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was a prolongation of the interatrial conduction time (from ST to LA [39 ± 1.5 vs. 46 ± 1.6 ms; $p=0.005$]) as well as of the interatrial conduction of the atrial extra stimuli (95 ± 4 vs. 112 ± 5 ms; $p=0.023$). In 68% of the dogs after atrial pacing was interrupted, AF was easily inducible by programmed atrial stimulation and was sustained for up to 7 minutes (average 2.4 ± 2 min). Intense inflammatory infiltration as well as interstitial edema and bands of cellular contraction were observed in the subepicardium on optical microscopy. On electron microscopy, there was an intense myofibrillar disorganization and an increase of the mitochondria size.

Conclusions: a) rapid atrial pacing with alkaline battery for 60 min induces AF that lasts after pacing is ceased; b) it causes electrical and histological atrial changes that increase atrial vulnerability that facilitates sustained AF induction; c) this is an easy, effective and cheap technique for the experimental study of AF.

P320

Response of high-sensitive C-reactive protein to catheter ablation of atrial fibrillation and its relation with rhythm outcome

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Background: High-sensitive C-reactive protein (hs-CRP) has been linked with atrial fibrillation (AF) but its role in predicting AF recurrences after catheter ablation is controversial. This study investigated the possible association between hs-CRP as well as hs-CRP changes and rhythm outcome after AF catheter ablation.

Methods: We studied 68 consecutive patients with AF undergoing catheter ablation. hs-CRP levels were measured using commercially available assays before and 6 months after catheter ablation. Serial 7-day Holter ECGs were used to detect AF recurrences.

Results: Early AF recurrence (ERAF, within one week) was observed in 38%, while late AF recurrence (LRAF, between 3 and 6 months) occurred in 18% of the patients. None of the baseline clinical or echocardiographic variables was predictive of ERAF or LRAF. Baseline hs-CRP measured 2.07 ± 1.1 $\mu\text{g/ml}$ and was not associated with ERAF and LRAF. At 6 months, hs-CRP levels were comparable with baseline values (2.14 ± 1.19 $\mu\text{g/ml}$, $p=0.409$) and were also not related with LRAF. However, patients with LRAF showed an hs-CRP increase from 2.03 ± 0.61 to 2.62 ± 1.52 $\mu\text{g/ml}$ ($p=0.028$). Patients with an hs-CRP change in the upper tertile (> 0.2 $\mu\text{g/ml}$) had LRAF in 32% as opposed to 11% ($p=0.042$) in patients in the lower (< -0.3 $\mu\text{g/ml}$) or intermediate (-0.3 — 0.2 $\mu\text{g/ml}$) tertile.

Conclusions: Changes in hs-CRP but not baseline hs-CRP are associated with rhythm outcome after AF catheter ablation. This finding points to a link between an inflammatory response and AF recurrence in this setting.

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Electrophysiological characterisation of the porcine right and left ventricle using myocardial slices

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Introduction: Porcine hearts are commonly used in cardiovascular research to simulate the size and the functional properties of the human heart. However, some important aspects, such as the differences between the electrophysiological properties of the left (LV) and right ventricle (RV) have not been characterised.

The purpose of this study was to investigate and compare electrophysiological properties of the porcine LV and RV at the multicellular level, using myocardial slices.

Methods: Vibratome-cut myocardial slices (350 μm thick) were prepared from the sub-epicardial and mid-myocardial regions of the LV and RV of pig hearts.

Slices were electrophysiologically assessed during electrical stimulation using a multi-electrode array (MEA) system. Extracellular field potentials, recorded from 60 MEA microelectrodes, were processed to measure field potential duration (FPD) — an index of action potential duration — and conduction velocity (CV). Frequency-dependent changes of FPD were assessed by pacing slices at 0.5, 1 and 2 Hz. To assess the effect of the direction of propagation on FPD and measure longitudinal and transverse CV, slices were paced at 1 Hz within or perpendicular to fiber orientation.

