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 con-
figuration 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=10), 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 (I\text{Ca}) amplitude, even when the effects of age, sex, ACE inhibitors, beta-blockers, angiotensin recep-
tor blockers, calcium antagonists, and left ventricular ejection fraction were taken into account. I\text{Ca} 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 af-
fected by age but fast I\text{Ca} inactivation was significantly slower in old patients. The tau for I\text{Ca} inactivation was 20.9±1.9 ms in old vs. 14.5±0.9 ms in young pa-
tients (p<0.01). Similarly the tau for slow I\text{Ca} inactivation was 120±12 ms in old vs. 73±3 ms in young patients (p<0.001). The caffeine releaseable sarcoplasmic reticulum (SR) calcium content also decreased with age (from 10±1.0±0.8 amol/mg in young to 7.3±0.7 amol/mg 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 age, constant 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<0.05). Moreover, age did not affect the ability of my-
ocyes to maintain a uniform beat-to-beat response when the stimulation fre-
quency 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 con-
tent linked to lower SERCA2 and calsequestrin-2 expression in human atrial my-
ocyes. These alterations may blunt atrial contraction and relaxation.

P5019 I BENCH
Human cardiac Kir2.1, but not Kir2.3, channel expression is regulated by Nav1.5
Department of Cardiology, University of Madrid, Madrid, Spain

Purpose: Human cardiac inward rectifying K+ current (I\text{K1}) 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. I\text{K1} plays a key role in the control
of resting membrane potential and action potential duration and, thus, excitabil-
ity and refractoriness. It has been described that an increase in the expression
of Kir2.3 is Ser-Ala-Ile. Thus, we analyzed whether Kir2.1 and Kir2.3 channel ac-
expression with Nav1.5 channels. The specificity of the effect is determined by
the channels whose sequence in Kir2.1 and 2.2 channels is Ser-Glu-Ile, while
Kir2.3 is Ser-Ala-Ile. Only expression of Kir2.1 homotetramers is increased by their co-
exist in CHO cells transiently transfected with wild-type (WT) and mutant Kir2.x
channels. The expression of Kir2.1 was increased by SITS but not by diethylamino
hydrogenase, two additional and essential components of the
\text{H^+}-ATPase. The significance of this protective effect is suggested to be involved in mediating this protective effect, presumably via a pre-synaptic and post synaptic coupled pathway.

P5020 I BENCH
Acetylcholine analogue mimics the protective effect of cardiac vagal nerve stimulation on ventricular fibrillation threshold
M. Kalla, M. Chotalia, G. Hao, D.J. Paterson, N. Herrera. University of Oxford, Department of Physiology, Anatomy and Genetics, Oxford, United Kingdom

Purpose: Implantable cardiac vagus nerve stimulators are a promising new treat-
mant for heart failure, which may improve quality of life and survival.
Animal studies also suggest an anti-fibrillatory effect of stimulating the cardiac vagal 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 carbachol (CCh), a stable analogue of the neurotransmitter acetyl-
choline, can mimic the effect of vagus nerve stimulation on ventricular fibrillation threshold (VFT), and whether this mechanism is dependent on muscarinic recep-
tor stimulation and/or generation of NO.

Methods: Experiments conformed to “Principles of laboratory animal care“ (NIH Publication No. 85-23, revised 1986) 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 pres-
sure 77.5±5.6mmHg, heart rate 282±8.34 bpm, n=6). VFT was reproducibly determined by pacing at a fixed cycle length (150ms) 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 (50mM/L). All data are presented as
mean±SEM with ANOVA for multiple comparisons.

Results: CCh (200µM, 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 which could be reversed with 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 (10µg, n=4) or the non-specific competitive inhibitor NOS inhibitor N-omega-nitro-L-arginine (200µg, n=4). Conversely, the protective action of L-NA could be reversed by the addition of the NOS substrate L-arginine (5mM). The NO donor sodium nitroprusside (SNP 10µg, n=8) mimicked the effects of CCh and significantly increased of 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 stim-
ulation and the generation of NO appear to be involved in mediating this protective effect, presumably via a pre-synaptic and post synaptic coupled pathway.