22 ms, $p<0.05$). Donepezil amplified spatial dispersion in a dose-dependent manner (0.5µM: +4ms, p=ns; 1µM: +10ms, $p<0.01$; 2µM: +33ms, $p<0.01$). Infusion of galantamin shortened APD_{90} (1µM: -10ms, p=ns; 5µM: -5ms, p=ns; 10µM: -14ms, $p<0.01$) but increased spatial dispersion (1µM: +18ms, 5µM: +12ms, 10µM: +19ms; $p<0.01$ each). Treatment with rivastigmine (n=13) prolonged APD_{90} (1µM: +2ms, p=ns; 5µM: +14ms, $p<0.01$; 10µM: +13ms, $p<0.01$) and reduced spatial dispersion (1µM: -3ms, p=ns; 5µM: -13ms, $p<0.01$; 10µM: -4ms. p=ns). With donepezil, 3 of 10 bradycardic hearts showed early afterdepolarizations (EAD) after lowering potassium concentration and 18 episodes of TdP occurred. Treatment with galantamine provoked EAD in 4 hearts and 40 episodes of TdP. No EAD or TdP were observed under rivastigmine infusion.
Conclusion: This is the first experimental study to show the ability of donepezil and galantamine to provoke TdP. Both drugs significantly increased spatial dispersion of repolarization, which is a known risk factor for drug-induced proarrhythmia. In spite of prolongation of repolarization under rivastigmine, spatial dispersion of repolarization was rather stable. Consequently, no triggered activity was observed with rivastigmine.

B-PO01-006

QUANTITATIVE PROTEOMICS PROFILING DETERMINES THE UNIQUENESS OF THE SINUS NODE

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Background: The sinus node is a collection of highly specialized cells that constitute the natural pacemaker activity of our heart. Although primarily comprised of myocytes and fibroblasts like the rest of the cardiac tissue, the protein expression landscape of the sinus node differs from the surrounding cardiac tissue, endowing it with its unique ability to regulate heart rate.
Objective: Here we performed quantitative proteomics experiments to profile protein expression in the pacemaker of the heart, and compared it to protein expression in the neighbouring atrial muscle.
Methods: Sinus node and right atrial biopsies were collected from 30 mice and proteomes analyzed by high-resolution mass spectrometry.
Results: We identified more than 7,000 proteins. Our dataset represents the most comprehensive investigation of the sinus node to date. In the sinus node, the expression of ~500 proteins was statistically significantly different from that in the atrial muscle, showing the uniqueness of the tissue. Significant differences were observed in ion channels, proteins involved in carbohydrate and lipid metabolism, contractile proteins, cytoskeletal proteins, protein of the natriuretic peptide system, proteins involved in exocytosis, extracellular matrix proteins, and proteins involved in cell adhesion and integrin signalling. As an example, 28 sarcolemmal ion channel subunits, the four major cardiac gap junction channel subunits, and eight mitochondrial ion channels were detected. The sarcolemmal channels include less described channels, such as the store-operated Ca^{2+} channel (Orai1 and STIM1), Trpm7, purinergic receptor channels (P2rx4 and P2rx7), the two-pore K^+ channel, TASK1, and various Cl^- channels. Of the sarcolemmal channels, the pacemaker channels (HCN1 and HCN4), two important L-type Ca^{2+} channel accessory subunits ($Ca_v a2d1$ and $Ca_v a2d2$) and the T-type Ca^{2+} channel ($Ca_v 3.2$) were significantly more abundant in the sinus node, whereas two K^+ channels (TASK1 and $K_{ir}3.1$) were more highly expressed in the atrial muscle.
Conclusion: In summary, the quantitative proteomics data presented here offer a highly detailed insight into the unique composition of the pacemaker of our heart.