Results: FPD was significantly shorter in slices from the RV versus slices from the LV when stimulated at 0.5 and 1 Hz, but was similar at 2 Hz (0.5 Hz: 295 ± 15 ms in RV, 354 ± 12 ms in LV, $n=5$, $p < 0.05$; 1 Hz: 273 ± 13 ms in RV, 341 ± 7 ms in LV, $n=5$, $p < 0.05$; 2 Hz: 230 ± 11 ms in RV, 267 ± 15 ms in LV, $n=5$, $p > 0.05$). All slices displayed rate dependence of FPD, with FPD prolongation at lower stimulation frequency in both RV and LV slices (ANOVA, $p < 0.05$ and $p < 0.01$, respectively). FPD/pacing cycle length relationship was steeper in LV than RV (mean lengthening: 88 ± 3 ms in LV, 66 ± 4 ms in RV, $n=4$, $p < 0.001$).

FPD was independent from the direction of propagation in both RV and LV slices (RV: 266 ± 32 ms & 273 ± 26 ms, LV: 344 ± 18 ms & 359 ± 24 ms, during longitudinal and transversal pacing respectively).

Average CV was 37 ± 5 cm/s in RV ($n=4$) and 24 ± 4 cm/s in LV slices ($n=4$). Longitudinal CV was faster than transversal CV in RV and LV slices (RV: 74 ± 9 & 19 ± 3 cm/s; LV: 46 ± 14 & 14 ± 1 cm/s).

Conclusions: We report significant electrophysiological differences between RV and LV in the pig heart, similar to that previously reported for the dog and human heart. These differences should be taken into consideration when assessing electrical remodelling and the effect of pharmacological drugs in porcine models of ischaemia and heart failure.

P322

The role of RGS4 in the electrophysiology of atrial cardiac myocytes

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Purpose: In an accompanying abstract we show that RGS4 deficient mice are predisposed to atrial fibrillation. We thus investigated potential electrophysiological substrates that might underlie this observation.

Methods: In vitro electrophysiological studies were performed using single atrial cardiomyocytes isolated from RGS4 $-/-$ and RGS4 $+/+$ littermates by enzymatic digestion. Cells were studied using the patch-clamp technique. Data are compared using Student t-test (* $p < 0.05$, ** $p < 0.001$).

Results: Paradoxically, atrial cells isolated from RGS4 $-/-$ animals show more rapid activation and deactivation kinetics of G-protein gated inward rectifying potassium (GIRK) current (lag + TTP $0.79 \pm 0.06^{**}$ ms, tau ac $218 \pm 22^{*}$ ms, tau inac $2056 \pm 250^{*}$ ms) than cells from RGS4 $+/+$ animals (lag + TTP 1.35 ± 0.12 ms, tau ac 370 ± 45 ms, tau inac 3512 ± 580 ms). Furthermore, the action potential parameters, measured as total depolarisation, APD50, APD90 and total duration, were not significantly different between the two groups. Finally, we examined rate dependence of action potential duration by constructing single-cell restitution curves. The slope of the restitution curves in its linear phase (30 to 70 ms) was steeper in the RGS4 $-/-$ mice (slope 1.07 ± 0.09) compared to RGS4 $+/+$ mice (slope 0.59 ± 0.05); this is a potential proarrhythmic substrate.

Conclusions: This study shows that deletion of RGS4 in mice leads to paradoxical changes in GIRK channel kinetics and no changes in action potential duration. However, the mice do have potentially proarrhythmic changes in restitution properties and this might underlie a predisposition to atrial fibrillation.

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RGS 4 KO mice are predisposed to atrial fibrillation (AF) and show disrupted sympathovagal balance, making RGS 4 a potential therapeutic target for AF

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Purpose: The electrophysiological basis of AF is not understood. Acetylcholine from the vagus nerve binds to muscarinic receptors causing dissociation of α - $\beta\gamma$ subunits of the inhibitory G-protein, activating G-protein gated inward rectifying potassium (GIRK) channels. The result is bradycardia, shortening of the APD and propensity for AF. Gi2 is the predominant cardiac G-protein and RGS 4 terminates Gi2.

