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where the mitochondrial permeability transition pore (mPTP) may play a role. We tested whether chronic exercise in aging mice was able to improve mitochondrial functions.
Methods: Young (n=20, 8 weeks) and old (n=18, 18 months) C57BL6 male mice underwent an evaluation of mPTP, either in sedentary conditions or after 4 weeks of chronic swimming. Following isolation of mitochondria, we assessed Ca2+-induced mPTP opening by a potentiometric approach (Calcium retention capacity, CRC, nmoles CaCl2/mg prot.) and assessed O2 consumption at state 3/state 4 by polarographic method (using complex I pyruvate+malate as a substrate) and respiratory control index (RCI = state 3/state 4 O2 consumption).
Results: RCI was similar in young and old sedentary mice and was not significantly increased by exercise. In old sedentary mice mitochondria, CRC was significantly lower compared to young mice. In old trained mice, CRC significantly increased to near young mice values (table, $p<0.05$).
In addition, in old mice, exercise resulted in less oxidatively modified protein (oxyblot) and increased aconitase activity than in the sedentary group ($P < 0.05$).

	State 3 (nmoles O2/ min/mg)	State 4 (nmoles O2/ min/mg)	RCI	CRC (nmoles CaCl2/ mg prot)	Aconitase (nmoles/ min/mg)
Young sedentary (n=10)	73±3	15±2	4.9±3.6	224±31	103±23
Young trained (n=10)	76±5	14±1	5.2±0.2	242±17	125±16
Old sedentary (n=9)	60±4 *	16±1	3.7±1.2	160±17*	65±23*
Old trained (n=9)	89±5 **	26±4	3.5±1.5	220±15**	77±15**

*p<0.05 vs young sedentary, **p<0.05 vs old sedentary.

Conclusion: These data suggest that chronic exercise may prevent the aging-associated damage of mitochondria and prevent mPTP opening in old mice.

P4291 Oxidized LDL induces hypoxia inducible factor-1 in monocytes and enhances angiogenesis in vitro and in vivo

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Background: Plaque neovascularization has been linked to the progression of atherosclerosis and increased plaque instability. We have recently shown that neovessel density is increased in lipid rich compared to fibrous plaque. However the mechanisms underlying the increased neoangenesis in lipid rich plaque areas are poorly understood. HIF-1-alpha is a key transcriptional regulator of multiple genes involved in angiogenesis.
Methods and Results: HIF-1-alpha DNA binding in human monocytes was stimulated by oxidized LDL (oxLDL, 40μg/mL) as revealed by nuclear shift assay. oxLDL-induced HIF-1-alpha expression was associated with increased expression of VEGF in monocytes as detected by immuno-fluorescence labeling. oxLDL treatment (20μg/mL) alone or co-culture with non-oxLDL treated monocytes using transwells had no effect on matrigel induced tube formation of human endothelial cells in vitro. However, co-culture of endothelial cells with monocytes that had been pretreated with oxLDL-significantly increased tube formation of endothelial cells 2.5-fold ($p<0.01$) in vitro. In an in vivo matrigel plug assay (n=16) in C57BL6 mice, the addition of oxLDL to the matrigel (20μg/mL and 200μg/mL) increased the formation of new vessels from preexisting skin vessels into the matrigel plug after six days in a dose dependent fashion ($p<0.05$). When performing immunofluorescence double labeling to detect endothelial cells and monocytes (using anti-CD31 and CD11b antibodies) we found a strong and significant increase in endothelial tubes with ox-LDL treatment interestingly in the absence of increased presence of inflammatory cells at day 6. This could point to rather a qualitative (inducing VEGF expression) rather than only quantitative change in monocytes in lipid-rich plaque induced by oxLDL.
Conclusion: oxLDL directly activates the transcription factor HIF-1 in monocytes and thereby increases monocyte-induced angiogenesis in vitro and in vivo. Our data suggest that oxLDL may be a major contributor to neoangiogenesis in lipid rich area of advanced atherosclerotic lesions.

