Elevated levels of CXCL-9, -10 and -11 are associated with left ventricular dysfunction

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Heart failure is a condition associated with high morbidity and mortality. Our current pharmacotherapeutic interventions can at best slow down the progression of the disease. At present, a proper molecular characterization of the disease process is lacking. Biomarkers that provide insight into the molecular mechanisms underlying the malfunctioning of the heart could be helpful in this respect. Because inflammation contributes to the symptoms of heart failure, we determined the circulating levels of three related chemokines, CXCL-9, -10 and -11, as well as NT-proBNP in serum from subjects with left ventricular dysfunction (LVD) and controls.
Methods: Subjects with either subclinical (n=17) or advanced (N=14) LVD as well as age- and sex-matched controls (n=31), were recruited from the large-scale family-based study on the genetic epidemiology of cardiovascular phenotypes (FLEMENGHO). Mean arterial blood pressure was significantly higher in the subclinical (104.8±8.6 mmHg) and advanced (101.1±11.7 mmHg) LVD groups vs. control (90.5±4.5 mmHg). One-way ANOVA, Odds ratios, Integration Discrimination Improvement and Net Reclassification Improvement were determined using SAS software.
Results: Increased serum levels of CXCL-9, -10 and -11 were observed in the LV dysfunction groups (1.5, 1.3 and 1.8-fold in the subclinical LVD and 2.2, 2.5 and 4-fold in the advanced LVD group, respectively; $p<0.01$, V0.001 and <0.01, respectively). NT-proBNP was 1.5 and 1.8-fold higher in the subclinical and advanced LVD groups; $p<0.01$). Adjustment for sex, age, BMI and mean arterial pressure resulted in a loss of significance for CXCL10 and NT-proBNP, whereas de difference for CXCL9 remained significant ($p=0.03$) and for CXCL11 a trend towards significance was observed ($P=0.06$). Unadjusted odds ratios associated with a doubling of the biomarker levels were 2.14 (1.38-3.31) for CXCL-9, 2.27 (1.30-3.95) for CXCL-10, 2.00 (1.23-3.24) for CXCL-11 and 2.52 (1.35-4.70) for NT-proBNP (all $p<0.005$). Sensitivities were 0.58, 0.65, 0.58 and 0.76 and specificities 0.88, 0.69, 0.84 and 0.69 for CXCL-9, -10 and -11 and NT-proBNP, respectively. When corrected for sex and BMI, adding the dichotomized biomarkers to a model already including established risk factors resulted in a significant Net Reclassification Improvement and Integrated Discrimination Improvement for all biomarkers except CXCL10.
In conclusion, including CXCL-9, -10 and -11 levels improves the discrimination of risk prediction models for LVD. The results of this study underscore the importance of using a panel of biomarkers to better characterize subjects with LVD.

Indications and dosages of atypical antipsychotics in Belgian nursing homes.

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Background and objective: The use of antipsychotics is highly prevalent in residential care, often used off-label for Behavioural and Psychological Symptoms of Dementia (BPSD). Nowadays, atypical or
second-generation antipsychotics have replaced the majority of lderantipsychotics. We wanted to explore the indications and dosages of antipsychotics used by nursing home residents, focusing on atypical antipsychotics.
Methods: Medication charts of 1,730 residents from 76 nursing homes in Belgium (2006) were collected and analysed, using the ATC classification. Drug name, indication and daily dosage were recorded. We compared the prescribed daily dose (PDD) to the
Defined Daily Dose (DDD) for atypical antipsychotics based on the WHO ATC/DDD index.
Results: Nursing home residents used in 32.9 % antipsychotics of which19.1% atypical and 16.1% typical antipsychotics. Concomitant use of 2 or more antipsychotics was found in 5% of cases. Risperidone and olanzapine were the main used atypical antipsychotics, 13.5% and 5.5%, respectively. Buterophynone derivates (12.1%) were the main used typical antipsychotics of which haloperidol counted for 5%. The main indications for prescribing antipsychotics were agitation within dementia (57%), and psychosis with or without dementia (23%).The median PDD for risperidone was 0,5 mg (range 0.25-9), lower than the recommended DDD of 5 mg; median PDD for olanzapine was 5 mg (1.25-25), lower than the recommended DDD of 10 mg; median PDD for quetiapine was 100 mg (25-400), also lower than the recommended DDD of 400 mg.
Conclusion: The DDD of antipsychotics is based on the main indication of psychosis. Also in Belgian nursing homes, antipsychotics are mainly used off-label for BPSD. The PDD is systematically lower than the DDD, indicating that GPs do take the altered drug disposition of the ageing body as well as the off-label indication into consideration.

The role of Phosphodiesterases on ageing-related vascular dysfunction

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Ageing is associated with a number of profound changes in the cardiovascular system such as the thickening, narrowing and stiffening of the large arteries, compromising blood supply, and therefore endangers health. This is believed to be a consequence of diminished endothelial signaling and subsequent decrease of cyclic nucleotides, such as cGMP, which acts as second messengers for vasodilator and growth responses in the vascular smooth muscle cells (VSMC). Phosphodiesterase (PDE) 1 and 5 are important proteins that modulate cGMP bioavailability by hydrolyzing this cyclic nucleotide that plays an important role in maintenance of vascular health. We observed reduced vascular cGMP signaling due to increased PDE activity in mouse models of accelerated vascular ageing and associated increased blood pressure.

Aims: To investigate the effect of aging on PDE1A, PDE1C and PDE5, in young and senescent human VSMC.

To investigate if SNPs in the PDE genes are associated with blood pressure (BP), Pulse pressure (PP), pulse wave velocity (PWV) and coronary artery disease (CAD) in a candidate gene association study.

Methods: mRNA was isolated from 5 independent cultures of young and senescent human VSMC to quantify expression of the PDE1A, PDE1C and PDE5 genes. Real-time qPCR was performed. Ct values of PDE subspecies were corrected for reference gene TBP. All data are expressed relative to the lowest passage, which was set at 1. WB was performed to ascertain the validity of qPCR data. We also searched for SNPs inside the PDE genes the publicly available GWAS meta-analysis results for BP, PP, PWV and CAD. A p-value of <3.12x10^-4 after applying Bonferroni correction for multiple testing (0.05/160) was considered significant.

Results: Senescent VSMC showed an 11- and 8-fold increase of PDE1A and PDE1C mRNA levels respectively, whilst the PDE5 level was only modestly increased. PDE1C protein levels corresponded with qPCR data. We found some SNPs in the PDE1A and PDE5 genes significantly associated with diastolic BP and one SNP in the PDE5 associated with CAD.

Conclusion: PDEs may be an interesting target to improve vascular functionality in particular in relation to increased diastolic blood pressure, and as a marker for vascular ageing.

The operationalization of electronic inn prescribing

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Introduction: The “International Nonproprietary Name (INN) prescribing project” in Belgium aimed to operationalize electronic INN prescribing for outpatient care in daily medical practice and medical informatics.Methods: The operationalization process consisted of three phases: (1) expert consultation, (2) review by regulatory authorities and (3) test phase with stakeholders and end-users.
Results: The INN prescribing project resulted into (1) operational rules for electronic INN prescribing, (2) the classification of the therapeutic arsenal, according to the operational rules and, (3) a reference database to be implemented in commercial medical software. The operational rules for electronic INN prescribing define valid INN groups as sets of equivalent medicinal products, described by three elements: the therapeutic moiety (the active part of the therapeutic ingredient) or combination of therapeutic moieties, the strength(with standardized denominators), and the method of administration (with simplified but standardized options). The operational rules also define two categories of exemptions for INN prescribing: INN groups where the first choice of treatment should be continued throughout the therapy period (NO SWITCH) and medicinal product groups not suitable for INN prescribing (NO INN).
Conclusion: Operationalizing INN prescribing for electronic prescribing was a difficult yet feasible assignment. The INN prescribing project resulted into universally applicable operational rules and a corresponding classification of the therapeutic arsenal and reference database. These outcomes can be used by other countries planning to implement electronic INN prescribing.

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Inhibiting connexin channels improves the viability and function of cumulus-oocyte complexes exposed to vitrification/warming

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There is a growing interest in vitrification and subsequent storage of oocytes and embryos but the process is still not optimal: only 49% of human vitrified and subsequently warmed mature oocytes develop to blastocysts upon fertilization, while in cattle this percentage is even lower. Previously we showed that the combined block of gap junctions (GJs)and hemichannels with connexin (Cx)-targeting peptides present during the processes of cryopreservation and subsequent thawing of human vascular grafts, strongly reduces cell death of endothelial and smooth muscle cells. GJs are channels based on Cx protein subunits that allow the passage of cell death messengers between cells, thereby expanding cell death as a result of bystander cell death. Cx hemichannels not incorporated into GJs are normally closed but can be opened by cooling/freezing-related stress, thereby forming a toxic pore that leads to cell death. Oocytes are surrounded by cumulus cells that prominently express Cx43 forming GJs as well as hemichannels. GJs composed of Cx37 exist between cumulus cells and oocytes. We here tested whether the Cx channel inhibiting peptide Gap27, targeting Cx37 and Cx43, has beneficial effects on the cell viability and function of cumulus-oocyte complexes after vitrification/warming in a bovine model system. We found that cell death of cumulus cells after vitrification/warming was reduced when Gap27 was present. Oocytes did not show cell death after vitrification/warming but when they were subsequently fertilized, the group previously treated with Gap27 displayed a strongly increased fertilization rate (~7 fold increase). Interestingly, application of Gap27 after vitrification/warming performed under control conditions also resulted in a protective effect on cumulus cells. Taken together, our data indicate that, although Cx channels are necessary for the physiology of the cumulus-oocyte complex, they adopt toxic functions during the vitrification/warming process, making them a novel and interesting target for improving cell function after exposure to biobanking stress.

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The influence of pregnancy on arterial stiffness and wave reflections

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Objective: Studies on changes in arterial stiffness and wave reflections during pregnancy are limited to cross-sectional studies. Our aim was to investigate maternal hemodynamic and cardiovascular adaptations at each trimester of pregnancy in a prospective longitudinal case-control study.
Methods: Cardiovascular measurements were performed at 12, 20 and 35 weeks of gestation, and included peripheral (Omron M6) and central (Sphygmocor) blood pressures, wave reflection and arterial stiffness measures (Sphygmocor and Esaote AU5 Wall tracksystem).
Results: 109 healthy women with a normal pregnancy (mean age 29.3y, range 21-42) and 26 healthy non-pregnant control subjects (mean age 28.4y, range 21-40) were included. Except for peripheral and central systolic blood pressure, all cardiovascular parameters showed significant ($p<0.05$) changes during pregnancy. Heart rate increased linearly during pregnancy. In contrast, diastolic blood pressure (DBP), mean arterial pressure (MAP), augmentation index (AIx@75) and aortic stiffness (PWV) showed a typical V-shaped pattern, characterized by a significant drop from 12 to 20 weeks of gestation (DBP: -2.6 mm Hg; MAP: -1.6 mm Hg; AIx@75: -10.0 %;PWV: -0.6 m/s), followed by a rise (DBP: +4.2 mm Hg; MAP +4.0 mm Hg) or smaller drop (AIx@75: - 7.8 % ; PWV: -0.4 m/s) at 35 compared to 12 weeks of gestation.
Conclusions: The present longitudinal case-control study confirms the results of previous cross-sectional studies on peripheral and central hemodynamics. In addition, it shows a drop in wave reflection and arterial stiffness which may be due to vasodilation in the second and third trimester of pregnancy.

Does long-term benzodiazepine use has an impact on cognitive deterioration?