Methods: In vivo studies were performed in RGS 4 KO and wild type (WT) mice. Electrophysiological studies (EPS) were undertaken in 8-week old anaesthetised mice with a 1.1F catheter inserted into the right atrium via the right internal jugular vein. ECG and EP parameters were recorded. AF induction was attempted with burst pacing for 25 seconds and repeated after injection of 0.5mg/kg carbachol. Telemetry probes were inserted intra-abdominally into 12-week old mice. After a two week recovery period, ECGs were recorded in conscious mice for 48 hours and studied for AF and atrial ectopics (AE). An ECG post carbachol injection was analysed. Heart rate variability (HRV) was measured from 12 to 2pm, when murine vagal tone is highest.

Results: ECG and EP parameters were comparable. RGS 4 KO mice developed AF (76.9 vs 38.5%, $P=0.04$). Mean duration of AF for WT was 70.2 ± 29.8 (1.9 to 770) versus 222.6 ± 164.9 (1 to 2867.4, $P=0.69$) seconds for RGS 4 KO mice. Although this did not reach significance, there was a trend to a longer duration of AF in RGS 4 KO mice. Carbachol did not increase AF in KO or WT ($P=0.28$), although a trend was apparent. Conscious RGS 4 KO mice were tachycardic (634.3 ± 52.6 vs 562.6 ± 36.3 bpm, $P=0.03$), and had an enhanced bradycardic response to carbachol (288.2 ± 37.9 vs 524.3 ± 24.2 bpm, $P=0.02$). ECG and HRV parameters of WT and RGS 4 KO mice without and with carbachol were comparable ($P > 0.05$). Carbachol-treated RGS 4 KO mice had disrupted HRV ($P=0.008$) compared to RGS 4 KO mice alone. This was not seen with WT treated with carbachol ($P > 0.05$). Carbachol-treated RGS 4 KO mice had several pauses, 2 to 7 AE, but no AF. WT mice treated with carbachol had a maximum of one AE. Without carbachol, no AE or AF was seen in RGS 4 KO or WT mice.

Conclusions: This is the first report of AF and HRV in the RGS 4 KO mouse in vivo. These are tachycardic, have an increased bradycardic response to carbachol and disrupted HRV. This signifies altered sympathovagal balance.

Mechanistic studies are underway to understand the electrophysiological basis of AF in the RGS 4 KO mouse. RGS 4 is a potential therapeutic target in the treatment of AF.

P324

Regulation of scn5a by microRNAs: miR-219 modulates scn5a transcript expression and cardiac rhythm in mice

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The human action potential is initiated by a fast-activating fast-inactivating Na^+ current modulated by Nav1.5 channel encoded by SCN5A, in association with its $\beta 1$ subunit encoded by SCN1B. The role of Nav1.5 in the etiology of many cardiac diseases strongly suggests that proper regulation of cell biology and function of the channel is critical for normal cardiac function. Hence, it is not surprising that numerous recent studies have focused on the regulatory mechanisms of its biosynthetic and degradation processes as well as its subcellular localization. However, the post transcriptional regulation by microRNAs remains unexplored. In this study we investigate the role of microRNAs on the post-transcriptional regulation of SCN5A/Nav1.5.

Functional studies in HL1 cardiomyocytes and luciferase assays in fibroblasts demonstrate that SCN5A is directly (miR-98, miR-106, miR-200, miR-219) and indirectly (miR-125 and miR-153) regulated by multiple microRNAs displaying distinct time-dependent profile and differentially expressed during cardiogenesis. Among all, only miR-219 increased Scn5a expression level, impaired Nav1.5 subcellular localization and altered contraction rhythm of HL1 cardiomyocytes. Transcription inhibition experiments deduced that miR-219 stabilized Scn5a transcript increasing thus Nav1.5 protein quantity and the current amplitude and shifting the voltage-dependent activation of INa in HL-1 cells as recorded by Patch-Clamp. Co-transfection experiments, demonstrated that miR-219 and miR-200 have an opposite, independent and additive effect modulating Scn5a gene expression. In vivo miR-219 injection did not affect normal mouse cardiac rhythm but it rescued it from the bradycardic effects of Flecainide intoxication. Chimeric miR-219 RNA molecules fine-tune the

