and this arrhythmia has been associated to alterations in intracellular calcium handling. The purpose of this study was to test the hypothesis that ageing per se alters calcium handling in human atrial myocytes.
Methods: Whole membrane currents were measured in the perforated patch configuration in human atrial myocytes from 74 patients free of atrial fibrillation and with normal left atrial size. Patients were categorized as young (<55 years, n=21), middle aged (between 55 and 75 years, n=42), and old (>75 years, n=11). Protein expression was determined with Western blot technique in right atrial samples from 7 young and 7 old patients.
Results: The expression of the alpha-subunit of the L-type calcium channel was lower in old patients, and statistical analysis showed that aging progressively and significantly ($p<0.01$) reduced the L-type calcium current (ICa) amplitude, even when the effects of age, sex, ACE inhibitors, beta-blockers, angiotensin receptor blockers, calcium antagonists, and left ventricular ejection fraction were taken into account. ICa decreased from 2.4±0.3 pA/pF in the young to 1.2±0.3 pA/pF in the old patient group ($p<0.01$). The current-voltage relationship was not affected by age but fast ICa inactivation was significantly slower in old patients. The tau for ICa inactivation was 20.9±1.9 ms in old vs. 14.5±0.9 ms in young patients ($p<0.01$). Similarly the tau for slow ICa inactivation was 120±12 ms in old vs. 73±3 ms in young patients ($p<0.001$). The caffeine releasable sarcoplasmic reticulum (SR) calcium content also decreased with age (from 10.1±0.8 amol/pF in young to 7.3±0.7 amol/pF in old patients, $p<0.05$). This was accompanied by a significant decrease in the expression of both SERCA2 and calsequestrin-2 protein levels. By contrast, age did not affect the frequency of spontaneous SR calcium release induced transient inward currents (1.4±0.3 in young vs. 1.1±0.8 events/min in old patients, p=0.5). Moreover, age did not affect the ability of myocytes to maintain a uniform beat-to-beat response when the stimulation frequency was increased.
Conclusions: Ageing is associated with depression of L-type calcium channel expression and current amplitude as well as a reduction of the SR calcium content linked to lower SERCA2 and calsequestrin-2 expression in human atrial myocytes. These alterations may blunt atrial contraction and relaxation.

P5019 | BENCH
Human cardiac Kir2.1, but not Kir2.3, channel expression is regulated by Nav1.5

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Purpose: Human cardiac inward rectifying K+ current (IK1) is mainly carried by Kir2.1 channels in ventricular cells, whereas the relative contribution of Kir2.2 and Kir2.3 seems to be greater in atrial cells. IK1 plays a key role in the control of resting membrane potential and action potential duration and, thus, excitability and refractoriness. It has been described that an increase in the expression of sodium channels (Nav1.5) increases Kir2.1 expression, a modulation that involves synapse-associated protein 97 (SAP97). SAP97 binds to the PDZ domain of the channels whose sequence in Kir2.1 and 2.2 channels is Ser-Glu-Ile, while in Kir2.3 is Ser-Ala-Ile. Thus, we analyzed whether Kir2.1 and Kir2.3 channel activities are modulated differentially by the presence of Nav1.5.
Methods: Kir2.x currents (IKir2.x) were recorded by using the patch-clamp technique in CHO cells transiently transfected with wild-type (WT) and mutant Kir2.x and Nav1.5 channels (1:1 ratio).
Results: In the presence of WT Nav1.5, IKir2.1 density was significantly increased compared to that in the absence of Nav1.5, both at potentials negative (-199±22 vs -121±12 pA/pF at -120 mV, $n>24$, $P<0.01$) and positive (19.7±4.5 vs 8.9±1.2 pA/pF at -50 mV) to the K+ reversal potential. Conversely, cotransfection of Nav1.5 neither with Kir2.3 channels alone nor with Kir2.1 plus Kir2.3 channels did modify IKir2.3 or IKir2.1/2.3 density ($n>16$). Importantly, when Glu426 of Kir2.1 was replaced by Ala (p.E426A), the increase induced by coexpression of Nav1.5 was abolished at all membrane potentials. Coexpression of Nav1.5 with p.A444E Kir2.3 channels markedly increased IKir2.3 density (-44.0±5.8 pA/pF at -120 mV, $n>12$, $P<0.01$). The increase of IKir2.1 and p.A444E IKir2.3 density was not observed when they were cotransfected with two Brugada syndrome-associated SCN5A mutations (p.D1690N and p.D1816VfsX7) that severely impair Nav1.5 channel trafficking.
Conclusions: Only expression of Kir2.1 homotetramers is increased by their coexpression with Nav1.5 channels. The specificity of the effect is determined by Glu426 in the PDZ domain of Kir2.1 and could lead to differential electrophysiological effects on atrial and ventricular cells on patients carrying SCN5A mutations.