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Aim: Long-term use of benzodiazepines and Z drugs (BZD) has been linked to cognitive decline and an increased risk of dementia. In this one year prospective cohort study, we explored a) the cognitive evolution of long-term BZD users compared to nonusers, and b) the impact of BZDs on cognitive decline.
Methods: Cognitively capable residents of 10 Belgian nursing homes were divided in BZD users and nonusers based on medication charts. We assessed cognition with the Mini Mental State Examination test (MMSE) at baseline and 12 months later. A decrease of ≥4 points on the MMSE (clinical relevant decrease) was used in multiple logistic regression. We collected baseline demographics, medication information and functional, psychometric and social characteristics potentially influencing cognition.
Results: We collected data of 131 BZD users and 95 nonusers (mean age in both groups 85 year and 77% female). In both groups the cognition decreased significantly, but without significant difference between the groups. Controlled for age, gender, education and BZD use, the strong, significant risk factors for clinical relevant cognitive decline were depression (OR 2.77) hearing (OR 3.83) and functional impairment (OR 1.18). Frequent reading was associated with less MMSE decrease (OR 0.46).
Conclusion: Although we observed a trend, we found no association between long-term BZD use and fast cognitive decline. The strong risk factors for fast decline were depression, hearing and functional impairment, the absence of a reading attitude, but not BZD use. However, BZD users had a higher prevalence of depression compared to nonusers.

Mechanisms involved in resveratrol-induced relaxation of isolated mice corpora cavernosa

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Introduction: Red wine consumption has been linked to cardiovascular benefits. This is partly due to the presence of the wine compounds resveratrol (Resv) and quercetin (Quer). These are natural occurring polyphenols with known vasorelaxant capacity. Vasodilators often have a potential to regulate penile erection and could therefore be beneficial for the treatment of erectile dysfunction (ED).
Aims: The goal of this study was to determine the relaxant effect of the polyphenols Resv and Quer on mice corpora cavernosa (CC)and the mechanisms involved.
Methods: Isometric tension measurements on isolated mice CC were performed in organ baths. Cumulative concentration-response curves (10–100 μM) were constructed for Resv and Quer.
Main outcome measures: Relaxation responses of CC to Resv and Quer in the presence/absence of inhibitors of different molecular pathways were measured.
Results: Despite the fact that both polyphenols are potent vasodilators of mice aorta, only Resv relaxes mice CC. The relaxant effect of Resv on CC was significantly diminished by pretreatment with SQ 22,536, an adenylyl cyclase (AC) inhibitor and 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer, a protein kinase A inhibitor. Also compound C, a 5’ adenosine monophosphate-activated protein kinase (AMPK) inhibitor significantly decreased the relaxant effect of Resv on CC.
Conclusion: The red wine compound Resv, but not Quer, relaxes mice CC in vitro through activation of AC, leading to increased cAMP levels as well as through AMPK activation. Whether Resv could be beneficial for patients suffering from ED remains uncertain and requires further research.

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Syntaxin 3 regulates granule exocytosis and cytokine secretion in neutrophils

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Neutrophils play a fundamental role in innate host responses and constitute the first line of defense against pathogens. To ensure an effective killing of infecting microorganisms, neutrophils dispose of a large arsenal of potent cytotoxic products released during the degranulation process. Neutrophils have additionally the ability to package a variety of cytokines into their different types of cytoplasmic granules for subsequent secretion by exocytosis, a secretion which leads to the magnification of the immune response. Molecular mechanisms governing protein exocytosis involve SNARE proteins, keys regulators of intracellular vesicular trafficking. Although it is now obvious that neutrophils express numerous SNARE isoforms, the specific mechanisms underlining the release of cytokines as well as contribution of SNARE proteins in this process are still elusive in neutrophils. Since cytokine secretion is known to contribute to the development of chronic inflammatory diseases, we intended to investigate the molecular mechanisms involved in cytokine secretion. Using a Cytometric Bead Array approach, we established a cytokine expression profile for differentiated HL-60 cells (dHL-60 cells) and human neutrophils underlining their ability to secrete a large variety of cytokines in pro-inflammatory conditions mimed by lipopolysaccharide. Then, we screened by microarray experiments the expression of SNARE isorforms. Syntaxin 3 (STX3), found up-regulated during the dHL-60 differentiation process, was selected for further functional studies. STX3 knock-down by specific siRNA triggered a decrease of IL12b, CCL4 and IL1 secretion in dHL-60 cells. In the same manner, STX3 siRNA affected the release of MMP-9 from gelatinase granules in which we show that STX3 is partly localized. According to our results, we can hypothesize that IL12b, CCL4 and IL1 are stored in gelatinase granules and secreted upon cell stimulation by a mechanism regulated by STX3. In conclusion, we provide a first evidence that STX3 is involved in trafficking pathways of certain cytokines in neutrophils.

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CGRP release in the trigeminovascular system of a mouse migraine model with a misssens mutation in the Cacna1a gene

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Migraine is a common disabling neurovascular disorder involving activation of the trigemino-vascular system (TGVS) and release of neuropeptide calcitonin gene-related peptide (CGRP). Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine and used as a model to investigate also common types of migraine. We used a knock-in mouse model that expresses the missense R192Q mutation in the Cacna1a gene, encoding for the α1subunit of Ca(v)2.1 Ca2+ channels which causes FHM1 in patients. In this study, we investigated the release of CGRP by the different components of the TGVS in the R192Q (RQ) mouse model and compared it with C57BL/6J (B6) mice Dura mater, trigeminal ganglion (TG) and trigeminal nucleus caudalis (TNC) from the TGVS were isolated from mice of 13-14 weeks-old and placed in organ baths with synthetic interstitial fluid. Tissues were triggered with 60 mM KCl and supernatant (600µL) was collected for CGRP measurements. KCl induced CGRP release in dura mater, TG and TNC in all tested mice. CGRP release was highest in TNC (B6:12.4±2.2 pg/mL, RQ:17.3±4.2 pg/mL and littermates (lit):16.3±2.4 pg/mL) while the CGRP release was comparable in dura mater (B6:1.7±0.8 pg/mL, RQ:1.4±0.5 pg/mL and lit:2.0±0.4 pg/mL) and TG (B6:1.2±0.4 pg/mL, RQ:1.0±0.7 pg/mL and lit:2.5±0.9 pg/mL). The CGRP release in the dura mater, TG and TNC of RQ mice was not different from that of littermates. In the (TGVS), KCl induced the highest CGRP release in the TNC, which suggests that the brainstem may be more sensitive to chemical stimulation than other structures of the (TGVS). The migraine gene mutation did not affect release of CGRP induced by KCl in the different components of the TGVS in our study .interestingly, an increase of CGRP release in the trigeminal ganglion of R192Q mice was found in younger mice of 4-6 weeks-old(1). This may suggest that Ca(v)2.1 Ca2+ channels mutation in neurons may cause a lower threshold to induce CGRP release at early age and that the threshold changed when animals get older. Future studies are needed to confirm this.

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Metabonomic profiling of intermittent hypoxia in a mouse model.

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Inflammation and oxidative stress (OS) are known to contribute to the pathophysiology of OSAS (obstructive sleep apnea syndrome). Different biomarkers are under evaluation but confounding factors exist. We evaluated metabolic alterations induced during acute and chronic intermittent hypoxia (IH) (a component of the OSAS) and identified potential urinary biomarkers. C57BL/6J mice were exposed to cyclic hypoxia (FIO_2 6% -21%, 30s/30s for 8h/d for up to 35 d). In comparison to sham animals, mice exposed to IH showed significant changes in the urine levels (collected over a 16h-period) of some intermediates of energy metabolism (decrease in citrate (Ci), succinate, isoleucine and pyruvate (Py) and increase in lactate and creatine (Cr)) (^{1}H NMR spectroscopy). These modifications were consistent with a perturbation of the aerobic metabolism and with a switch toward the anaerobic pathway. Partial least square discriminant analysis (PLS-DA) revealed also a decrease in methionine and taurine (Ta), as well as a fluctuation of allantoïn (Al) suggesting the presence of OS. To evaluate the kinetic of these changes, a comparison between urine samples collected in early and late days (d0-4 vs d7-34) was performed. Principal component analysis (PCA) revealed that the perturbations of citrate cycle were significantly more important in the early phase (decrease in Ci, Py and increase in acetoacetate, trans-aconitate, Cr). Recovery towards normal urine composition over time indicated a compensatory phenomenon which was associated with an adaptive response against the OS. Indeed, in the early phase, elevation in Al evidenced the increased OS. Return to normal Al levels in association with a decrease in Ta indicated an increase of anti-oxidative defense at late days. In conclusion, IH alters the aerobic metabolism and induces cellular OS. However, adaptive and compensatory processes are noticed over time and must be considered when interpreting experimental data. Al could be a potential biomarker to evaluate an imbalance OS in OSAS patients difficult to treat.

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Different administration protocols of UM206 and their effects on remodeling and cardiac function following myocardial infarction

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Activation of the Wnt/frizzled signaling pathway has a key role in wound healing and cardiac remodeling following myocardial infarction (MI). We have previously shown that Wnt/frizzled signaling blockade with UM206 immediately after MI suppresses adverse remodeling and prevents heart failure (HF) development in a mouse MI model. Here, we investigated the role of UM206 after short-time early treatment and long-term late treatment, in order to unveil the optimal anti-HF regimen. Swiss male mice were subjected to MI by left anterior descending coronary artery permanent ligation. Two different regimens were followed: a) short-time early treatment: 2 weeks of UM206 treatment followed by 3 weeks without treatment and b) long-term late treatment: 3 weeks without treatment followed by 5 weeks of UM206 treatment. UM206 was administered via an osmotic mini-pump (6μg/kg/day) and controls were administered normal saline. Early UM206 treatment led to improved end diastolic volume (178±6 mm3) compared to saline (214±20 mm3, $p<0.01$) or to late UM206 treatment (278±24 mm3, $p<0.01$). Both effects were less pronounced compared to the full UM206 treatment (132±30 mm3). Ejection fraction was improved in early (24.4±0.1%) and late (23.2±2.6%) treatment compared to saline (16.5±0.2, $p<0.01$ and 11.0±3.2%, $p<0.05$ respectively) but full treatment was the optimal (31.4±0.4%), $p<0.001$). Both short-time early and long-term late UM206-treatment regimens demonstrate a cardioprotective effect by halting the progression to HF. Nevertheless, full UM206 treatment immediately after MI is the one eliciting the most beneficial effects for the injured myocardium.

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Intercellular communication via connexin channels and radiation-induced bystander effects in brain endothelial cells.

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Evidence is accumulating that intercellular communication pathways can propagate radiation-induced effects from directly irradiated to non-irradiated neighboring cells, a phenomenon known as bystander effects. Intercellular communication can be mediated by channels composed of connexins (Cxs), i.e. gap junction channels that connect the cytoplasm of neighboring cells, and hemichannels that form a pore in the plasma membrane and contribute to paracrine signaling. We previously demonstrated that both types of Cx channels can propagate cell death in an in vitro glioma cell model. Most notably, the transfer of the Ca2+-mobilizing messenger IP3 through gap junctions appeared to be crucial for communicating cell death. We here aimed to explore the role of Cx channels in bystander effects (DNA damage and cell death) in brain endothelial cells (ECs) exposed to ionizing radiation. To address this issue, we optimized two in vitro models in which X-rays were applied to a well delineated zone of an adherent EC monolayer. We investigated kinetics of radiation-induced DNAdamage (γ-H2AX foci) and cell death in the irradiated and surrounding non-irradiated area in response to 1 and 20 Gy X-rays. Both doses resulted in the appearance of γ-H2AX foci in the non-irradiated area, whereas apoptosis was detected only after application of high dose X-rays. Moreover, we found that DNA damage preceded apoptosis and that the presence of bright fluorescent, persistent foci coincided with the apoptotic response. Interestingly, inhibition of Cx channels with carbenoxolone reduced the appearance of bystander foci in the non-irradiated area. We conclude that Cx channels play a role in radiation-induced bystander damage of brain ECs. EC dysfunction and the resulting vascular damage, in particular at the blood-brain barrier, contribute to the primary side effects of brain tumor radiotherapy because of the inevitable exposure of healthy surrounding brain tissue. Hence, inhibiting the intercellular communication mechanisms that underlie these bystander effects, might reduce these side effects.