modulation of Scn5a as demonstrated in HL1 cells and confirmed by luciferase assays. Loss of function miR-219 molecules injection increase the RR interval and correct the bradycardiac effect of flecainide once administrated together as demonstrated by ECG recording in mice.

This study demonstrates the involvement of multiple microRNAs on the regulation of SCN5A. Particularly, miR-219 increased Nav1.5 protein quantity by stabilizing its transcript increasing thus the cellular sodium gating. In vivo, miR-219 over-dose did not affect the normal heart rhythm but it modulated the effect of flecainide. Fine-tune modulation can be achieved by discrete altering a reduced number of nucleotides within the miRNA molecules. Thus, our data suggest that microRNAs, such as miR-219 constitute promising therapeutical tools to treat sodium cardiac arrhythmias.

P325

Anisotropic extracellular resistances influence action potential upstroke within subepicardial layers in the intact isolated rabbit heart

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Upstroke velocity of the cardiac action potential (AP) is an important index of tissue excitability. Previous studies have demonstrated that in epicardial tissue preparations, surface AP upstroke velocity is greater when electrical propagation is in the transverse direction relative to the longitudinal. However, the electrophysiological basis for these differences is debated. Furthermore, no previous studies have examined AP upstroke velocity in the transmural axis during the normal endo-epicardial activation sequence. In this study we report transmural AP characteristics from layers up to 600µm below the epicardial surface and compare the data with a computational model of cardiac electrical propagation.

Hearts from male New Zealand white rabbits were Langendorff-perfused at 37°C and paced at a cycle length of 300ms. Preparations were loaded with the ratiometric dye di-4-ANEPPS and optical APs were recorded using both widefield epifluorescence and two-photon (2P) microscopy. BDM (10mM) and blebbistatin (10µM) were used to minimise motion artefacts.

During normal endo-epicardial activation (right-atrial (RA) pacing), mean 10-90% AP rise times for 2P recordings prolonged steadily with increasing tissue depth (3.4 ± 0.2 ms vs. 6.8 ± 0.9 ms; 50 vs. 500µm from surface, $P < 0.05$, $n=11$) while epifluorescence recordings demonstrated consistently longer rise times (7.5 ± 0.4 ms, $n=11$). This prolongation of AP rise time was not observed beyond 500µm below the surface. Surface microelectrode recordings revealed significant differences in rise times between RA, transverse and longitudinal activation (3.6 ± 0.6 , 6.1 ± 0.6 and 8.7 ± 1.2 ms; RA vs. transverse vs. longitudinal, $n=5$), consistent with previous studies. In contrast to RA pacing, AP rise times at depth did not significantly change from those recorded 50µm below surface with either transverse or longitudinal conduction, suggesting the effect is not an optical artefact. Comparison of these data with the output from an augmented monodomain cardiac tissue model revealed that the inclusion of an extracellular resistance compartment along the fibre axis and a lower resistance component at the tissue surface as described in the literature reproduced the increasing rise time with depth seen experimentally (0.9 vs. 2.0 ms; 100µm deep vs. 500µm deep), and resulted in the appearance of differences in AP rise time between transverse and longitudinal epicardial activation. Collectively these data suggest that AP upstroke characteristics are influenced by the anisotropic extracellular resistances within the ventricle and the low resistance pathway at the surface.

P326

Ventricular remodeling in a murine model of cardiac selective and inducible deletion of HCN4 gene

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Purpose: Heart rate disorders are responsible for ventricular remodeling and enhanced susceptibility to cardiac arrhythmias. In experimental bradycardia electrical remodeling of cardiac myocytes (CM) includes a reduction of the delayed rectifier K⁺ currents, which are responsible for action potential repolarization delay and increased propensity to develop potentially lethal arrhythmias.

