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n=3, P=0.0008). To account for any strain difference, this comparison was repeated in 129SV strains which produced a similar effect (ca. 2.3-fold increase in atrial NAD(P)H oxidase activity with apelin gene deletion versus wildtype littermate controls, n=3, P<0.05). In contrast, NAD(P)H oxidase activity in human right atrial myocytes isolated from RAA in NSR showed no significant change in superoxide release with the topical addition of 5nM apelin. In isolated rat neonatal cardiomyocytes, the expression of APJ, the endogenous receptor for apelin, revealed reductions in both mRNA and protein following overnight culture. Despite variable effects of apelin on oxidative stress, infusion of 200nM apelin increased conduction velocity by ca. 31% in a cultured rat neonatal cardiomyocyte preparation (in m/s: 2.1±0.08 versus 1.6±0.04; n=8, P=0.006).

**Conclusions:** These findings suggest that apelin exerts direct functional effects on conduction velocity and modulates atrial NAD(P)H oxidase activity which may play an important role in the redox regulation of atrial electrophysiology and vulnerability to human AF.

### 5155 Atrial sources of reactive oxygen species vary with the duration and substrate of atrial fibrillation: implications for the antiarrhythmic effect of statins



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**Background:** An altered nitric oxide (NO)-redox balance has been implicated in the pathogenesis of atrial fibrillation (AF). Statins inhibit NADPH oxidases and decrease the occurrence of post-operative AF but are not as effective in the secondary prevention of AF; the molecular mechanisms underlying these inconsistent findings are poorly understood.

**Results:** By using goat models of pacing-induced AF (n=34) and of atrial structural remodeling secondary to atrioventricular block (AVB, n=9), and right atrial samples from 130 patients undergoing cardiac surgery (72 in SR before and after surgery, 32 developed post-operative AF and 26 had long-standing permanent AF), we found that the mechanisms responsible for the NO-redox imbalance in AF differ between left and right atria and with the duration and substrate of AF. Rac1 and NADPH oxidase activity and the protein level of the cytochrome-forming subunits of the oxidase (i.e., NOX2 and p22phox) were significantly increased in the left atrial myocardium of goats after 2 weeks of AF and in right atrial samples from patients who developed AF after cardiac surgery, in the absence of differences in neutrophils infiltration (as assessed by CD18 staining and immunoblots). By contrast, in the presence of long-standing AF in goats and humans or of AVB in goats, expression of mitochondrial oxidases, ROS production and "uncoupled" NOS activity (secondary to reduced atrial BH4 content or increased arginase activity) accounted for the bi-atrial increase in superoxide production. Under these conditions, NADPH oxidase activity and protein and gene expression of p22phox and NOX2, NOX4 and NOX5 did not differ from SR. The increase BH4 oxidized products (BH2) that accompanied the increase in ROS production from NADPH oxidases was not sufficient to cause NOS uncoupling, in the absence of a significant reduction in tissue BH4 concentration. The latter was only observed in the presence of long-standing AF. Ex vivo atorvastatin (50 µg/L for 30 min) caused a mevalonate-reversible inhibition of atrial Rac1 activity, p67phox and p47phox membrane translocation and superoxide production in patients who developed AF after cardiac surgery but it did not affect atrial superoxide production, NOS uncoupling or BH4 content in patients with permanent AF.

**Conclusions:** These findings indicate that upregulation of NADPH oxidases in the atrial myocardium is an early but transient event in the natural history of AF. Changes in the sources of ROS with atrial remodeling may explain why statins are effective in preventing the development of AF but not in its management.

### 5156 (W) Downregulation of Cx40-protein in Cx40A96S point-mutated mice results in atrial conduction disturbances and prolongation of atrial fibrillation



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**Introduction:** We analyzed the germ-line mutation Cx40A96S of the connexin Cx40 gene as a previously described mouse model for human idiopathic atrial fibrillation (AF). We now aimed at the evaluation of AF predisposing pathomechanisms associated to this point-mutation.

**Methods:** In mice heterozygous (Cx40A96S/Het) and homozygous (Cx40A96S/Hom) for the Cx40A96S mutation and wild type littermates (WT), we performed telemetric Holter ECG recordings, in vivo (transvenous catheterization) and ex vivo (epicardial mapping) electrophysiological investigations (EPI) as well as rt-PCR and western blotting for gene expression analyses.

**Results:** Microinjection of Cx40A96S expressing HeLa cells revealed significantly reduced diffusion of neurobiotin, resulting in a 96% loss of coupling properties in all investigated clones as compared to Cx40 WT. In the Holter-ECGs, we saw significantly longer P-wave durations, PQ-Intervals and QRS-intervals in the homozygous (n=5) and heterozygous (n=5) transgenics versus WT (n=3). In vivo EPI (n=13 per group) showed that inducibility of long-lasting episodes was sig-

nificantly elevated in the Cx40A96S/Hom and Cx40A96S/Het (animals with AF episodes >30sec per group: 75% in Cx40A96S/Hom and 78% in Cx40A96S/Het versus 20% in WT; >60sec: 70% and 68% vs. 17% in WT; p<0.05). 2 of 15 Cx40A96S/Het and 3 of 14 Cx40A96S/Hom developed sustained AF >10min vs. none in the WT. Epicardial mapping showed significantly reduced atrial conduction velocities in the mutants. Homozygous mutant mice developed reduced atrial refractory periods (ARP: 25.7±6.1ms versus 19.5±5.9ms in WT). Semiquantitative rt-PCR (n=4 per group) revealed no relevant changes of RNA expression for the voltage gated ion channels Nv1.5, Kv1.5 and Kv4.2, and Kir2.1. rt-PCR and western blotting showed age dependent consecutive reduction of Cx40 mRNA and protein expression in homozygous Cx40A96S mice.

