one of the main regulators of cardiomyocyte hypertrophy and highly elevated in cardiac samples from HF patients. Moreover, mice overexpressing miR-132 developed hypertrophy and fibrosis and died from HF at an early stage. Hypertrophy and fibrosis in the remote myocardium are also mechanisms involved in adverse remodelling in patients post-MI often leading to heart failure. Therefore, we wanted to validate whether miR-132 intervention would be beneficial in this process.

Methods: and Results: Pharmacological inhibition of miR-132 by LNA-based oligonucleotides (antisense or scrambled) was done at day 7 and 14 post-MI in a mouse model of experimental MI. Hemodynamic parameters, measured by echocardiography and left ventricular (LV) pressure volume catheter, were assessed at the study end point 4 weeks post-MI. These results show that post-MI antimiR-132 treatment ameliorated cardiac function: both left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) were reduced after miR-132 silencing in post-MI mice. We then observed by Longitudinal Strain Rate (LSR) analysis that antimiR-132 treatment led to a recovery of the function in the remote areas after MI. At the molecular and histological level, repression of key genes involved in adverse remodeling highlighted the protective cardiac effects of antimiR-132 treatment. After confirming the efficacy of the treatment in a mouse model, we tested this concept in a clinically relevant large animal model. Domestic pigs had a balloon-mediated occlusion of the left anterior descending artery (LAD) for 90 min followed by reperfusion. AntimiR-132 or placebo treatment were delivered by anterograde slow infusion into the coronary artery by a catheter at day 3 and intravenously 4 weeks post-MI. Based on magnetic resonance imaging (MRI) data, 8 weeks post-MI, the volumes and cardiac mass were well preserved and the expression of hypertrophic genes was decreased after antimiR-132 delivery. In addition, we specifically investigated regional function of the post-MI myocardium using segmental contraction analysis. Segmental velocity was improved in most of the remote area segments. The regional improvements could also be observed by NOGA catheter measurements, giving a functional assessment based on electrophysiological properties.

Conclusions: The presented data show that antimiR-132 treatment is beneficial post-MI and attenuates adverse cardiac remodeling by improving global cardiac performance and reducing hypertrophy. These data provide further evidence of this miRNA's translational and clinical relevance and encourages the future therapeutic testing of miR-132 inhibitors on the road of clinical application in HF patients.

P2305

Regulation of beta adrenoceptor evoked inotropic responses by inhibitory g protein, adenylyl cyclase isoforms 5 and 6 and phosphodiesterases

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Purpose: Our data indicate that inhibitory G protein (Gi) inhibits adenylyl cyclase (AC) independently of the receptor. The two major subtypes of AC in the heart are AC5 and AC6. Compartmentalization of β1-adrenoceptor- (β1AR) versus β2AR differs depending on the subcellular localization of the AC subtypes. Deletion of AC6 impairs left ventricular responsiveness to βAR ligands and it is unknown if AC5 or 6 differentially regulate β1AR- versus β2AR-mediated inotropic responses. Determine if intrinsic Gi inhibition is AC subtype selective and whether there is a differential role of AC5 and AC6 to mediate β1AR- and β2AR-evoked inotropic responses. In addition, determine if there is an interplay between Gi and phosphodiesterases 3,4 (PDE3,4).

Methods: We measured β1AR- and β2AR-mediated changes in contractility in left ventricular muscle strips from wild type (WT), AC5 and AC6 knockout (KO) mice. First, with or without pertussis toxin (PTX) to inactivate Gi and/or after inhibition of PDE3 or PDE4.

Results: AC6KO mice revealed increased noradrenaline potency (EC50) at the β1AR compared to WT and AC5KO. Furthermore, AC6KO mice revealed an adrenaline-evoked β2AR-inotropic response only after PDE3 or PDE4 inhibition whereas both were required in WT and AC5KO. A β2AR-mediated inotropic response was also observed after PTX treatment alone in all groups

Conclusion: Gi tonically inhibits AC since PTX enhances both β1AR- and β2AR-mediated inotropic responses despite Gi not coupling to β1AR. PDE4 seems to be the primary PDE regulating the β1AR response in all groups. Inhibiting Gi and PDE3 or PDE4 appears to synergistically enhance adrenaline-evoked β2AR-inotropic response in WT. We therefore propose that inhibiting Gi and PDEs allows cAMP to leak from the β2AR to the β1AR contractile compartment.

P2306

Angiotensin 1-7 blunts in vitro induced acute heart failure.

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Background: Angiotensin 1-7 (Ang 1-7) comprises consistent evidences regarding cardiovascular regulatory benefits due to Ang II receptor AT1 modulation via mass receptor.

Aim: Evaluation of the Ang 1-7 cardiac effects in the in vitro induced acute heart failure.