The aim of this study was to evaluate ventricular remodeling occurring in a novel model of bradycardia (Baruscotti M., PNAS 2011), based on inducible and cardiac specific ablation of HCN4 gene encoding for the f current in sinoatrial node cells.

Methods: Cardiac HCN4 knockout was induced by i.p. injections of tamoxifen (200 mg/Kg) for 5 consecutive days in adult HCN4lox/lox;Cre mice. Control mice underwent the same treatment. Heart rate was monitored daily. Electrophysiological study was performed using the single-cell patch-clamp technique. Gene expression was studied by quantitative RT-PCR on ventricular tissue.

Results: Tamoxifen injections produced a progressive development of bradycardia that reduced heart rate by 25% in the HCN4lox/lox;Cre mice but not in control animals. Bradycardia was associated to 75% reduction of cardiac HCN4 mRNA level. Patch-clamp recordings on ventricular CM showed a marked prolongation of action potential duration in HCN4lox/lox;Cre bradycardiac mice with respect to control animals (80 ± 0.4 vs 30 ± 0.1 ms at 90% of repolarization). Quantitative mRNA expression of subunits underlying repolarizing currents showed downregulation of the inward-rectifier K⁺ channel subunit Kir2.1 (-60%), the slow delayed-rectifier K⁺ channel subunit Kcnq1 (-50%), and the transient outward K⁺ channel subunits Kv4.2 (-80%), Kv4.3 (-50%) and KChIP2 (-90%) in HCN4lox/lox;Cre bradycardiac mice with respect to control animals. The rapid delayed-rectifier K⁺ channel subunit erg and the regulatory subunit MinK were unchanged, while the regulatory subunit Mirp-1 was upregulated.

Conclusions: The HCN4lox/lox;Cre transgenic mouse represents an innovative and reliable model of inducible bradycardia useful to study rate-related electrical remodeling of CM. In accordance to different model (Gross GJ, J Cardiovasc Electrophysiol 2006), we identified a prominent repolarization delay of ventricular action potential as major electrophysiological modification in bradycardiac HCN4lox/lox;Cre mice. Expression studies point to a crucial role for the downregulation

of the inward-rectifier, the slow delayed-rectifier, and the transient outward K⁺ channels to underlie electrical remodeling in bradycardiac HCN4lox/lox;Cre mice.

P327

The KATP channel subunit, Kir6.2, is regulated by a Ca²⁺-dependent isoform of Protein Kinase C

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Purpose: ATP-sensitive potassium (KATP) channels couple cell metabolism to membrane potential in many cell types to control vascular smooth muscle tone and excitability in neurons and muscle. Protein Kinases are involved in the modulation of channel activity and are particularly important in regulating smooth muscle tone. In previous work, Kir6.1/SUR2B channels have been shown to be inhibited by Protein Kinase C (PKC) whilst Kir6.2/SUR2B channels are activated or have no response. In this study we show that Kir6.2/SUR2B channels can be inhibited by PKC in a Ca²⁺-dependent manner and identify a specific site for potential PKC phosphorylation.

Methods: Human embryonic kidney cells (HEK293) stably transfected with SUR2B and Kir6.1 or Kir6.2 were subjected to Rubidium flux and whole cell patch-clamp electrophysiology. An in vitro phosphorylation assay and phosphopeptide mapping were used to determine potential PKC phosphorylation sites.