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Unraveling the signaling and dimerization properties of CXCR3 isoforms and interplay with CXCR7 in the context of lioblastoma

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Chemokines are a superfamily of chemo-attractant cytokines playing critical roles in many pathophysiological processes including cancer. The biological effects of chemokines are mediated through seven transmembrane receptors coupled to G proteins (GPCR). Chemokine receptors are usually coupled to Gi but may also trigger further signaling through β-arrestins.Tumor and stromal cells express diverse patterns of chemokines and chemokine receptors. For instance, multiple evidences have linked the CXCR4-CXCL12 axis to initiation and progression of various types of cancer, including gliobastomas. This aggressive malignant brain tumor derived from glial cells is currently incurable, thus requiring innovative treatments. Recently, CXCR7 has been identified as another high-affinity receptor for CXCL12 but also CXCL11, a chemokine initially reported to bind exclusively to CXCR3. Interestingly, CXCR7 displays a propensity to form homo- and hetero-dimers with CXCR4. CXCR3 binds CXCL11, CXCL10 and CXCL9 inducing or inhibiting cell migration and proliferation or death depending on the cell type.These opposite behaviors were proposed to be a consequence of the existence of three splice variants, CXCR3-A, CXCR3-B and CXCR3-alt. Some studies put forward the possibility that CXCR3-A and CXCR3-B may be coupled to different G protein subtypes, triggering distinct signaling pathways. Nevertheless, no direct evidences of such differential coupling have been reported. Similarly to CXCR7, CXCR3-A was recently shown to heterodimerize with CXCR4. Several important questions regarding CXCR3 biology remain elusive. For instance, the precise signaling pathways of the different CXCR3 splicing isoforms, their ligand selectivity as well as the impact of receptor homo- and hetero-dimerization on ligand binding and signaling properties require deep investigation. The aim of this project is to decipher these molecular mechanisms in the context of glioblastoma pathophysiology.

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CBS/H2S Pathway Prevents Kidney Injury During Pharmacologically-induced Torpor in Natural Hibernators

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Introduction: Pharmacologically reducing the demand for oxygen is a promising strategy to minimize unavoidable hypoxia-induced injury such as ischemia/reperfusion injury during transplantation. Hibernating animals are able to suppress their metabolism and undergo repetitive cycle of cooling (torpor) and rewarming of body temperature without reperfusion injury. We recently observed that one of the protective mechanisms consists of endogenous H2S production by the enzyme cystathionine-β-synthase (CBS). Previously, we induced torpor in mice pharmacologically by injecting 5'-Adenosine monophosphate (5'-AMP). It is unknown whether 5'-AMP influences the CBS/H2S pathway. Therefore in this study, we investigated whether pharmacologically induced torpor leads to activation of CBS/H2S pathway and organ protection in natural hibernators.
Methods: i-button temperature loggers were implanted intraperitoneally (i.p.) in Syrian hamsters (Mesocricetus auratus) to record core body temperature (CBT) during the experiment. Following recovery from surgery, at one day before start of the experiment, hamsters were transferred to a climate controlled room set to 5°C. After one day of acclimatization, hamsters were injected i.p. with saline or CBS inhibitor, aminooxyacetic acid (AOAA; 100 mg/kg) and then i.p. injection of 5'-AMP (3.0 µmol/kg). Ten hours following injection of 5'-AMP hamsters were euthanized. Blood was drawn and kidneys were harvested for molecular and histological analyses.
Results: CBT dropped within 30 minutes of 5'-AMP injection and reached 7-8°C after 10 hours of injection in all groups of hamsters. Blocking CBS with AOAA caused increased renal damage in both AOAA groups compared to saline group. Furthermore, the expression levels of CBS and AMPK (AMP-activated protein kinase) were significantly decreased in both AOAA groups compared to saline group.
Conclusions: 5'-AMP-induced torpor is independent of CBS activation. However, it may be that the increased damage in AOAA groups is caused by blocking CBS, making CBS potentially promising for several clinical applications.

Ligand-independent Identification of orphan GPCR ß-arrestin binding

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G protein-coupled receptors (GPCRs) are involved in many physiological processes and constitute the target of around 30% of marketed therapies. Nonetheless, ~100 human GPCRs have no known ligand and are "orphan". This project focuses on defining signaling pathways of orphan receptors, in order to select adequate assays for ligand identification through screening. GPR27, a rhodopsin-like orphan, has been prioritized in our study due to its involvement in the regulation of insulin secretion, suggesting an important role in metabolism.In order to study signaling pathways of orphan GPCRs in a ligand-independent fashion, we developed a luciferase complementation assay to assess the binding of GPCRs to ß-arrestin. We designed a constitutively active mutant of GPCR Kinase 2 (GRK2mod) able to induce a phosphorylation of the intracellular tail, a phenomenon inducing ß-arrestin recruitment. In addition, we used the truncated mutant of ß-arrestin1 (ß–arr1T) generating more stable complexes and thus detecting weaker interactions. We validated our approach on the Rhodopsin-like ß2 adrenergic receptor (ß2AR), closely related to GPR27 and characterized by a transient arrestins interaction (Type A). We could detect this transient ß2AR-arrestin interaction with our assay by using ß–arr1T and GRK2mod. Secondly, we successfully confirmed the persistent (type B) interaction of ß-arrestin with CXCR7, a rhodopsin-like chemokine receptor. When applied to GPR27, we identified an inability of this receptor to interact with ß-arrestin, even in the presence of ß-arr1T and GRK2mod. This is similar to other Rhodopsin-like receptors such as the ß3 adrenergic receptor and thus we classified GPR27 as "atypical" for its interaction with arrestin. This methodology can be used to categorize all the Rhodposin-like orphan receptors with regard to ß-arrestin interaction (Type A, B or atypical). Furthermore, the investigation of GPR27 signaling pathways will be deepened by using other GRKs and using similar assays to identify its signaling partners.

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T-wave area as an additional predictor of response to cardiac resynchronization therapy.

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Purpose: Chronic heart failure patients with a left ventricular (LV) conduction delay, mostly due to left bundle branch block (LBBB), generally derive benefit from cardiac resynchronization therapy (CRT). However, approximately 30% of patients do not improve clinically after CRT. We investigated whether T-wave analysis can improve patient selection.
Method: Baseline 12-lead electrocardiograms (ECGs) and baseline and follow-up echocardiograms were recorded in 258 CRT recipients. CRT response was defined as absolute increase in LV ejection fraction (LVEF) of ≥ 5% after 6 months of CRT. Vectorcardiograms (VCGs) were constructed from the measured 12-lead ECGs using an adapted Kors method.
Summary of results: Logistic regression models indicated repolarization variables as good predictors of CRT response. The VCG-derived T-wave area had the best ability to predict CRT response, even better than QRS-wave area (odds ratio (OR) per 10 μVs increase 1.17 ($p < 0.001$) vs. 1.12 ($p = 0.001$) respectively). After dividing the study cohort in LBBB (n = 154) and non-LBBB patients (n = 90), T-wave area had especially predictive value in the LBBB patient group (OR were 2.77 ($p = 0.004$) vs 1.09 (NS) respectively). The ORs for echo-response in LBBB patients versus non-LBBB patients was 3.21 ($p < 0.001$). The ORs remained the same after adjustment of multiple covariates, such as gender, ischemia, age, hypertension, coronary artery bypass graft and the usage of diuretics and beta blockers.
Conclusion: In patients with LBBB morphology of the QRS complex, a larger baseline T-wave area is an important additional predictor of LVEF increase upon CRT.

Patient Resistance Artery Relaxing Responses to angiotensin AT2 Receptor Agonist Depend on the Type of Contractile Stimulus

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Aim: Test whether stimulation of angiotensin type 2 receptors (AT2R) reduces effects of various vasoconstrictor stimuli in resistance-sized arteries of cardiovascular disease patients.
Methods: From biopsies of the parietal pericardium, obtained during open cardio-thoracic surgeries and stored overnight in physiological salt solution (PSS), 4 arterial segments were micro dissected, mounted in wire myographs, and studied at a diameter (202 ± 6 µm) corresponding to a distending pressure of 100mmHg. Relaxing responses were recorded during submaximal contractions and compared to appropriate "time controls".
Results: 10 µM acetylcholine reduced contractile responses to 32mM K+, suggesting presence of functional endothelium. Angiotensin II (0.1 – 100 nM) caused transient contractions that could be prevented by 3 nM valsartan (AT1R antagonist) and that tended to be reduced by 10 µM PD123319 (AT2R antagonist). 24 mM K+, 0.1 µM U46619 (thromboxane A2 analogue) and 2 nM endothelin-1 (ET-1) caused submaximal contractions of comparable amplitude. Increasing concentrations of the AT2R agonist Compound 21 (C21, 0.1 – 100 nM) did not modify these contractile responses to K+ (n = 14) or ET-1 (n = 7). However, 100 nM C21 significantly reduced the contraction induced by U46619 by 53± 11 % (n = 9) and this relaxation was not influenced by valsartan.
Conclusion: A non-peptidergic AT2R agonist can relax intact pericardial resistance arteries of patients with ischemic heart disease and/or cardiac valve problems. This effect is either selective for mechanisms stimulated by thromboxane or inhibited by depolarization and ET-1, a potent stimulus of oxidative stress.

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Identification of modulators for SUCNR1 by screening of a SOSA library with a bioluminescent cAMP assay

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Succinic acid (SA), a metabolic component that takes part in the citric acid cycle, has been described as the cognate agonist for the orphan receptor SUCNR1 (GPR91). This receptor belongs to the G Protein-Coupled Receptor family (GPCR) that play an essential role in regulating many physiological functions and represent 30% of targets for currently marketed drugs. Several studies on KO models suggested different roles through the activation of SUCNR1. Nevertheless, the characterization of the pharmacology and physiology of SUCNR1 is limited by the lack of pharmacological tools.In order to identify ligands modulating SUCNR1 Gαi activity, we adapted a specific cAMP level measurement assay based on a modified luciferase fused with cAMP binding domain (Glosensor® Promega). Upon cAMP binding, conformational changes induce luminescent signal. This assay allows end-point and real time measurements. We designed a protocol compatible with the screening of 96-well plates distributed molecules libraries. We screened with our assay the Sigma LOPAC library (composed of 1280 active compounds that might have an activity on new targets at high concentration) on HEK293 expressing SUCNR1. In parallel, we docked a part of the ZINC database ‘‘lead-like’’ molecules against SUCNR1 built by homology modeling from the β2-adrenergic receptor (SA binding site described by He et al.). We performed a primary agonist screening on 1280 compounds of a SOSA library at two different concentrations (100-10μM) with a cAMP assay (Z’=0,4-0,6). For the primary screening, we set up a criterion of activity higher than 20% compared to WT cells. 114 compounds fitting these parameters were subjected to a secondary screening performed in triplicates. Results analysis provided 16 putative modulators of SUCNR1 Gαi activity. We followed a similar strategy in another screening to find candidates with an antagonist profile. The virtual screening has sorted 30 compounds with substantial affinity that are under thorough pharmacological investigations to confirm activity.

Neutrophil Gelatinase-Associated Lipocalin in rats with myocardial infarction; expression in the brain related to depression?