P4292 Targeting bone marrow endothelial nitric oxide synthase (eNOS) by the eNOS enhancer AVE9488 increases circulating endothelial progenitor cells after myocardial infarction

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Purpose: Endothelial progenitor cells (EPCs) play a major role in neoangiogenesis after myocardial infarction (MI). EPCs are located in a microenvironment of bone marrow stromal and endothelial cells, and are translocated to the circulation upon matrix metalloproteinase-9 (MMP-9) and/or vascular endothelial growth factor (VEGF)/nitric oxide (NO) mediated pathways.
Methods and Results: We studied the effects of AVE9488, a novel eNOS expression enhancer, on EPC number and function, as well as on mobilising pathways in bone marrow early after MI. Treatment with AVE9488 (25ppm, for 3 days starting immediately post-MI) versus placebo increased levels of circulating EPC 5.2-fold ($p<0.001$) and significantly improved EPC migratory capacity. eNOS expression (0.726±0.06 versus 0.403±0.05 densitometric units/μg protein, $p<0.05$) and activity (20.4±4.4 versus 7.15±0.9 nmol/μg protein x103, $p<0.05$) were increased by AVE9488 in bone marrow of MI rats. VEGF expression in bone marrow was increased by AVE9488 versus placebo MI rats (0.121±0.01 versus 0.076±0.01 pg/μg protein, $p<0.05$), whereas plasma VEGF levels were unchanged. Phosphorylation of Akt1 in bone marrow was significantly increased in AVE9488-treated animals (0.519±0.09 versus 0.310±0.09 densitometric units/μg protein, $p<0.05$), whereas malondialdehyde, an index of reactive oxygen species formation, was reduced (0.560±0.12 versus 1.362±0.30 μmol/μg protein, $p<0.05$). MMP-9 activity was not affected by AVE9488 (448±36 versus 438±82 densitometric units/μg protein). Long-term AVE9488 treatment resulted in improved neovascularisation and cardiac function after experimental myocardial infarction.
Conclusions: Treatment with the eNOS enhancer AVE9488 reduced ROS formation and increased eNOS expression and activity in rat bone marrow early after MI with a subsequent marked increase EPC number and function. Thus, the eNOS enhancer AVE9488 is an interesting novel approach for post-MI treatment.

P4293 Connexin43 regulation during hypoxia and hypoxia/reoxygenation conditions in atrial cardiac cells: pathogenetic implications

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Background: In chronic atrial fibrillation (AF) and severe heart failure, downregulation of connexin43 (Cx43) and up-regulation of connexin40 (Cx40) have been observed in left atrium. We tested different possible triggers (hypoxia, inflammation, vagal and adrenergic stimulation) of this gap junctional remodeling in atrial cardiomyocyte cell line (HL-1) and reversibility of this process after reoxygenation.
Methods: HL-1 cells, differentiated atrial cardiomyocytes, were exposed to hypoxia (3% O2) for 24 and 48 hours. Futhermore, cells subjected to simulated ischemia (SI) by replacing the cell medium with an "ischemia buffer" that contained 118 mM NaCl, 24mM NaHCO3, 10 mM NaH2PO4,2.5 mM CaCl2-2H2O, 1.2 mM MgCl2, 20 mM sodium lactate, 16mM KCl, 10 mM 2-deoxyglucose (pH 6.2) were incubated in normoxic and hypoxic conditions for 3-6-9-12 hours. Western blot analysis using anti-Cx43 and anti-Cx40 polyclonal antibodies (Santa Cruz,CA) was performed. Cx43 levels were also evaluated during 24 and 48 hours of hypoxia followed by reoxigenation (8-24-30-48 hours) and after exposure to C-reactive Protein (CRP: 1-5-10-20-50 μg/ml), to Angiotensin II (Ang II: 1, 2.5, 5 μM), IL-6 (1, 10, 100 ng/ml), TNF-alpha (1, 5, 10 ng/ml), Acetylcholine and Epinephrine (1, 10, 100 μM), for 24 and 48 hours.
Results: After 24 and 48 hours of hypoxia, total Cx43 protein decreased by 75% and 90% respectively ($p<0.001$). A significant reduction of Cx43 protein level was observed already within 6 hours of simulated ischemia (p= 0.001), in addiction to hypoxic conditions. After 24 hours of hypoxia, total Cx43 protein levels increased gradually during reoxygenation to reach the basal level within 48 hours. In contrast, after 48 hours of hypoxia, Cx43 protein remained at low level during reoxygenation. Cx43 expression during CRP, IL-6, Ang II, TNF-alpha treatment for 24 and 48 hours was unaltered as during vagal and adrenergic stimulation. Hypoxia, CRP, IL-6, TNF-alpha, vagal and adrenergic stimulation did not significantly change the expression of Cx40.
Conclusions: Hypoxia per se, and even more ischemia, downregulate Cx43 protein expression in atrial cardiomyocytes. Protein downregulation is reversible depending on hypoxia duration. These alterations might contribute to the generation of an arrhytmogenic substrate.