B-PO01-007

CARDIAC FIBROSIS AND VENTRICULAR ARRHYTHMOGENESIS IN HYPERTENSIVE HEART DISEASE PROGRESSION

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Background: The progression of hypertensive heart disease (HHD) is associated with increased risk of arrhythmia and sudden cardiac death. While structural remodeling and fibrosis are strongly implicated, the mechanisms involved are not fully understood.
Objective: Investigate the influence of fibrosis on electrical function in HHD progression.
Methods: Arrhythmic susceptibility (AS) was assessed in Langendorff perfused, spontaneously hypertensive rat (SHR) hearts at 6, 12 and 18 months (n=6,6,5). Rate-dependent changes in CV and APD were estimated using optical mapping. Extracellular matrix organisation and 3D myocyte morphology were quantified from confocal images of optically cleared LV short-axis slices.
Results: Age-related variation was seen in all electrical measures. CV fell, APD increased, while transverse CV decreased and APD dispersion increased at high rates. These changes were most marked between 6 and 12 months. Cell

cross-sectional area was significantly larger at 12 months, with proliferation of interstitial fibrosis (Fig 1A). Patches of replacement fibrosis were evident at 12 and 18 months, but there was substantial overlap across these ages (Fig 1B). There was a similar trend with AS and age (Fig 1C), but a very strong association between AS and extent of patchy fibrosis (Fig 1D). AS was also strongly associated with APD dispersion (R^2 = 0.66, p < 0.001). Finally, physical coupling between myocytes was reduced with fibrosis (R^2 = 0.69, p < 0.001), consistent with slower transverse conduction and heterogeneity in impulse propagation.
Conclusion: The progression of electrical dysfunction with HHD in SHR is associated with altered extent and organization of fibrosis.

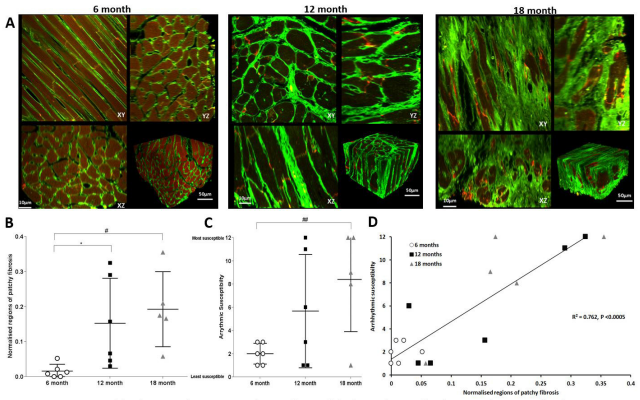

Figure 1. A: 3D reconstructions of cleared SHR ventricular tissue. XY, XZ and YZ views, bottom right hand corner shows 3D volume (isotropic resolution, 0.41 µm). Cardiomyocytes (maroon, dark red), Cx43 (bright red), remaining segmented WGA signal, cell membrane and ECM (green). B: Normalised regions of patchy fibrosis for the three age cohorts. The regions are connected patches of fibrosis acquired from complete LV short axis section at 6.22 µm resolution. The significance of differences between 6 and 12 month-old animals is indicated by * p <0.05, and between 6 and 18 month-old animal by # p <0.05. C: Arrhythmic susceptibility for all hearts at 6, 12 and 18 months. Significance between 6 and 18 month-old animal indicated by ## p <0.005. D: Arrhythmic susceptibility compared with normalised regions of patchy fibrosis across all hearts.

B-PO01-008

APPLICATION OF INTERMITTENT NEGATIVE UPPER AIRWAY PRESSURE AS A NOVEL RAT MODEL FOR OBSTRUCTIVE SLEEP APNEA AND ATRIAL FIBRILLATION