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Heart failure increases the risk to develop depression, which worsens prognosis. We recently showed that Neutrophil Gelatinase-Associated Lipocalin (NGAL), an indicator of prognosis in heart failure patients, is also found to be associated with depression in these patients. The objective of this study was to investigate the role of NGAL in depressive symptoms in a rat model of myocardial infarction. Male Wistar rats were subjected to coronary artery ligation (MI) or sham surgery. Three weeks ater,depressive symptoms were obtained from the open field test. Plasma levels of NGAL were measured with Elisa, and NGAL expression in the brain was obtained using immunohistochemistry. Plasma NGAL levels were increased related to infarct size ($r=0.78$, $p=0.013$, $N=9$). NGAL expression in the paraventricular nucleus of the hypothalamus was increased in MI compared to sham rats (45±7 vs 26±3 NGAL+ cells per high power field), irrespective of plasma NGAL levels. NGAL levels in cerebrospinal fluid were not altered. The increased number of NGAL positive cells in the paraventricular nucleus of the hypothalamus was negatively correlated to exploratory behavior in the open field ($r=-0.77$, $p=0.006$). A decrease in exploratory behavior in the open field can be considered a sign of depressive behavior. Data indicate that while circulating NGAL may rather represent severity of heart failure, locally expressed NGAL in the brain may be associated with depressive symptoms after myocardial infarction in rats.

Defective autophagy in vascular smooth muscle cells promotes neointima formation via induction of senescence

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Autophagy, a life-sustaining process for lysosomal degradation of long-lived or damaged organelles and proteins, has been implicated in the pathogenesis of multiple cardiovascular diseases. Recently, we have gathered evidence for the occurrence of autophagy in VSMCs in the diseased vasculature. Hence, we investigated the role of autophagy on SMC survival and phenotype and its significance in the development of arterial stenosis. Deletion of the essential autophagy gene Atg7 in VSMCs (Atg7F/FSM22α-Cre+ VSMCs) promoted the induction of stress-induced premature senescence as shown by cellular hypertrophy, a G1 proliferative arrest and increased collagen synthesis. Moreover, Atg7F/FSM22α-Cre+ VSMCs were resistant to oxidative stress-induced cell death as compared to controls. This effect was attributed to nuclear translocation of the transcription factor Nrf2 resulting in upregulation of several anti-oxidative enzymes including GSTα and NQO1. To investigate the effect of defective autophagy in VSMCs on neointima formation, the left common carotid artery was ligated for 5 weeks in Atg7F/FSM22α-Cre+ and control mice. Atg7F/FSM22α-Cre+ mice showed more stenosis (98±1%) as compared to controls (49±7%).Lesions were characterized by increased collagen deposition, a typical feature of cellular senescence. We conclude that autophagy is crucial for VSMC function, morphology and survival. Moreover, defective autophagy in VSMCs contributes significantly to post-injury neointima formation via the induction of senescence, implying that autophagy inhibition in VSMCs would be unfavorable as therapeutic strategy.

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Amiodarone and dronedarone increase KIR2.1 ion channel expression by backward trafficking inhibition

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Drug induced ion channel trafficking defects can be pro- or anti-arrhythmic. KIR2.1 inward rectifier channels become internalized via the clathrin mediated endocytosis pathway and degraded in lysosomes. Amiodarone and dronedarone are class III antiarrhythmics used in atrial and ventricular fibrillation therapy. Amiodarone is effective in long-term arrhythmia treatment. Studies demonstrate detrimental effects of amiodarone and dronedarone on cargo trafficking through the late endosome/lysosome compartments. We demonstrated earlier that some inhibitors of backward trafficking, i.e. chloroquine and dynasore, functionally increase inward rectifier current. We hypothesize that both amiodarone and dronedarone impair KIR2.1 degradation (backward trafficking) thereby inducing increased levels of KIR2.1 protein and IK1.Inward rectifier current density in isolated rabbit ventricular cardiomyocytes was measured by patch clamp electrophysiology. KIR2.1 trafficking in HEK293 cells was studied by confocal laser scanning immuno-fluorescence microscopy and Western blot following pharmacological intervention with amiodarone (2-20 μM) and dronedarone (2-10 μM) at concentrations that do not affect inward rectifier currents in the acute phase. Quantitative RT-PCR was used to determine KIR2.1 mRNA levels. Amiodarone and dronedarone dose dependently increased full-length KIR2.1 expression (1.4±0.1, 1.7±0.1, 2.1±0.2 and 3.5±0.5 fold for 2, 5, 10 and 20 μM amiodarone; 1.4±0.1, 2.2±0.3 and 5.5±1.2 fold for 2, 5 and 10 μM dronedarone, respectively), while mRNA levels were unaffected. KIR2.1 protein upregulation was observed within 6 hours, while maximal responses were achieved after 24 hours. CLSM fluorescence microscopy indicated dose dependent accumulation of KIR2.1-GFP in the late-endosomal compartment. Finally, increased IK1 was observed following 24 h dronedarone (2 μM) or amiodarone (5 μM) treatment. Amiodarone and dronedarone inhibit degradation of KIR2.1 inward rectifier channels and increased IK1 by interfering in backward trafficking at the level of the late endosome.

Postoperative cognitive dysfunction in the elderly: involvement of neuroinflammation

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Postoperative cognitive dysfunction (POCD) is a complication that may occur after surgery, at all ages, but becomes persistent mainly in the elderly. Although the underlying mechanisms are still to be determined, our previous studies indicate that aged rats show long term surgery-induced changes in affective behaviour that may be associated with a prolonged increase in microgliosis following surgery.To investigate effects of surgery on cognitive performance, we subjected 3 months (young, n=12) and 25 months (aged, n=12) old rats to abdominal surgery with ischemia reperfusion of the upper mesenteric artery and assessed performance in the Morris water maze (MWM) and novel object recognition (NOR) test in postoperative week 2. Age matched rats that remained naïve (n=24) served as control. To further study the mechanisms underlying POCD we analysed IL-1B, microglia activation, BDNF and DCX levels in the hippocampus of young rats in the first 3 weeks following surgery. Compared to young rats, aged controls showed decreased spatial learning and memory. Whereas surgery impaired spatial memory in both young and aged rats, it led to a decreased NOR in aged rats only. Young rats display an increase in IL-1B and microglia activation in the first week after surgery only, and a decreased concentration of BDNF and DCX in the hippocampus that lasts for at least two weeks. Concurrent with POCD in humans, our rodent model surgery leads to more extensive and generalized cognitive impairment in aged compared to young rats. Our results in young rats indicated that postoperative neuroinflammation may lead to cognitive impairment by modulation BDNF and neurogenesis (DCX). Further analysis of these pathways in young and aged rats after surgery is necessary to gain a better understanding of the mechanisms involved in POCD in the elderly.

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Sumatriptan non-responders: assessment of a possible Biomarker

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Sumatriptan is a frequently applied, acutely acting anti-migraine treatment, yet about 20-30% of migraine patients the do not respond to this drug. TRPV1 channels on sensory nerve endings of the trigeminal nerve release the vasodilator calcitonin gene-related peptide (CGRP) during migraine attacks, while, supposedly, stimulation of the presynaptic 5-HT(1B)/1D receptor by sumatriptan inhibits this release. We developed a model to study trigeminal CGRP release in humans. Capsaicin (CAP), the active ingredient of hot chili peppers, stimulates TRPV1 channels, causing CGRP-dependent vasodilatation, whereas electrical stimulation (ES) induces TRPV1 channel-independent vasodilation. We investigated the effect of sumatriptan and placebo on the rise of dermal blood flow (DBF) of the forehead skin (innervated by the trigeminal nerve) by CAP application (0.6 mg/ml) and ES (0.2-1.0 mA, each current stimulation lasting 1 min) before and after subcutaneously placebo and sumatriptan injection one week apart in a randomized, double-blind, placebo controlled cross-over study, including healthy male (n=11, age ± SD: 29 ± 8 yrs) and female (n=11, 32 ± 7 yrs) subjects. DBF was measured with a Laser Doppler Imager. DBF responses to CAP were significantly attenuated after sumatriptan (mean decrease ± SEM in DBF: 82 ± 18 A.U., $p < 0.001$) but not after placebo (mean decrease in DBF: 21 ± 12 A.U., $p = 0.1026$), whereas DBF responses to ES were not affected by sumatriptan or placebo. Compared to placebo sumatriptan increased blood pressure by 6 ±2/11 ± 2mmHg, $p < 0.001$). We conclude that sumatriptan inhibits the forehead DBF response to CAP but not to ES. Sumatriptan may inhibit the release of CGRP via the stimulation of the presynaptic 5 HT1B/1D receptor and/or by a direct effect on TRPV1 channels. ES appears to be a nonspecific stimulus, most likely because besides CGRP it also released other neuropeptides.

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Blood pressure development after perinatal stimulation of the renin angiotensin system in Cyp1a1Ren2 rats

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In male transgenic cyp1a1Ren2 rats the mouse renin 2 (mRen2) gene is placed under the transcriptional control of the cyp1a1 gene. Adding indole-3-carbinol (I3C) to the food will lead to liver enzyme induction of cyp1a1 and hence to elevated circulating mouse renin. When I3C is added transiently, from weeks 4-8 of age, then hypertension develops, which is sustained into adult life even after I3C is withdrawn from the food. Maintenance of hypertension in this rat model is associated with increased renal resistance, renal inflammation and irreversible renal damage (Heijnen et al. 2013). Conversely if the renin angiotensin system (RAS) is transiently inhibited in spontaneously hypertensive rats (SHR), again at young from 4-8 weeks by pharmacological treatment, then hypertension development is permanently inhibited and associated with a reduction of organ damage (Bauman et al. 2007). These studies suggest that, at least in rat models for hypertension, RAS activation/inhibition at young age is a crucial determinant for the blood pressure level in later life.
The current study was designed to examine if RAS activation during uterine development would also lead to permanent cardiovascular adaptations in Cyp1a1Ren2 rats. Since I3C is able to cross the placenta, pregnant dams were treated with I3C during gestation up to 10 days after birth of the pups. The male offspring was followed up for 12 weeks. Tail cuff blood pressure was measured repeatedly and renal arterial resistance was measured under anesthesia at 12 weeks. To determine if intra-uterine I3C treatment would induce the cyp1a1 gene and enhance the transcription of RAS components, livers were harvested in rats one day after birth. In these animals cyp1a1 mRNA levels were 26 x higher than in non-treated controls. Remarkably ren2 (rat) levels were significantly down regulated to 0.74±0.05 x the level observed in non-treated controls. ACE mRNA levels were unaltered (0.98±0.11 x control). The age-dependent related increase (between weeks 5-12) in body weight and systolic blood pressure was not different between perinatally I3C and non-I3C treated control rats. This was confirmed by intra-arterial measurements at 12 weeks of age. However, left kidney weight (in g/kg body weight) was significantly elevated (I3C, 3.72±0.18, n=9 versus control 3.47±0.18, n=7 in g/kg) in perinatally I3C-treated rats. Also renal vascular resistance (in mmHg.min.gram/ml) was significantly ($p=<0.05$) higher in the perinatally I3C-treated animals (21.4±5.0, n=9) than in the controls (16.4±2.4, n=7). These data suggest that, in cyp1a1mRen2 rats, perinatal activation of the RAS system can induce a mild change in the cardiovascular phenotype later in life characterized by an increased renal size and increased renal vascular resistance. The magnitude of these changes was not sufficient to result in systemic hypertension.

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A resurgent current in Kv3.1 channels reconciles fast deactivation with complete action potential repolarization

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The shape of the action potential (AP) can be tuned by expressing a selected subset of voltage-dependent potassium (Kv) channels. Members of the Kv3 family have been linked to high-frequency AP firing because of their high threshold of channel gate opening and fast closure kinetics. Although fast closure prevents the membrane from being repolarized to strongly (affecting subsequent AP generation), the probability of incomplete membrane repolarization increases. We show here that Kv3.1 channels bypass this problem by displaying a very steep voltage dependence in their channel kinetics and having very fast voltage-sensor domain (VSD) relaxation that develops before channel opening and slows down VSD return upon repolarization.Therefore, upon short membrane depolarizations the Kv3.1 channel displays a previously uncharacterized resurgent (hooked tail) current upon membrane repolarization (in the voltage range between -30 and 0 mV). This resurgent current disappeared when the membrane depolarization was sufficiently long to open the channels completely. This unique Kv3 behavior could be transferred onto the Shaker channel by transplanting the S3S4 linker of the VSD and is not linked to an underlying inactivated state as is the case for the hERG channel. We conclude that the presence of a resurgent current in Kv3 channels is an important feature for high-frequency AP firing as it secures complete membrane repolarization during the falling phase of the AP that normally works as a negative feedback mechanism on channel closure.