Results: Rubidium flux experiments with Kir6.1/SUR2B and Kir6.2/SUR2B showed that both were inhibited by the activation of PKC. In the presence of the Ca²⁺ chelator, BAPTA-AM, inhibition by PKC was observed only in Kir6.1/SUR2B cells suggesting that the PKC effect on Kir6.2/SUR2B is Ca²⁺ dependent. This was confirmed using whole cell patch clamp recordings, where KATP current inhibition was only seen when free intracellular Ca²⁺ was 100 nM or higher. A serine residue at position 372 was identified by phosphopeptide mapping to be a potential PKC phosphorylation site. In vitro phosphorylation was reduced when Serine 372 was mutated to an alanine residue compared to wild type. Currents evoked from cells expressing Kir6.2-S372A/SUR2B were not inhibited by PKC activation in the presence of 300nM free intracellular Ca²⁺.

Conclusions: The results in this study show that Kir6.2/SUR2B is regulated by PKC and that this is Ca²⁺-dependent. In addition, Serine 372 in the Kir6.2 subunit has been identified as a novel site for PKC phosphorylation.

P328

Does cardiomyocyte specific deletion of plasma membrane calcium ATPase 1 lead to altered microRNA expression in heart failure?

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Background: MicroRNAs are newly discovered small non-coding RNAs that can regulate hypertrophic gene expression at the post-transcriptional level through targeted mRNA 'silencing' in heart failure. Abnormal Ca²⁺ handling impairs cardiac function and our research group has shown that deletion of the calcium extrusion pump, plasma membrane Ca²⁺ ATPase1 (PMCA1), leads to dilated cardiomyopathy and heart failure. This study aims to determine whether deletion of PMCA1 leads to altered microRNA expression in heart failure.

Methods and Results: PMCA1 cardiomyocyte-specific knockout (PMCA1cko) mice were generated using Cre/LoxP technology. A microarray plate revealed a number of microRNA changes in PMCA1cko mice (n=5) compared to PMCA1lox/lox (controls) (n=7) which, by reverse transcription and qPCR, confirmed a marked down-regulation ($p < 0.05$) of miRNAs let7e (75%), let7i(50%), 101(76%), 101a(61%), and 93(57%). PMCA1cko and PMCA1lox/lox mice were subjected to haemodynamic stress by transverse aortic constriction (TAC) inducing cardiac hypertrophy. After TAC, PMCA1lox/lox mice demonstrated a downregulation of miR-101a by 50% ($p=0.06$) compared to sham operated PMCA1lox/lox mice but there was an overexpression by 61% (< 0.05) in the PMCA1cko mice (n=3). Rcan1.4, a marker of NFAT activity, was upregulated ($> 300\%$) in PMCA1cko mice (n=7) under basal conditions.

Conclusions: Deletion of PMCA1 has been shown to directly or indirectly regulate transcription of microRNAs let7e, 7i, 101, 101a and 93, whilst under pathological hypertrophic conditions, deletion of PMCA1 leads to upregulation of miR101 and 101a. Initial studies suggest MicroRNAs 101 and 101a may regulate pathological hypertrophy through suppression of the calcineurin-NFAT pathway, one of the most well characterised pathways in heart failure.

Excitation-contraction coupling, Cardiomyopathy

P329

Chronic stress is required to evoke left ventricular dilation in the E361G mouse model of dilated cardiomyopathy

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Around 20-35% of cases dilated cardiomyopathy (DCM) are due to genetic mutations. A mutation in cardiac actin—ACTC E361G, has been shown to cause DCM in humans. This mutation has been stably expressed at around 50% of total actin in a transgenic (TG) mouse-line. Previous work on this TG mouse has shown that the molecular phenotype constitutes an uncoupling of Ca²⁺-sensitivity from the level of troponin I phosphorylation that may cause a blunting of the lusitropic response to stress, with consequent heart failure (HF). Despite an absence of whole animal phenotype under basal conditions, E361G mice show a blunted response to acute dobutamine treatment which would lead to a reduced cardiac reserve. We hypothesise this reduced cardiac reserve may cause the mice to develop HF when exposed to chronic stress. TG and NTG mice were chronically exposed to angiotensin II (1.4mg/kg body weight/day) for 4 weeks using subcutaneously implanted osmotic mini-pumps. Cardiac parameters were measured using echocardiography prior to

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