P5020 | BENCH
Loss of dihydrolipoylsuccinyltransferase (DLST) function leads to defective energy production and severe bradycardia in vivo

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It is well known that mitochrondria play a pivotal role for the energy supply of cells. In particular, the main energy equivalent adenosintriphosphate (ATP) is provided by the citric acid cycle which is localized in mitochondria. Mitochondriopathies in humans lead to myopathies, various cardiac disorders including arrhythmias and affect the central nervous system. However, due to early lethality and few reported cases, the disease remains obscure.
By the means of a large-scale ENU-mutagenesis screen, we isolated the recessive zebrafish mutant Schneckentempo. The zebrafish embryos exhibit a bradycardiac heart rhythm (99 beats per minute vs. 140 beats per minute) and pericardial edema whereas heart development appears to be normal. By positional cloning, gene knock-down analyses by Morpholino-modified antisense oligonucleotides and rescue experiments we determined that the Schneckentempo phenotype is caused by a splice-site mutation leading to a frame shift and a premature stop in the dihydrolipoylsuccinyltransferase (DLST) gene. This enzyme is part of the α-ketoglutarate dehydrogenase complex of the citric acid cycle in mitochondria involved in energy production. Interestingly, we were able to show that in Schneckentempo mutant embryos the ATP content is significantly reduced, suggesting that reduced energy supply is the molecular cause for the observed bradycardiac phenotype in Schneckentempo mutants. To validate these findings, we performed targeted knock-downs of, oxoglutaratdehydrogenase and dihydrolipoyldehydrogenase, two additional and essential components of the α-ketoglutarate dehydrogenase complex. Intriguingly, we find that the knock-down of each enzyme of the α-ketoglutarate dehydrogenase complex, as well as the pharmacological blockade of the citric acid cycle is accompanied by significantly reduced ATP production and the development of severe bradycardia in these embryos. Therefore, we show here for the first time, that loss of DLST leads to defective energy production and severe bradycardia in vivo.

P5021 | BENCH
Acetylcholine analogue mimics the protective effect of cardiac vagal nerve stimulation on ventricular fibrillation threshold

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Purpose: Implantable cardiac vagus nerve stimulators are a promising new treatment for heart failure, which may improve both quality of life and ejection fraction. Animal studies also suggest an anti-fibrillatory effect of stimulating the cardiac vagus nerve that may involve a nitric oxide (NO) dependent pathway, although the exact site of action in the cardiac-neural axis is still debated. We investigated whether carbamylcholine (CCh), a stable analogue of the neurotransmitter acetylcholine, can mimic the effect of vagus nerve stimulation on ventricular fibrillation threshold (VFT), and whether this mechanism is dependent on muscarinic receptor stimulation and/or generation of NO.
Methods: Experiments conformed to "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1996) and the Animals (Scientific Procedures) Act 1986 (UK). Hearts were isolated from adult male Sprague Dawley rats (300-350g) and Langendorff perfused with tyrode solution in constant flow mode (11ml/min, baseline perfusion pressure 54.75±7.27mmHg, LV developed pressure 77.5±5.6mmHg, heart rate 282.8±3.4bpm, n=6). VFT was reproducibly determined by pacing at a fixed cycle length (150msec) for 20 beats followed by a 5sec 50Hz burst at increasing current amplitude (mA) until sustained VF was induced. VF was cardioverted to sinus rhythm by perfusion with 1ml of high concentration potassium chloride solution (50mmol/L). All data are presented as mean±SEM with ANOVA for multiple comparisons.
Results: CCh (200nM, n=9) significantly ($p<0.05$) reduced baseline heart rate from 292±8 to 224±6bpm). [In paced hearts] Independent of this heart rate change, CCh also caused a significant increase in VFT similar to vagus nerve stimulation that could be reversed with washout of the drug (control 1.5±0.25 vs. CCh 2.4±0.4 mA vs. wash out 1.14±0.18 mA). The effect of CCh on VFT was completely abolished by atropine (10μM, n=4) or the non-specific competitive NOS inhibitor N-omega-nitro-L-arginine (L-NA 100μM, n=6). The inhibitory action of L-NA could be reversed by the addition of the NOS substrate L-arginine (5mM). The NO donor sodium nitroprusside (SNP 10μM, n=8) mimicked the effects of CCh and significantly increased VFT (baseline 1.4±0.125 vs, SNP 2.9±0.83 vs. wash out 1.18±0.23 mA).
Conclusions: These data demonstrate that the protective effect of cardiac vagal nerve stimulation on VFT is mimicked by CCh in the rat. Muscarinic receptor stimulation and the generation of NO appear to be involved in mediating this protective effect, presumably via a pre-synpatic and post synaptic coupled pathway.

P5022 | BENCH
The electrocardiographic T-wave is mainly explained by temporal repolarization differences along various anatomical axes

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Purpose: T-wave abnormalities are used to identify risk for life-threatening arrhythmias. However, the exact genesis of the T-wave is still unclear. Therefore, we conducted an experimental study to correlate the ventricular repolarization pattern derived from intramural electrograms with the corresponding T-wave on the surface map.
Methods: Pig hearts (n=9) were perfused according to Langendorff and paced from the atrium (cycle length 650 ms). Transmural needles (32-45 needles, 4 electrode terminals each) were inserted and local activation and repolarization