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The NO-cGMP and NOX-ROS balance in post-stroke blood brain barrier stabilisation and anti-inflammation and therapeutic applications

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In many settings of ischemia, vasodilation to increase perfusion is a suitable approach; yet in stroke it bears the risk of systemic hypotension, shunting of blood from the ischemic to healthy areas, increased infarcts and eventually reduced survival. One potentially innovative mechanism-based approach are sGC activators, as they represent disease-specific vasodilators that are potentiated under conditions of oxidative stress and have microvascular selectivity. Moreover, inhibitors of NADPH oxidases have the potential of preventing breakdown of the blood brain barrier (BBB). Here we show that in the experimental mouse stroke model of transient middle cerebral artery occlusion, post-stroke treatment with the sGC activator, BAY 60-2770, increased cerebral blood flow, prevented breakdown of the BBB and decreased apoptosis and infarct volumes. This was associated with an increase in a biomarker of sGC-cGMP signalling, P-VASP. As expected this increase in cGMP was not affecting oxidative stress directly but surprisingly exerted a potent anti-inflammatory response on microglia and neutrophils and inhibited neuronal apoptosis. Systemic blood pressure, a potential confounder, was not affected. Importantly, also survival was dramatically enhanced and despite the severity of the model 50% of the animals survived after 7 days. When analysing specific sources of ROS that may lead to sGC oxidation and haem loss, we observed that in NADPH oxidase 4 (NOX4) KO mice, P-VASP levels were dramatically increased versus wild-type mice. Thus in stroke NOX4-derived oxidative stress may contribute to sGC oxidation and haem loss and subsequent apo-sGC activation represents a disease-specific therapeutic option. Alternatively NOX4 is a potential detrimental source of ROS in stroke. We further delineated the responsible cell-type by generating endothelial and vascular smooth muscle specific KO mice. The protection of global KO mice was nearly completely preserved in eNOX4 KO suggesting that endothelial ROS and cGMP have antagonistic effects in stroke and could be synergistically targeted.

Evaluation of the cardiomyotoxic effects of doxycycline overdose in calves using 2-dimensional speckle tracking.

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Background: Accidental doxycycline overdose in calves is suspected to be associated with left ventricular (LV dysfunction. However, classical Doppler echocardiography failed to document such a dysfunction in an experimental model.Two-dimensional-speckle tracking echocardiography(2DST) proved to be useful in the evaluation of LV dysfunction in numerous species. The goal of the present study was to evaluate the cardiotoxic effects of experimental overdose of doxycycline in calves using 2DST.
Material and methods: Ten healthy male Holstein calves divided in 2 groups. In group 1, 5 calves (mean age 58.0+/-16.3 days; mean body weight 72.2+/-13.0 Kg) received 25 mg/kg of doxycycline (5 times the recommended dose) orally for 5 days. In group 2, 5 calves (mean age 56.4+/-15.7 days; mean body weight 73.4+/-7.0 Kg) received a placebo orally for 5 days. Electrocardiography (ECG) and 2DST were performed at day 0 and day 8. ECG traces were analysed for occurrence of arrhythmias. 2DST measurements included global, segmental, and averaged peak values for radial and circumferential strain (SR, SC), radial and circumferential strain rate (SrR, SrC), rotation (Rot), rotation rate (RotR) and radial displacement (DR). The observer was blinded for the treatment at all time.
Results: All calves completed the study. ECG recordings were unremarkable in both groups. Heart rate was neither significantly different between groups nor before and after treatment. LV function was affected in calves receiving an overdose of doxycycline as shown by a significant decrease in segmental SR ($p<0.05$), SC ($p<0.05$) and DR ($p<0.05$) for several segments in treated calves when compared to the placebo group. The SrC for peak E at the level of the septal segment and the Rot at the level of the inferior segment were also significantly decreased(all $p <0.05$) after treatment.
Conclusion: Doxycycline overdose in calves seems to induce a left ventricular dysfunction in systole, and to a lesser extent in diastole.

Relaxations in pericardial resistance arteries of CVD patients to endothelium-dependent vasodilators and insulin

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Endothelial dysfunction is proposed to be involved in the development of cardiovascular diseases (CVDs) but marked relaxing responses to shear stress can be observed in coronary resistancearteries (rAs) of CVD patients (Liu et al. Circ. Res. 108, 566,2011). We tested whether responses to endothelium-dependent agonists are also maintained in more peripheral rA of CVD patients. A biopsy of the parietal pericardium was obtained during coronary artery bypass and/or cardiac valve related surgery. From this tissue, we isolated rA segments (Æ at 100 mmHg: 198 ± 8 µm; n = 134, N = 37) that were investigated in wire myographs. During contraction induced by an EC50 concentration (2 nM) of endothelin-1 (ET-1, Emax: 1.5 ± 0.1 N/m), acetylcholine (EC50 and Emax: 148 ± 28 nM and 70 ± 3 %), bradykinin (EC50 and Emax: 1.8 ± 0.1 nM and 110 ± 1 %) and insulin (EC50 and Emax: 10 pM and 105 ± 13 %) induced potent and marked relaxing responses, in spite of the CVD profile of the patients. The relaxing responses to the endothelium-dependent agonists were not significantly reduced by the combined presence of 100 µM L-NAME (NOS-inhibitor), 10 µM indomethacin (COX-inhibitor), 1 µM UCL 1684 and 1 µM TRAM-34 (inhibitors of small- and intermediate conductance calcium-activated K+-channels) plus 10 µM ODQ (inhibitor of soluble guanylatecyclase). Our observations suggest compensatory upregulation of alternative vasodilator pathways in peripheral resistance arteries of CVD patients. The mechanisms of these pathways remain to be established.

Study of sexual development in ewe lambs under altitude conditions in a non-seasonal country

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In order to study sexual development in ewe lambs, of four different Colombian breeds, one native (Colombian Creole) and three foreign adapted under altitude conditions (Romney Marsh, Hampshire, Corriedale), a longitudinal study was designed to estimate onset of puberty and regularity of estral cycles. Seven ewe lambs of each breed were followed up daily from four to twelve months of age with the aid of a teaser ram in order to estimate time at puberty defined as the day of first mount by the ram while regularity was considered the sequence of at least three consecutive regular estral cycles (14-20 days) with assessment of Blood Progesterone levels (BPL) and sexual behaviour. Blood samples were collected once a week and BPL was measured by competitive ELISA (DS-EIA-Steroid-Progesterone). In all breeds, mean onset of heat (first mount) occurred at 6±1.88 months, beginning of ovarian activity assessed by BPL (mean age 6.7±1.6 months) and regular heats were recorded at 8.8±1.51months. Median BPL were higher in Creole (2.44 [0.63-3.27]ng/ml) and Hampshire ewes (2.34 [0.60-3.21]ng/ml) than in Romney (2.15 [0.51-3.23] ng/ml) and Corriedale (2.22 [0.42-3.17] ng/ml) ($p<0.05$).First mount and the beginning of ovarian activity assessed by BPL was coincident in 61% of all ewe lambs (Criolla 42%, Romney 85%, Hampshire 71% and Corriedale 42%). The time lapse between first heat and onset of regularity was characterised by a majority of short cycles (<14 days) in Criolla and Romney (48%) whereas extra-long cycles (27-37 days) predominated in Hampshire 38% and Corriedale 47%. The percentage of adult body weight at which the ewe lambs accepted first mount was 61±12.7% and regularity of cycles was reached at 72±9.5%.In conclusion, this study did not to evidence breed related differences of onset by puberty and onset of regular ovarian cyclicity in four Colombian sheep breeds. Behavioural and hormonal detection of ovarian cyclicity were in agreement in around 60% of oestrus detection. The native Creole breed was characterized by a predominance of short cycles.
Key words: Puberty.

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n-alkanols operate outside the K+ pore and inhibit Kv channels by immobilizing voltage sensor movement.

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Voltage-gated K+ (Kv) channels transition from closed to open upon changes in the membrane potential, which they detect with their voltage-sensing domains (VSDs) whose response generates a transient gating current. General anesthesia's such as 1-alkanols (e.g. 1-butanol and 1-hexanol) inhibit Kv channels from the intracellular side through a yet unidentified mechanism.Both 1-butanol and 1-hexanol inhibited the ionic currents of the Shaker Kv channels in a concentration dependent manner with IC50 values of approximately 50 mM and 5 mM, respectively (both having a Hill factor of 1). Testing their effect on the gating currents of the non-conducting Shaker-W434F channel indicated that both compounds immobilized approximately 20% of the gating charge and markedly accelerated the deactivation kinetics. This indicated that 1-alkanols prevented the final VSD movements that are linked with channel opening and carry the final 20% of gating charge. These results were similar to the effect of 4-amminopyride (4-AP) but competition experiments showed that both compounds have structurally different binding sites. Likewise, the polycyclic ether toxin gambierol, which immobilizes VSD movement totally, competed only partially with 1-alkanols. The presence of three distinct binding sites and unique gating modifying mechanisms was strengthened by testing all three compounds on the Shaker-P475A mutant. Whereas Shaker-P475A remained sensitive to gambierol, the affinity for 4-AP was drastically reduced. Remarkably, both 1-butanol and 1-hexanol shifted the late gating charge component towards more hyperpolarized potentials effectively activating the ionic currents of Shaker-P475A instead of inhibiting them. We conclude that 1-alkanols act a gating modifiers and prevent Shaker Kv channels from passing the late subunit-cooperative transition step leading to channel opening through a unique mechanism and distinct binding site different from that of 4-AP and gambierol.

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Autophagy deficiency in smooth muscle cells alters vascular function without affecting blood pressure

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Autophagy degrades intracellular components via the formation of autophagosomes which fuse with lysosomes to protect the cell against the accumulation of damaged cytosolic material. Although defective autophagy in cardiomyocytes leads to cardiac disfunction, the consequences for smooth muscle cell (SMC) function remain poorly understood. SMC contractility and calcium homeostasis (organ chambers), arterial stiffness (myograph) and peripheral blood pressure (tail cuff) were investigated in aortic segments of 3.5 month old mice containing a SMC-specific deletion of the essential autophagy gene Atg7 (Atg7F/F SM22α-Cre+) and compared with corresponding controls (Atg7+/+ SM22α-Cre+). Vascular reactivity experiments revealed that L-type calcium channels of Atg7F/F SM22α-Cre+ mice were more sensitive to depolarization (EC50: 17.9±0.4 vs. 20.7±0.4 mM KCl, $p<0.001$, n=5). However, there was no difference in sensitivity for the L-type calcium channel blocker diltiazem (logEC50 Atg7F/F vs. Atg7+/+: -6.08±0.08 vs. -6.22±0.09 logM, p=0.245, n=5).Inositol triphosphate-mediated transient contractions after addition of phenylephrine, were significantly higher in Atg7F/F SM22α-Cre+ mice (AUC: 146±19 vs. 84±9 mN*s; $p<0.01$, n=5). In addition, expression of the sarco/endoplasmic reticulum calcium ATPase-2 (SERCA2) was also increased. Interestingly, higher basal cytosolic calcium concentrations were observed in SMCs of Atg7F/F SM22α-Cre+ mice, which could be abolished by the non-selective cation channel blocker 2-aminoethoxy diphenylborate. Despite these vascular differences, there were no effects observed on peripheral systolic and diastolic blood pressure (Atg7F/F vs. Atg7+/+: 107±5 over 68±1 vs. 110±9 over 70±8 mmHg, n=4). However, stress-strain experiments showed that arterial stiffness was slightly increased in segments of Atg7F/F SM22α-Cre+ mice.Overall, our study indicates that SMC autophagy plays an important role in the vasculature and that an autophagy defect in SMCs leads to changes in vascular function without affecting blood pressure.