**Conclusions:** The Cx40A96S mutation leads to a severe impairment of the channel function in Cx40 containing gap junctions and loss of Cx40 protein in aging mice. Cx40A96S pointmutated mice show a perpetuation of induced AF episodes following atrial burst stimulation due to shortening of the ARP and severe atrial conduction disturbances, explainable a) by loss of function of the mutated Cx40 in homozygous younger and b) additional loss Cx40 protein in older mice.

### 5157 Effects of beta-adrenoceptor stimulation on human atrial voltage-dependent K<sup>+</sup> currents



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**Purpose:** The electrophysiological effects of β-adrenergic stimulation on atrial myocytes obtained from patients in sinus rhythm (SR) and chronic atrial fibrillation (CAF) have not been compared until yet, even when it has been proposed that β-adrenergic stimulation has profound influence in the genesis and maintenance of atrial fibrillation. Therefore, we analyzed the effects produced by isoproterenol (Iso, 1 nM), a β-adrenoceptor agonist, on the transient outward (I<sub>to</sub>), the ultrarapid (I<sub>Kur</sub>) and the slow delayed rectifier (I<sub>Ks</sub>) K<sup>+</sup> currents recorded in human atrial myocytes obtained from SR and CAF patients.

**Methods:** Currents were recorded in enzymatically dissociated myocytes obtained from right (RAA) and left (LAA) atrial appendages from SR and CAF patients using the patch-clamp technique.

**Results:** In SR myocytes Iso slightly inhibited the I<sub>to</sub> (by 10.1±6.6% in RAA and 15.8±3.3% in LAA myocytes at +30 mV, P>0.05). In CAF myocytes, the Iso-induced I<sub>to</sub> inhibition reached a 24.9±6.2% in RAA (P<0.05 vs SR) and was even significantly greater in LAA (36.5±4.9%) cells. In RAA and LAA myocytes from SR and CAF patients, I<sub>Kur</sub> was not significantly modified. Moreover, in SR myocytes Iso did not modify the I<sub>Ks</sub> (4.5±2.6% augmentation in RAA and 6.6±1.4% in LAA myocytes at +30 mV) which was almost undetectable (25.0±4.5 pA at +30 mV). Conversely, as we previously demonstrated I<sub>Ks</sub> amplitude significantly increased in both RAA and LAA CAF myocytes (59.7±8.3 pA at +30 mV, P<0.01 vs SR), and, under these conditions β-adrenergic stimulation increased the I<sub>Ks</sub> by 51.8±6.2% in RAA and by 78.0±12.4% in LAA myocytes (P<0.05 vs CAF RAA). Moreover, in both SR and CAF myocytes atenolol, a selective β<sub>1</sub>-adrenoceptor antagonist, abolished Iso effects on I<sub>to</sub> and I<sub>Ks</sub>. Furthermore, a real-time q-PCR analysis demonstrated that the β<sub>1</sub>-adrenoceptor mRNA expression was significantly higher in CAF than in SR samples and that this CAF-induced up regulation was significantly more marked in the LAA than in the RAA.

**Conclusions:** We concluded that CAF potentiates the β<sub>1</sub>-adrenergic effects on I<sub>to</sub> and I<sub>Ks</sub> an effect produced by means of an up-regulation of the β<sub>1</sub>-adrenoceptors which was greater in LAA than in RAA myocytes. The CAF-induced increase in the I<sub>Ks</sub> amplitude and in the β<sub>1</sub>-adrenergic stimulating effects could contribute to the shortening in the duration of the atrial action potential and refractoriness observed in CAF.

### 5158 Frequency-dependent protective role of simvastatin in electrophysiological remodeling of AF and in terminating experimental AVRT mediated via KATP opening and endogenous Nitric Oxide (NO)



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**Purpose:** The purposes of the present study are to determine (1) whether Simvastatin modifies the rate-dependent AV nodal conduction and refractoriness to terminate experimental AVRT and AF and (2) to determine protective signaling mechanism of Simvastatin via opening of KATP, blocking of MPTP and inhibiting endogenous Nitric Oxide (NO).

**Methods:** Selective stimulation protocols and mathematical formulations were used to assess basic AV-nodal conduction and refractoriness (AVERP & AVFRP) and to induce simulated experimental AVRT model in various cycle lengths in isolated perfused rabbit AV nodal preparation in 5 groups (N=45). AF was simulated by high-rate atrial pacing with random coupling intervals (75-150ms). The stimulation protocols were carried out during control conditions and in the presence of various concentrations of Simvastatin (0.5, 1, 5, 10 µM), Cyclosporine (1 µM), Glibenclamide (5 µM), L-NAME (50 µM).

**Results:** Simvastatin in concentration-dependent manner prolonged nodal ERP, FRP and Wenckebach cycle length. Simvastatin suppressed simulated AV reentrant tachycardia in concentration-dependent manner by increasing the positive

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