P4294 Nitric oxide increases human cardiac Kir2.1 currents

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Cardiac Kir2.1 channels generate the inward rectifier K+ current (IK1) that controls the resting membrane potential (RMP) and participates in shaping the final phase of the human action potential (AP). Nitric oxide (NO) directly and indirectly regulates many cardiac functions, and excessive or defective myocardial production of endogenous NO has been implicated in some cardiac diseases. This work was undertaken to determine the effects of NO on Kir2.1 channels. Currents were recorded from transiently transfected Chinese hamster ovary cells at room temperature by using the whole-cell and cell-attached patch-clamp. The NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP, 200 μM), which released 192±15 nM NO as measured by using a NO-sensitive electrode in the cell chamber, increased both the inward (17.7±5.9% at –120 mV) and the outward (53.3±9.8% at –50 mV) Kir2.1 currents recorded at extracellular ([K+]o) and intracellular ([K+]i) K+ concentrations of 4.0 and 140 mM, respectively. Moreover, SNAP rightwardly shifted the reversal potential (EK,C=-79.5±3.1 vs EK,SNAP=-76.3±3.7 mV, $P<0.05$), and slowed the activation process (TauC=1.6±0.2 vs TauSNAP=2.7±0.5 ms, $P<0.05$) without modifying the time course or the current decay. Similar results were obtained in the presence of another NO donor (diethylamine NONOate) or a NO saturated solution. The NO-induced increase

was of the same magnitude when the [K+]o was 1, 10 or 140 mM, whereas it was completely prevented by the potent reducing agent dithiothreitol. This result suggests that the effects could be mediated by the S-nitrosylation of the thiol group of a cysteine residue. Indeed, the NO effects were completely abolished in a Kir2.1 mutant in which 6 cysteines were mutated (C54V, C76V, C89I, C101L, C149F, and C169V), confirming that NO effects were mediated by the S-nitrosylation of one/various of these residues. Single-channel analysis revealed that SNAP significantly increased the open probability (from 0.17 to 0.23) and the opening frequency (from 1.5±0.3 to 6.1±2.2 Hz) without modifying the single-channel current amplitude (1.7 vs 1.6 pA). Furthermore, SNAP hyperpolarized the RMP of atrial AP recorded in both wild type and NO synthase 3 defective (NOS3-/-) mice (from -86.0±2.7 to -89.5±2.6 mV, $P<0.05$) and shortened the duration of the AP measured at 90% of repolarization (from 81.0±16 to 65.9±11 ms, $P<0.05$). These results demonstrate that NO, at physiological concentrations (200-800 nM), produces the S-nytrosilation of the Kir2.1 protein which, in turn, increases the IK1 that is important in the control of the RMP and the duration of the cardiac AP.

P4295 Na+/H+ exchanger-1 inhibitors decrease myocardial superoxide production by a direct mitochondrial action