Electrophysiological and hemodynamic effects of Vernakalant and Flecainide in dyssynchronous canine hearts

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Introduction: Ventricular conduction disturbances, in particular left bundle branch block (LBBB), are frequently observed in patients with heart failure who are prone to atrial fibrillation (AF). Vernakalant and Flecainide are used to convert AF to sinus rhythm. We compared the electrophysiological and hemodynamic effects of these drugs in canine dyssynchronous hearts.
Methods: Experiments were performed in dogs with LBBB, n=6 with Vernakalant and n=6 with Flecainide. Extensive epicardial mapping and hemodynamic measurements were performed before and after drug infusion. The degree and direction of dyssynchronous activation were quantified using the epicardial contact mapping electrodes to calculate the vector amplitude (VA-CM) and vector direction (VD-CM). For both drugs a two-dose regime was used in order to achieve clinically used plasma levels.
Results: Both drugs caused a uniform prolongation of ventricular conduction, as derived from an increase in VA-CM (30±2% after Flecainide vs. 16±10% after Vernakalant) but with a similar activation pattern (Figure) and unchanged VD-CM (-1±3% vs -1±1%). QRS width increased significantly more after Flecainide compared to Vernakalant (34±15% vs. 17±9%), while PR interval equally prolonged (17±15 vs. 17±13%). Flecainide and Vernakalant similarly reduced LV dP/dtmax (15±9 vs. 17±4% respectively).
Conclusion: In the dyssynchronous canine heart Vernakalant has less effect on ventricular conduction than Flecainide, but with an equal atrial/AV-nodal conduction delay. Both drugs had a similar, moderate negative effect on ventricular contractility. Accordingly, increased dyssynchrony cannot entirely explain the reduced contractility by Vernakalant.

Role of the monocytic inflammatory factors IL17D, IL27, IL32 and calprotectin in the gene regulatory response of HUVEC

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In a multicellular organism, e.g. human body, cell-to-cell communication is absolutely essential and the individual cells communicate with each other using various secreted proteins.Those proteins are also regulators of host responses to infection, immune responses and inflammation. In case of dysregulated protein secretion due to stress (e.g.) injury, chronic inflammatory diseases), the interaction between blood monocytes and vascular endothelial cells can result in enhanced monocyte recruitment, attachment and infiltration to the intima of the vessel wall. This phenomenon is for example described as the first step of the atherosclerotic process. The aim of the study is to investigate in a simplified approach the modulatory effects of secreted proteins involved in monocyte-endothelial cell communication. Based on a microarray analysis of TNF-α (20 ng/mL)-treated monocytic THP-1 cells, four proteins (IL17D, IL27, IL32,calprotectin) were used to stimulate primary human umbilical vein endothelial cells (HUVECs). Our results indicated that among the four proteins, IL27 induced the most significant differential gene expressions in HUVECs in a concentration dependent manner (10-30-100 ng/mL) of several cytokines (CXCL10, CXCL11, IL6, IL7, IL15). In order to identify potential calprotectin modulatory effects, time course experiments of HUVECs stimulated with IL27 and/or calprotectin were performed to further investigate the differential gene expression of the mentioned cytokines. These findings will be integrated to a study of the secretome using LC-MS/MS. This whole approach should give better insights in the inflammatory role of IL27 and calprotectin on secreted proteins by vascular endothelial cells in a inflammatory context.

Regulation of the neutrophil NADPH oxidase by the S100A8/A9 proteins

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Neutrophils have a pivotal role in the innate immune response and are designed to kill invading pathogens through the release of cytosolic granule contents and production of reactive oxygen species (ROS). While the inability of neutrophils to produce ROS leads a severe immune disease called Chronic Granulomatous Disease; an excessive and/or inappropriate activation of neutrophils results contributes to host tissue damage. ROS production by neutrophils is specifically catalyzed by the NADPH oxidase, a multicomponent enzyme system composes of a core membrane complex named cytochrome b558 (Nox2/p22) and cytosolic regulators. Recently, we and others have shown that the calcium binding proteins S100A8 and S100A9 are regulators of the NADPH oxidase. However, the precise mechanism of their interaction remains elusive. Our results show that in ex vivo as well as in vitro conditions the NADPH oxidase activity of Nox2 is increased in the presence of S100A8/A9 proteins. We further identify that the potentiation of Nox2 NADPH oxidase activity by the dimer S100A8/A9 is triggered by S100A8 but not by S100A9 proteins and requires calcium. Furthermore, S100 proteins co-localize with the cytochrome b558 and interact with it at the plasma. Using recombinant full length and truncated S100 proteins, we showed that the four strategic 87-HEES-90 amino acid residues of the S100A8 C-terminal sequence is essential in the interaction with the cytochrome b558 and therefore to it stimulation. Our data all together demonstrated an important role of the S100A8/A9 proteins in the regulation of the NADPH oxidase activity and suggest that S100 proteins may be implicated in pathological states while disregulated as observed in the synovial fluid of patients suffering from Rheumatoid arthritis. Therefore, deciphering the molecular mechanism of the NADPH oxidase regulation is an important step forward in order to be able to modulate its activity during inflammation processes and thus, to prevent the oxidative stress leading to pathological situations.

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NO-donating oximes induce erection through mechanisms other than those involved in arterial vasodilation

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Erectile dysfunction (ED) as well as many cardiovascular diseases are associated with impaired NO-bioavailability. Recently, oxime derivatives have emerged as vasodilators due to their NO-donating capacities. However, whether these oximes offer therapeutic perspectives as alternative NO-delivery strategy for the treatment of ED is unexplored.
Aims: This study aims to analyze the influence of formaldoxime (FAL), formamidoxime (FAM) and cinnamaldoxime (CAOx) on corporal tension and to elucidate the underlying molecular mechanisms.
Methods: Organ bath studies were carried out measuring isometric tension on isolated mice corpora cavernosa (CC), thoracic aorta and femoral artery. After contraction with norepinephrine (NOR), cumulative concentration-response curves of FAL, FAM and CAOx (100 nmol/L–1 mmol/L) were performed.
Main outcome measures: FAL-/FAM-induced relaxations were evaluated in the absence/presence of various inhibitors of different molecular pathways.
Results: FAL, FAM and CAOx relax isolated CC as well as aorta and femoral artery from mice. ODQ (sGC-inhibitor), DPI (non-selective flavoprotein inhibitor) and 7-ER (inhibitor of CYP450 1A1 and NADPH-dependent reductases) substantially blocked the FAL-/FAM-induced relaxation in the arteries, but not in CC. Only a small inhibition of the FAM response was observed with ODQ.
Conclusions: This study shows for the first time that NO-donating oximes relax mice CC. Therefore they are new molecules with potential for the treatment of ED. However, the underlying mechanism(s) of the FAL-/FAM-induced corporal relaxation clearly differ(s) from the one(s) involved in arterial vasorelaxation.

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Investigating the potential role of L-type calcium channels in the genesis of Parkinson's disease

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Dopaminergic neurons (DA) of the substantia nigra pars compacta (SNc) and ventral tegmantal area (VTA) are autonomous pacemakers. This activity is responsible for the sustained release of dopamine necessary for the proper functioning of target structures, such as the striatum. Most of the movement-related symptoms of Parkinson's disease are caused by a lack of dopamine due to the loss of dopamine-producing cells in the SNc.

Although it is well known that lack of dopamine causes the motor symptoms of Parkinson's disease, it is not clear why the dopamine-producing brain cells deteriorate. It has been suggested that the mechanisms of pacemaking are different in the SNc and VTA, with SNc DA neurons relying more on L-type calcium channels and VTA DA neurons on voltage dependent and leakage Na^+channels. Calcium entry through L-type calcium channels could cause oxidative stress in SNc DA neurons. Thus, a different calcium channel density/function in the SNc and in the VTA could be an explanation for their differential vulnerability in Parkinson's disease.

We decided to test this hypothesis by rigorously quantifying the density of L-type calcium channels in the soma DA neurons using nucleated patch-clamp recordings. This technique allows us to isolate the somatic membrane from the rest of the cell and thus to have a better control of the voltage. Moreover, the current amplitudes are bigger (+/- 40 pA in our conditions) than in cell-attached or conventional outside-out recordings, making pharmacological studies easier.

Our first results show a measurable amount of calcium current in the soma of SNc and VTA DA neurons. A part of this current seems to be sensitive to nifedipine suggesting that L-type calcium channels are present in the soma of these neurons. Moreover, the mean L-type calcium current amplitude seems to be higher in the SNc neurons than in the VTA.

Effect of exercise training on adiponectin multimeric forms in a mouse model of metabolic syndrome

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Adiponectin (Ad) is a hormone secreted by adipose tissue that regulates numerous metabolic processes. Ad prevents lipotoxicity, improves insulin sensitivity and exhibits anti-inflammatory and anti-atherosclerotic properties. This 30kDa-protein multimerise into low (LMW), medium (MMW) or high molecular weight (HMW) forms (Ad-mers). Ad has been intensely studied in various diseases including the metabolic syndrome. A reduced Ad plasmatic level ($[Ad]_{pl}$) was described in obesity with a selective depletion of HMW Ad-mers which are considered as the most active forms. Exercise training was suggested to increase $[Ad]_{pl}$ but these data as well as the effect on Ad-mers distribution are still controversial.
Here, we studied the effect of muscle conditioning on Ad-mers distribution in a model of metabolic syndrome. One group of C57BL6J mice (n=18) was fed with HFHS (High Fat-High-Sugar) diet and a second (n=19) with standard diet for 10 weeks. Nine mice from both groups were submitted to a training session on a treadmill, 5 days a week during the last 8 weeks. HFHS diet increase caloric intake, body weight, glycaemia (glucose tolerance test) and fat mass (MRI). Although no Ad change was observed at the mRNA level in the adipose tissue (RT-qPCR) and at the protein level in the blood (ELISA), HFHS diet induced a decrease of HMW forms (non-denaturant PAGE-SDS and Western blot). Exercise training promotes decreased glycaemia and a better caloric intake control in trained HFHS mice, resulting in a body weight similar to controls. No significant $[Ad]_{pl}$ variation, Ad-mers distribution or expression in adipose tissue was observed after training.
In conclusion, the HFHS mice recapitulate the key troubles of metabolic syndrome and exhibit a switch of Ad-mers distribution in favour of the less active forms. Exercise training reverses some adverse effects of the HFHS diet and the control of food intake as well as the glucose sensitivity were improved. Further analyses are needed to attribute this beneficial effect to Ad-mers levels.

Overexpression of APP23 in the brain of ApoE-/- mice enhances atherosclerotic plaque vulnerability via amyloid beta 1-40.

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Atherosclerosis is a chronic inflammatory disease and leads to development of plaques in large and medium-sized arteries. It is frequently associated with Alzheimer's disease (AD). Therefore, convergent disease processes are assumed to be the basis of both pathologies. Amyloid precursor protein (APP) plays a major role in the development of AD and can be proteolytically cleaved to amyloid beta 1-40 (Aβ1-40). The purpose of this study was to investigate whether selective overexpression of APP23 in the brain can influence plaque vulnerability in ApoE-/- mice and if Aβ1-40 plays a role in the disease process. This could reveal a link between atherosclerosis and AD. ApoE-/- (n=7) and APP23/ApoE--/-- (n=7) mice were fed a Western type diet for 10 weeks. The brachiocephalic artery (Abr) was collected for histological analysis. ELISA was performed to determine plasma concentrations of Aβ1-40. Western blotting was used for analysis of iNOS and APP expression in J774 macrophages treated with 0.05μM and 5μM Aβ1-40 for 24h. Plaque size in the Abr was smaller in APP23/ApoE--/- mice when compared to ApoE-/- mice. Collagen content was reduced from 41±2% in ApoE-/- mice to 31±3% in APP23/ApoE--/-- mice (p=0.015). The fibrous cap was thinner due to APP23 overexpression (ApoE-/- mice: 7.6±1.5 μm vs. APP23/ApoE--/-- mice: 4±0.7μm; p=0.024). The amount of macrophages present in the plaque was 7±1% in ApoE-/- mice and 12±2% in APP23/ApoE--/-- mice (p=0.098). APP expression tended to be higher in plaques of APP23/ApoE--/-- mice. Plasma analysis revealed that Aβ1-40 was present at higher concentrations in APP23/ApoE--/-- mice (112±4 pg/ml) when compared to the control group (93±4 pg/ml; p=0.006). Western blots of J774 macrophages showed that treatment with Aβ1-40 increased the expression of both iNOS and APP.
Conclusion: APP23 overexpression in the brain of ApoE-/- mice results in smaller, but more vulnerable atherosclerotic plaques. Furthermore, our data suggest a role for Aβ1-40 as a link between atherosclerosis and AD.