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Background: Obstructive sleep apnea (OSA) is associated with increased occurrence of atrial fibrillation (AF). Obstructive respiratory events lead to intermittent hypoxia (IH) and ineffective inspiration against the occluded upper airways result in intrathoracic pressure changes and increasing cardiac transmural pressure gradients.
Objective: To develop a novel animal model for AF and OSA mimicked by intrathoracic pressure changes on top of IH.
Methods: In spontaneously breathing sedated rats (2% isoflurane), IH (n= 9) was applied by intermittent increase in the respiratory dead volume. Reproducible and standardized obstructive respiratory events were induced by defined intermittent negative upper airway pressure (INAP = inverse CPAP) applied via a customized mask which was connected to a negative pressure device (n= 9). One minute of IH or INAP was followed by a rest period of nine minutes for four hours every second day. Blood pressure was measured by telemetry. Rats with comparable anesthesia were used as controls (CTR). After three weeks, left ventricular pressure was measured invasively and inducible AF-duration by atrial burst stimulation was determined before sacrifice. Left (LA) and ventricular (LV) tissue was processed for histological and biochemical analyses.
Results: Blood pressure and end-diastolic left ventricular pressure were not affected by IH or INAP. Intermittent desaturation ($\leq$ 77% O2) and post-apneic hyperventilation was comparable in INAP- and IH-rats, but INAP-rats showed significantly higher breathing efforts during apnea compared to IH-rats (IH: 3.44±0.13 Vs. INAP: 4.47±0.14 mbar; p<0.01). LA interstitial fibrosis formation (+135% vs. CTR, p=0.01) and LA-myocyte diameters (+107%, p=0.03 vs. CTR) were increased in INAP-rats, but unchanged in IH-rats. This was associated with longer inducible AF-durations in INAP-rats (p=0.02 vs. CTR; INAP: 11.65 seconds; CTR: 0.98 seconds) but not in IH-rats (p=0.31 vs. CTR; IH: 1.28 seconds).
Conclusion: Application of INAP in rats mimics important components of OSA beyond IH and allows the study of the progressive arrhythmogenic substrate in the atrium independent of the development of hypertension or overt diastolic dysfunction.

B-PO01-009

KCNJ2-RELATED CPVT CAUSES DECREASED REPOLARIZATION RESERVE AND PERTURBATIONS IN BASELINE AND PEAK CALCIUM FOLLOWING ADRENERGIC STIMULATION

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Background: KCNJ2 mutations can cause a Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) phenotype, but the arrhythmia mechanism remains unknown. We previously reported KCNJ2 mutation R67Q from a patient with adrenergic-induced polymorphic and bidirectional ventricular tachycardia and created a R67Q knock-in mouse that mimics the patient phenotype with adrenergic-dependent bidirectional VT.
Objective: To elucidate the arrhythmic mechanism of KCNJ2 mutation R67Q in an in vivo model.
Methods: ECG at baseline and following administration of epinephrine and caffeine was performed in anesthetized WT-Kir2.1 and R67Q-Kir2.1 mice. Isolated ventricular myocytes were analyzed by voltage clamp, current clamp, and calcium imagaing using standard protocols.
Results: Following epinephrine and caffeine, R67Q-Kir2.1 mice had prolonged PR intervals compared to WT-Kir2.1. The corrected QT interval (QTc), lengthened in WT-Kir2.1 mice compared to baseline after epinephrine and caffeine. The QTc in R67Q-Kir2.1 mice was longer at baseline than WT-Kir2.1, increased after stimulation (NS) but WT-Kir2.1 QTc was significantly longer after stimulation. Baseline IK1 recorded from WT-Kir2.1 and R67Q-Kir2.1 VMs showed no significant difference but following isoproterenol (ISO), outward current at -50mV increased by 20.69 ± 3.69% in WT-Kir2.1 VMs, whereas R67Q-Kir2.1 decreased by 23.69 ± 8.27% (p<0.001). APD recorded from WT-Kir2.1 and R67Q-Kir2.1 VMs was similar at baseline at 4 and 6Hz. APD90 was prolonged in R67Q-Kir2.1 (47.15 ± 22.45s) VMs but not WT-Kir2.1 (25.7s) following ISO. Using ratiometric fura2-AM dye, baseline calcium in R67Q-Kir2.1 VMs was increased compared to WT-Kir2.1 (1.315 vs. 1.04 Fura Ratio) (p<0.05). Peak calcium did not increase in response to ISO in R67Q-Kir2.1 VMs (2.5 ± 0.08 to 2.75 ± 0.17) compared to WT-Kir2.1 (1.58 ± 0.16 to 3.72 ± 1.5) (p<0.05).
Conclusion: Our data indicate that the R67Q-Kir2.1 mutation results in adrenergic-dependent loss of outward IK1, increased baseline calcium and failure to augment calcium cycling under adrenergic stress. Loss of repolarization reserve combined with impaired calcium handling thus leads to triggered activity and arrhythmia susceptibility.