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The possibility of a direct mitochondrial action of Na+/H+ exchanger-1 (NHE-1) inhibitors leading to a decrease in reactive oxygen species (ROS) production was explored in cat cardiac slices. Anion superoxide (O2-) production was measured by the lucigenin-enhanced chemiluminescence method. Values are the difference from the non stimulated control (arbitrary units/min/mg dry weight) after 15 min minutes in the presence of lucigenin.
1nM angiotensin II induced after 30 min a NADPH oxidase-dependent (NOX) increase in O2- production (1nM Ang II, 49.6±8.9, n=34; 300μM apocynin, -7.7±17.0, n=7) that was prevented by three different NHE-1 inhibitors: 10μM cariporide (7.4±6, n=12), 1μM BIIB723 (5.7±13.9, n=3) and 5μM EMD87580 (-2.2±12.1, n=4) ($p<0.05$, ANOVA). These drugs were devoid of ROS scavenger activity. The source of the NOX-dependent O2- released seemed to be the mitochondria through the "ROS induced-ROS release" mechanism since it was blunted by the mitochondrial KATP channel (mKATP) blockers 100μM 5-hydroxydecanoate (2.5±14.3, n=10) and 50μM glibenclamide (-20.8±4.9, n=6), by inhibition of complex I of the electron transport chain with 10μM rotenone (-11.9±23.5, n=8) and the mitochondrial permeability transition pore (MPTP) by 2μM cyclosporin A (-8.7±9.1, n=4) ($p<0.05$, ANOVA). O2- production induced by the opening of mKATP with 100μM diazoxide (53.3±9.3, n=19) was also prevented by 10μM cariporide (12.3±5.6, n=5), giving further support to a direct mitochondrial action of NHE-1 blockers. Cariporide also decreased Ca2+ induced-mitochondrial swelling by 36.7±3.3% (n=7), approximately to the same extent than 2μM cyclosporin A (41.5±4.4%, n=7) and 10μM bongkrekic acid (34.2±2.0%, n=6) ($p<0.05$, ANOVA), without showing any additive effect. Ang II-increase of O2- stimulated ERK1/2 and p90RSK phosphorylation measured by Western blot (315±7% and 173±11% respectively vs. non stimulated control, n=4, $P<0.05$, ANOVA), and it was also prevented by cariporide (116±25% and 111±13% respectively, n=4, $p<0.05$, ANOVA) This gives additional support to the presence of a direct mitochondrial action of NHE-1 inhibitors preventing ROS release. We report a direct mitochondrial action of NHE-1 inhibitors that may explain their beneficial effect in several cardiac diseases such as ischemia/reperfusion injury and cardiac hypertrophy and failure.

P4296 Rho kinase inhibition reduces angiotensinII- and urotensinII- stimulated cardiac hypertrophy and collagen synthesis via attenuation of reactive oxygen species production

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Objective: Cardiac hypertrophy and extracellular matrix deposition are important features in cardiac remodelling post myocardial infarction leading to heart failure. The role of Rho kinase (ROCK) and reactive oxygen species (ROS) in Angiotensin II (AngII) and Urotensin II (UII) stimulated hypertrophy and collagen synthesis has not been explored. The aim of this study was therefore to examine the effect of ROCK inhibition on myocyte hypertrophy, fibroblast collagen synthesis, and its mechanism of action by examining the involvement of ROS.
Methods: Neonatal rat cardiac myocytes (NCM) and fibroblasts (NCF) were isolated from 1-day old Sprague-Dawley rats. NCF and NCM were serum starved for 48hours prior to treatment with the ROCK Inhibitor GSK576371 (GSK; 0.1-10μM) for 2 hours, followed by 48 hours stimulation with AngII or UII (both 0.1μM). Collagen synthesis in cardiac fibroblasts was assessed by ^{3}H-Proline incorporation, and NCM hypertrophy were assayed for ^{3}H-Leucine incorporation. ROS production was measured by lucigenin-enhanced chemiluminescence (5μM) 1 or 24 hour after stimulation.
Results: AngII- and UII-stimulated NCM hypertrophy and NCF collagen synthesis was dose-dependently inhibited by GSK. Furthermore, AngII- and UII-stimulated ROS production in NCM and NCF was dose-dependently inhibited by GSK (Table). At 24hr both AngII and UII did not increase NCF ROS production (data not shown).
Conclusions: These results demonstrate that ROCK plays an important role in cardiac hypertrophy and collagen turnover. A potential mechanism, whereby ROCK may attenuate pathogenic cardiac remodelling, may involve inhibition of ROS production in these cells.

P4297 Tumor necrosis factor-alpha mediates downregulation of the fatty acid oxidation pathway in cardiomyocytes exposed to angiotensin II