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Regulation of the neutrophils NADPH oxidase by the calcium-binding proteins S100A8 and S100A9

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Neutrophils play a fundamental role in host defense by neutralizing pathogens through the generation of reactive oxygen species by the NADPH oxidase (NOX2). Dysfunction of NOX2 contributes to inflammatory processes, which can promote the development of diseases such as atherosclerosis or rheumatoid arthritis. The activation of NOX2 is regulated by the integration of diverse signaling pathways, such as calcium signaling and kinase activation. Moreover, several studies in cell-free systems have provided evidence for the implication of two calcium-binding proteins of the S100 family, S100A8 and S100A9, in the regulation of NOX2 activity. These proteins could constitute the molecular switch between the receptor-activated, calcium-dependent signaling cascade and the activation of NOX2. The aim of our study was to characterize S100A8/A9-dependent mechanisms of NOX2 regulation in neutrophil-like HL60 cells. First, we confirmed the involvement of S100A8/A9 in the regulation of NOX2 activity by using a siRNA approach and we determined the requirement of calcium in this process by measuring simultaneously intracellular calcium concentration and H2O2 production. By studying the membrane recruitment of S100A8/A9, an important step for NOX2 activity, we found that calcium release from the intracellular stores is necessary but not sufficient for the translocation of S100A8/A9. By opting for both a pharmacological and an siRNA approach, We were also able to involve calcium-independent phopholipase A2 (iPLA2) and the sphingosine kinases (SphKs) as two novel determinants in S100A8/A9 translocation and subsequent NOX2 activation. In conclusion, we showed that NADPH oxidase activity is regulated by calcium-dependent S100A8/A9 translocation, through iPLA2 and SphKs.

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The effect of stabilized angiotensin-(1-7) analogue, Cyclic angiotensin-(1-7), on progenitor cell recruitment and cardiovascular function

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In animal models, angiotensin-(1-7) (Ang-(1-7)) has beneficial effects through its own AT1-7/Mas receptors improving cardiac function and remodelling after myocardial infarction (MI). Ang-(1-7) might have a beneficial angiogenic effect after MI, since infusion increases the number of endothelial progenitor cells in the heart. Ang-(1-7) also promotes the *in vitro* differentiation of endothelial progenitor cells, and tube formation. Despite these promising results, Ang-(1-7) may not be optimal for use in patients because it is rapidly metabolised in plasma and tissue. For optimal clinical use, metabolically stable 4-Ser,7-Cys-thioether-bridged angiotensin-(1-7), called cyclic angiotensin-(1-7) (cAng-(1-7)), has been developed.
Angiogenic progenitor cell recruitment was measured 24h after a bolus injection of 50µg/kg cAng-(1-7) in healthy C57BL/6 mice by flow cytometric counting of cKit+, Sca-1+ and Flk1+ cells in blood and bone marrow. In addition, mice underwent coronary ligation or sham surgery and either saline, 5 or 50µg/kg/day cAng-(1-7) infusion. After 3 or 9 weeks of treatment, cardiac function was measured by intraluminal pressure-volume catheter. Aortic endothelial function was measured by Mulvany myograph. Mesenteric arteries were mounted in organ baths to access the myogenic tone. Cardiac vascular density, fibrosis and myocyte dimensions were assessed. Furthermore, we studied the dose-related effects of cAng-(1-7) on tube formation by HUVEC. cAng-(1-7) increased blood cKit+, Sca-1+ and Flk1+ cells, which tended to decrease in bone marrow. Besides cAng-(1-7) reduced cardiomyocyte hypertrophy compared to saline, but showed no effect on heart weight, infarct size, fibrosis and vascular density after 3 weeks of treatment. Furthermore cardiac and thoracic aortic function were not improved by cAng-(1-7). Tube formation by HUVEC was reduced by cAng-(1-7) in a dose dependent manner.
Our results suggest that cAng-(1-7) given early after MI might not lead to improved angiogenesis, despite an increased recruitment of hematopoietic and endothelial-like cells. This might explain why there is no swift beneficial effect of cAng-(1-7) on cardiac performance despite a reduction of myocyte hypertrophy.

Long term FTY720 treatment attenuates vascular tone to S1P in resistance vessel of WK rat

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The immunosuppressant drug and S1P analog FTY720 (Fingolimod) has pleiotropic effects on the vasculature and heart, leading to changes in blood pressure and transient bradycardia in patients with MS. Although the acute effects of FTY720 on vascular function has been studied before in experimental models, the effects of long term FTY720 treatment on vascular function of resistance vessels is yet unknown. We therefore aimed at investigating myogenic constriction of mesenteric arteries in long term FTY720 treated rats. M&M Healthy Wistar rats were treated for six weeks with FTY720. At termination, blood pressure was recorded and myogenic constriction of intact and denuded small mesenteric arteries was determined in a vessel perfusion set up.
Results: Myogenic constriction of intact arteries in both control and FTY720 treated animals was low, but addition of S1P augmented myogenic constriction. Denudation of the arteries inhibited the responses to S1P in control rats by approximately 40%. However, in FTY720 treated animals, responses to S1P were completely blocked. Myogenic responses to PE, ACh and SNP were similar in all groups.
Conclusion: Long term treatment with FTY720 leads to unresponsiveness of the smooth muscle layer of mesenteric arteries to S1P. However in healthy animals, the impaired responsiveness to S1P of the smooth muscle layer can be compensated through increased S1P mediated endothelium dependent constriction

Effect of exercise training on adiponectin multimeric forms in a mouse model of metabolic syndrome

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Adiponectin (Ad) is a hormone secreted by adipose tissue that regulates numerous metabolic processes. Ad prevents lipotoxicity, improves insulin sensitivity and exhibits anti-inflammatory and anti-atherosclerotic properties. This 30kDa-protein multimerise into low (LMW), medium (MMW) or high molecular weight (HMW) forms (Ad-mers). Ad has been intensely studied in various diseases including the metabolic syndrome. A reduced Ad plasmatic level([Ad]pl) was described in obesity with a selective depletion of HMW Ad-mers which are considered as the most active forms. Exercise training was suggested to increase [Ad]pl but these data as well as the effect on Ad-mers distribution are still controversial. Here, we studied the effect of muscle conditioning on Ad-mers distribution in a model of metabolic syndrome. One group of C57BL6J mice (n=18) was fed with HFHS (High Fat-High-Sugar) diet and a second (n=19) with standard diet for 10 weeks. Nine mice from both groups were submitted to a training session on a treadmill, 5 days a week during the last 8 weeks. HFHS diet increase caloric intake, body weight, glycaemia (glucose tolerance test) and fat mass (MRI). Although no Ad change was observed at the mRNA level in the adipose tissue (RT-qPCR) and at the protein level in the blood (ELISA), HFHS diet induced a decrease of HMW forms (non-denaturant PAGE-SDS and Western blot). Exercise training promotes decreased glycaemia and a better caloric intake control in trained HFHS mice, resulting in a body weight similar to controls. No significant [Ad]pl variation, Ad-mers distribution or expression in adipose tissue was observed after training.
In conclusion, the HFHS mice recapitulates the key troubles of metabolic syndrome and exhibits a switch of Ad-mers distribution in favour of the less active forms. Exercise training reverses some adverse effects of the HFHS diet and the control of food intake as well as the glucose sensitivity were improved. Further analyses are needed to attribute this beneficial effect to Ad-mers levels.

Dopamine protects against DNA damage during cellular hypothermia and rewarming in cultured smooth muscle cells

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Introduction: Ischemia is a condition suffered by cells and tissues when deprived of blood flow due to inadequate nutrient and oxygen supplementation. This is mainly due to the rapid generation of reactive oxygen species (ROS) at the start of reperfusion and characterized by apoptotic cell death. Similarly, many mammalian cell types are vulnerable to prolonged and profound hypothermic storage related to a burst of ROS upon rewarming, which may be attenuated by treatment with dopamine. Cooling/rewarming is thus thought to represent ischemia/ reperfusion condition in cell lines. As oxidative stress is causing DNA damage, here we investigated whether cooling/rewarming injury leads to DNA strand breaks by Comet assay.
Methods: Smooth muscle aortic cells (SMAC) were cultured in 6 well plates until confluency, cooled at 4°C for 24 hours with or without subsequent rewarming to 37°C. Untreated cells (37°C) served as a control. Dopamine (30 uM) was added throughout the whole procedure, or during parts of the cooling-reperfusion protocol. Single and double strand DNA breaks, DNA cross-links and oxidative damage in single cells was assessed by Comet assay. Per condition > 60 Comets were analyzed and damage was expressed as the percentage of DNA in the Comet's tail (% TailDNA) using ImageJ software.
Results:Compared to control cells, the %TailDNA was substantially increased both by cooling and cooling/rewarming, as reflected by an increase in the median %TailDNA from 8.4% to 87.3% and 76.4%,respectively. Dopamine treatment throughout the protocol strongly reduced DNA damage, as evidenced by a substantial decrease in the median of %TailDNA to 49.9%. Notably, when treatment with dopamine was restricted to 30 min. prior to cooling, reduction in DNA damage was absent.
Conclusion: Our results demonstrate that dopamine protects SMAC cells from DNA damage induced by hypothermia/rewarming by protection of cells in the hypothermic and rewarming phases. Thus, dopamine represents a pharmacologically promising compound for several clinical applications that require hypothermia and

Intermittent pacing therapy attenuates infarct remodeling

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Background and Objective: Despite early revascularization strategies, left ventricular (LV) remodeling and dysfunction after acute myocardial infarction (AMI) remain therapeutic targets of great clinical significance. Intermittent pacing therapy (IPT) of the LV has been shown to reduce infarct size applied before or during early reperfusion. However, it is presently unclear whether IPT is capable of limiting LV remodeling independent of its protection against acute myocardial necrosis. To investigate this, IPT was performed starting 3 days post reperfusion in a porcine model of reperfused acute myocardial infarction.
Methods: Fifteen pigs underwent 2h ligation of the left circumflex coronary artery followed by reperfusion after which pacing devices were implanted and connected to LV-leads that were positioned in the peri-infarct zone. After three days, LV function, infarct size and infarct geometry were assessed with MRI and animals were stratified into Control and IPT groups (3 x 5 min VVI b.i.d. until follow-up). Thirty-five days post AMI, follow-up MRI was obtained and pigs were sacrificed. Infarct tissue was quantified for myofibroblast presence with α-smooth muscle actin (αSMA) histology and infarct remodeling-related gene expression was studied with quantitative polymerase chain reaction experiments (RT-qPCR). Blood plasma was longitudinally studied for markers of extracellular matrix (ECM) turnover and inflammation.
Results: IPT had no significant effect on global LV remodeling and function or infarct mass five weeks post AMI, but markedly influenced scar geometry. In all pigs, there was a significant reduction in infarct mass over time. In control pigs this reduction was principally due to a 26.2±4.4% reduction in infarct thickness, while in IPT pigs it was mainly due to a 35.7±4.5% decrease in the number of infarcted segments with no significant change in infarct thickness. No changes were observed in circulating markers for ECM turnover or inflammation, but myofibroblast content of the infarct-zone was higher in IPT (10.9±2.1%) compared to control pigs (5.4±1.6%; $P \leq 0.05$). Higher myofibroblast presence was confirmed with increased expression of αSMA but no further differences in expression of markers for infarct remodeling were found. Higher myofibroblast presence could not be explained with Wnt/Frizzled expression. Taken together,IPT applied 3 days post reperfusion significantly limited infarct expansion and changed infarct composition, suggesting that IPT positively influences healing of the infarct zone, likely by increasing myofibroblast content in the infarcted tissue.