Material and methods: Acute heart failure (AHF) was induced using the model of isolated rat pumping heart perfused by Krebs solution without glucose during 20 min according to Neely-Rovetto model (glucose is a single energetic substrate in this model) – control series. In another series heart has been perfused without glucose, but Ang 1-7 was added till final concentration of 10-7 M – medicated series. Left ventricle (LV) functional parameters were assayed during inotropic stimulation by norepinephrine (NE) and endothelin 1 (ET-1) in concentration of 10-6 M, or ischemia-reperfusion impact (15 min of total ischemia followed by 20 min of reperfusion) reproduced in Langendorff isovolumic isolated heart.

Results: Cardiac output (CO) significantly decreased after 20 min perfusion of isolate heart without glucose by 25,9% ($29,4 \pm 1,3$ vs $39,7 \pm 2,1$ ml/min). Action of Ang 1-7 led to a less decline of CO compared to control ($34,8 \pm 1,6$ vs $29,4 \pm 1,3$ ml/min, $p < 0,05$). NE stimulation induced an increase of control CO by 10,7% associated by LV end-diastolic pressure (LVEDP) elevation of 30,3% while in medicated series response was better: CO increased by 14,4% and LVEDP boosted only by 17,6% (($19,3 \pm 1,6$ (Ang 1-7) vs $27,4 \pm 1,7$ (control) mm Hg, $p < 0,05$). Stimulated by ET-1 control isolated heart responded by a negative inotropic effect, and both systolic LV pressure and CO fallen respectively by 13,2% and 9,6%. Ang 1-7 insured a positive inotropic response during ET-1 action leading to CO and LV systolic pressure increase respectively by 10,5% and 11,7%. Ang 1-7 also improved the dynamics of LVEDP during ischemia-reperfusion. Thus, LVEDP was in medicated series significantly less than control index at finish of both ischemia ($41,3 \pm 3,2$ vs $55,4 \pm 4,4$ mm Hg) and reperfusion ($17,2 \pm 1,4$ vs $28,7 \pm 2,2$ mm Hg) periods.

Conclusion: Angiotensin 1-7 is a component of renin-angiotensin-aldosterone system which has a benefic action on acutely developing heart failure due to energy privation, manifested by improvement of inotropic response of NE and reinstated positive inotropic of ET-1 action as well as significant diminution of LVEDP during ischemia-reperfusion syndrome.

P2307

Bioenergetic properties of inotropic drugs

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Background: Cardiogenic shock is an unmet clinical need since catecholamines increase mortality in patients with acute heart failure. Newer inotropes target sarcomeres to increase contraction. Levosimendan sensitizes troponin C to calcium (Ca), while EMD 57033 (a "classical" Ca2+ sensitizer) and the novel inotrope omecamtiv mecarbil (OM) act downstream of troponin C at the level of actin-myosin interaction. Since the mitochondrial redox state is under the control of ADP (oxidizing NADH for ATP production) and Ca (stimulating the Krebs cycle to regenerate NADH), and Ca-sensitization may increase work (=ADP) at any given Ca, we evaluated the bioenergetic properties of these inotropes.

Methods and results: Cardiac myocytes were isolated from guinea pig and mouse hearts and field-stimulated at 1-5 Hz and 37°C. EMD 57033 shortened diastolic and systolic sarcomere lengths without affecting cytosolic Ca (measured by indo-1). The redox states of NAD(P)H and FAD (autofluorescence) were oxidized by EMD. This elevated mitochondrial ROS emission (determined by DCF) during β-adrenergic stimulation with isoproterenol (Iso). Levo alone increased sarcomere shortening only modestly at 1 and 10 µM, but its potency and efficacy were substantially increased by pre-incubation with low β-adrenergic stimulation (Iso; 1 nM). Under these conditions, the increase in sarcomere shortening was explained by increases in cytosolic Ca, while the redox states of NAD(P)H and FAD remained stably reduced. At concentrations that cover therapeutic plasma concentrations in clinical trials, OM (0.1-3 µM) prolonged the time and amplitude of sarcomere shortening, but also the time of relaxation, and increased baseline diastolic tension. These effects on sarcomere function were associated with slight (though significant) oxidation of NAD(P)H/FAD, while the mitochondrial membrane potential (measured by TMRM) remained unchanged. Low (1 nM) or intermediate (30 nM) Iso concentrations prevented OM-induced oxidation. However, 30 nM Iso aggravated diastolic dysfunction and provoked arrhythmias in >50% of cases in OM-treated myocytes. In isolated cardiac mitochondria, OM (3 µM) did neither affect respiration, ROS production nor redox state. Conclusions: Pure Ca sensitization with EMD 57033 increases systolic function, but at the same time impairs diastolic function, oxidizes the mitochondrial redox state and elevates ROS emission. Levosimendan requires a PDE-inhibitory effect to increase Ca and sarcomere shortening, but this Ca elevation matches energy supply to demand and prevents mitochondrial oxidation. At concentrations that improve systolic function, OM impairs diastolic function and slightly oxidizes mitochondrial redox state, which may predispose to arrhythmias during substantial (but not low) concomitant β-adrenergic stimulation.