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Purpose: Experimental observations suggest that maladaptive remodeling of the left ventricle is associated with reduction of fatty acid oxidation. In mice with overexpression of angiotensinogen in the myocardium, downregulation of the fatty acid oxidation pathway coincides with the onset of heart failure. We now investigated the effects of angiotensin II (AngII) on fatty acid oxidation in long-term cultured adult rat cardiomyocytes (ARC). We hypothesized that AngII-induced release of tumor necrosis factor-α (TNF-α) may be involved.
Methods: ARC were exposed either to AngII (100 nM) or to TNF-α (10 ng) for up to 14 days. To investigate the role of TNF-α released in response to prolonged exposure to AngII, TNF-α was neutralized using anti-TNF-α antibodies (0.2μg/ml).
Results: AngII and TNF-α both markedly downregulated protein expression of enzymes of fatty acid oxidation (carnitine palmitoyltransferase-I -41% and -81%, respectively; medium chain acyl CoA dehydrogenase -40% and -82%), whereas, markers of hypertrophy were increased (ANF +548% and +2465%, BNP +749 and +1340%, cell surface area +20% and +30%). 14C-palmitate oxidation was reduced by 62% and 84% in the presence of AngII and TNF-α, respectively. However, there was a marked difference in the time-course. The onset of changes was immediate in the presence of TNF-α, but delayed to day 7 in the presence of AngII. In ARC treated with AngII, mRNA and protein expression of TNF-α started to increase on day 7, concomitantly with the onset of metabolic changes. The induction of TNF-α was dose dependent and could be blocked by inhibition of the NADPH oxidase by apocynin and translocation of nuclear factor kappaB (NFkB) by SN50. The addition of TNF-α neutralizing antibodies to the culture-medium of AngII-stimulated ARC completely abolished downregulation of the fatty acid oxidation pathway and attenuated the hypertrophic response.
Conclusions: AngII induces TNF-α synthesis in ARC in a dose-dependent manner via NADPH-dependent activation of NFkB. TNF-α mediates downregulation of the fatty acid oxidation pathway and, enhances the hypertrophic response to AngII.

P4298 nNOS/PMC4b complex modulates beta-adrenergic signalling in the heart

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Neuronal nitric oxide synthase (nNOS) is a major regulator of β-adrenergic signal transmission in the heart. We have previously shown that nNOS, which is a calcium and calmodulin dependent enzyme, is tightly regulated by isoform 4 of the plasma membrane calcium ATPase (PMCA4), which binds to nNOS via a PDZ domain. Recently, we have shown that PMCA4b overexpression reduced β-adrenergic responsiveness in vivo via an nNOS dependent mechanism. In the

Abstract P4296 – Table 1

	AngII (10^{-7}M)				UII (10^{-7}M)			
GSK (M)	0.0	10^{-7}	10^{-6}	10^{-5}	0.0	10^{-7}	10^{-6}	10^{-5}
NCM Hypertrophy[1]	125.9±1.4***	107.3±3.7$^{\#}$	107.7±3.0$^{\#}$	93.5±3.5$^{\#\#}$	117.6±2.1*	91.0±13.2	114.1±6.7	95.2±9.8$^{\#}$
NCF collagen Synthesis[1]	209.2±26.9**	200.8±29.3	127.9±20.2$^{\#}$	85.2±6.5$^{\#\#}$	125.8±5.8*	86.2±5.3$^{\#\#\#}$	63.3±6.3$^{\#\#\#}$	53.3±7.7$^{\#\#\#}$
NCM ROS (1 hour)	111.5±2.8**	99.7±4.3$^{\#}$	102.9±1.5$^{\#}$	98.9±1.3$^{\#\#}$	120.9±1.6***	109.7±2.4$^{\#\#}$	103.7±3.3$^{\#\#}$	104.6±3.3$^{\#\#}$
NCM ROS (24hr)	124.7±3.9**	114.9±3.0	105.9±1.3$^{\#\#}$	91.6±4.9$^{\#\#\#}$	125.4±2.1**	99.4±2.5$^{\#\#}$	98.0±4.2$^{\#}$	97.5±3.3$^{\#\#}$
NCF ROS (1hr)	116.8±1.5***	107.,8±1.7$^{\#\#\#}$	106.±2.1$^{\#\#\#}$	82.21±1.2$^{\#\#\#}$	129.2±2.0***	120.8±1.7$^{\#\#}$	136.5±1.4	108.2±1.9$^{\#\#\#}$

Data is presented as % of unstimulated control (mean ± SEM). ***p<0.001, **p<0.01, *p<0.05 vs control; $^{\#\#\#}$ p<0.001, $^{\#\#}$p<0.01, $^{\#}$p<0.05 vs stimulated control. [1]NCM and NCF were transfected with UII receptor adenovirus 48hr before stimulation.