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The maintenance mechanism of ventricular fibrillation determines defibrillation threshold of electric shock therapy by affecting

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Introduction: Early afterdeplorization (EAD) formation is one of the major mechanisms of the onset of cardiac arrhythmias, including fibrillation, and is associated with various cardiac pathologies. However, it remains incompletely understood how fibrillation is maintained and how to optimize the efficacy of electric shock-based defibrillation strategies.Recently, we identified the requirements for EAD-based fibrillation in an in silico model of human cardiac tissue by modeling of a wide range of clinical conditions. The aim of this work is to investigate termination of EAD-based fibrillation by electric shocks.
Methods: We used a bidomain version of the TNNP-TP06 (ten Tusscher, Panfilov 2006) model for human cardiac cells and simulated EAD-and non-EAD-based fibrillation. Next, we systematically determined the defibrillation threshold (DT) and efficacy of electric shock therapy, including the underlying ionic mechanisms.
Results: We show that there is a paramount difference in DT of EAD and non-EAD-based fibrillation . EAD defibrillation requires a 4 to 6 times higher DT. This result is valid in a wide range of conditions for both EAD and non-EAD types. In total more then 2000 simulations were performed. We have also investigated the mechanism of defibrillation failure, and found that is was related to the calcium dynamics of the system. Similar experiments were performed in models of fibrillation using monolayers of rat ventricular cardiomyocytes.
Conclusions: Ventricular fibrillation mediated by EADs required a 4-6 times higher DT than rotor-based fibrillation. Mechanistically, changes in intracellular calcium dynamics may be responsible for these striking differences. Further exploration and eventual implementation of these insights could help to further improve the efficacy of electrical defibrillation.

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Control of Nematode Peripartum increase in ewes and offspring at high tropical conditions in Colombia

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During peri-partum period there is a breakdown of immunity to gastrointestinal nematodes, a critical factor in the epidemiology of gastrointestinal nematode infections for small ruminants. The present study tested the benefit of anthelmintic treatment at peripartum in ewes and their offspring. 46 pregnant ewes were divided into 3 groups. 15 ewes(T1) received at 120 days of gestation, a single moxidectine (M) injection at a dose of 200µg/Kg, 15 ewes(T2) received the same dose of M at 48 hours after lambing and 16 ewes(C) remained untreated. A longitudinal study was implemented and characterized by clinical observations and nematode detection from fecal egg counts(FEC) before and after lambing. Lambs born to these ewes underwent fecal sample analysis after lambing until 60 days. In contrast to treated ewes, untreated ewes maintained significantly elevated FEC after lambing(T1 279+146epg, T2 660+197epg and C 1747+406epg) and thehighest mean value was reached at 15 days after lambing(3596+339epg, $p<0.01$). Clinical parameters such as diarrhea, body conditions, FAMACHA and body weight did not show significant differences between groups($p>0.05$). All lambs increased FEC within 60 days post-lambing, however, lambs born from C ewes reached the maximum FEC value at 45 days after lambing(2733+679 versus 1115+249 in T1 and 1023+229 in T2 lambs, $p<0.01$). Lambs' clinical parameters did not show significant differences between groups($p>0.05$). This study suggests that peripartum treatment against nematodes with moxidectin was efficient in ewes raised in Colombia. Periparturient rise of FEC was reduced although no clinical differences between treated and untreated animals were detected. The peripartum treatment of ewes had a protective effect on their lambs by delaying nematode infection. As all animals were kept on a same pasture during this study, an environmental impact of the treatment could be excluded. It is most likely that delayed nematode infection and FEC excretion in lambs was due to prolonged moxidectin absorption by milk from treated ewes.

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Beat-to-beat variability of repolarization in antiarrhythmic drug evaluation

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Introduction: Beat-to-beat variability of repolarization (BVR) quantified as short-term variability (STV) is a surrogate parameter that has been introduced and extensively studied by our department. Preclinical evaluation of this parameter has been primarily performed in the cAVB dog (chronically AV-blocked dogs) in a proarrhythmic setting with satisfying results. Over the years, also several antiarrhythmic drug efficacy evaluations have been performed in which also STV was determined. Here, we aim to evaluate the use of STV in antiarrhythmic efficacy testing. Methods: All antiarrhythmic experiments in the cAVB dog model and in isolated cAVB cardiomyocytes in which STV was analyzed were included. Experiments were conducted in a preventive or suppressive setup, for some antiarrhythmic drugs both settings were applied in the same animals. An animal was considered inducible when 3 or more TdP episodes of least 5 beats occurred. Drug efficacies are categorized as low (< 50%), intermediate (50-90%), or high (> 90%) based on the percentage of animals that remain or become free of TdP due to the treatment.
Results: In the cAVB dog, suppressive antiarrhythmic drug testing yielded 2 low (K201, AVE0118), 4 intermediate (levcromakalim, ranolazine, lidocaine, W7), and 3 highly (flunarizine, verapamil, SEA0400) effective drugs. In all categories, STV values corresponded to the antiarrhythmic efficacy whereas repolarization prolongation did not. Preventive drug testing resulted in 2 drugs in each category (low K201 and AVE0118; intermediate ranolazine and lidocaine; high flunarizine and verapamil), classification corresponded to the suppressive experiments of the respective drugs. In cellular experiments 4 drugs were 100% effective (flunarizine, SEA0400, ranolazine, W7) 2 of these drugs were categorized as intermediate in the in vivo experiments.
Conclusions: We showed that 1) in vivo prevention and suppression experiments provide similar results; 2) antiarrhythmic effects are comparable in vivo and in cellular experiments, and that 3) STV seems to be superior to QT/APD in

The combined IKr and INa blocker flecainide provides strong antiarrhythmic efficacy against class III-induced TdP arrhythmias

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Introduction: Flecainide displays IKr (IC50 1-2 μM) and INa peak and late (IC50 4-80 μM) blocking activity to be successful in the treatment of atrial fibrillation, but harboring the risk for ventricular proarrhythmia. Here we examined the antiarrhythmic efficacy against class III-induced ventricular arrhythmias with emphasis on ventricular activation and repolarization parameters in our cAVB dog model.
Methods: AV block was created by radiofrequency ablation in 12 mongrel dogs. The experiments were performed in anesthetized animals after completion of the electrical remodeling process (≥ 3 weeks). During the experiments the animals were paced from the right ventricular apex (CL 1000 ms). An animal was considered inducible when 3 or more Torsade de Pointes arrhythmias (TdP) episodes occurred.
Results: Dofetilide (25μg/kg/5') prolonged repolarization (QT: 390±43 to 613±56 ms $p<0.05$*; LVMAP: 262±26 to 441±116* ms) and increased STV (0.9±0.4 to 2.2±1.2* ms) without any effect on conduction, and induced TdP in 8/12 cAVB dogs. One dog died from persistent dofetilide-induced arrhythmias. Subsequent flecainide administration (3mg/kg/5') shortened QT and LVMAP to respectively 472±53 and 330±82 ms ($p<0.05$ vs dofetilide) at the expense of considerable QRS widening (109±22 to 139±27* ms).Flecainide suppressed the majority of TdP arrhythmias (6/7*), and single and multiple ectopic beats (5/7). In the non-inducible animals (n=4), flecainide suppressed multiple ectopic beats, while single ectopic beats persisted in 2 dogs. Accordingly, flecainide significantly decreased STV (2.2±1.2 to 0.7±0.4* ms). Of note, flecainide induced a severe decrease in blood pressure in the majority of animals and was in the absence of dofetilide not tolerable.
Conclusions: The combined IKr and INa blocker flecainide provided a robust antiarrhythmic effect against dofetilide-induced TdP arrhythmias. However, profound negative inotropic effects preclude 1) further increases in dose, and 2) preventive antiarrhythmic efficacy testing.

CXCL1 promotes arteriogenesis through enhanced monocyte recruitment into the peri-collateral space

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Aims: The mechanisms of monocyte recruitment to arteriogenic collaterals are largely unknown. We investigated the role of chemokine (C-X-C-motif) ligand 1 (CXCL1) and its cognate receptor, chemokine (C-X-C-motif) receptor 2 (CXCR2) inarteriogenesis.
Methods and Results: After femoral artery ligation in Sprague Dawley rats, either native collaterals were explanted or placebo, CXCL1 or CXCR2 blocker was administered via an osmopump. Perfusion recovery was measured with Laser Doppler, leukocyte populations were analysed by FACS and hind limb tissue sections were stained for macrophage marker CD68. In vitro, fluorescent CXCL1 or THP-1 monocytic cells were flown over shear stressed endothelium. CXCL1 mRNA expression in collaterals was dramatically upregulated already 1 hour after ligation. CD68 mRNA was upregulated from 12 hours until 3 days after ligation. CXCL1 treatment augmented perfusion recovery at 3 and 7 days ($p<0.05$) after femoral artery ligation and a significant increase in number of peri-collateral macrophages was evident concomitantly ($p<0.05$). Conversely, CXCR2 antagonist treatment caused a decrease in hind limb perfusion recovery both at 7 and 10 days post-ligation ($p=0.01$) and also significantly reduced the number of peri-collateral macrophages ($p<0.05$). In vitro, CXCL1 tethered to and was taken up by endothelial cells under shear stress conditions and consequently enhanced THP-1 adherence as compared to control ($p<0.05$). In contrast, CXCR2 antagonist compromised monocytic THP-1 adherence to endothelial cells ($p<0.05$).
Conclusion: CXCL1 binds to endothelial cells and subsequently leads to an increase in the number of peri-collateral macrophages, thus improving the arteriogenic response after arterial ligation.

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Inhibition of ER-stress induced autophagy preserves proteostasis and protects against cardiomyocyte dysfunction in AF

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Introduction: Atrial Fibrillation (AF) is characterized by its self-perpetuating nature, which is rooted in structural remodeling of cardiomyocytes, resulting in electric dissociation, contractile dysfunction and limited success of cardioversion.
Recent view on its pathogenesis suggest an important role for the derailment of protein homeostasis (proteostasis). Excessive autophagy, by degradation of proteins and organelles, could play an important role in derailed proteostasis in AF.
Methods: HL-1 mouse atrial cardiomyocytes (in vitro) were subjected to tachypacing, after which cells were used for western blot and qPCR. Atrial tissue from a canine in vivo model for AF and atrial tissue from patients with permanent AF were used for western blot. Calcium transient measurements were performed with HL-1 mouse atrial cardiomyocytes (in vitro) and Drosophila melanogaster (in vivo) treated with autophagy modulating drugs or subjected to HSPA5 overexpression.Results: Tachypacing of HL-1 atrial cardiomyocytes resulted in gradual and significant activation of autophagy, due to endoplasmic reticulum (ER) stress. The findings were extended to the canine model of AF and to human AF. In human AF, autophagy levels correlated with cTnI, cTnT, α-tubulin and correlated inversely with the amount of myolysis. In both HL-1 atrial cardiomyocytes and Drosophila melanogaster treatment with autophagy inhibitors (bafilomycin A1 and pepstatin A) or the ER stress inhibitor 4-phenyl-butyric-acid (4-PBA) showed prevention of calcium transient loss. In contrast, the mTORC1 inhibitor rapamycin and ER stress inducer tunicamycin were not protective. Furthermore, overexpression of the ER stress protein HSPA5 showed protection against calcium transient loss in HL-1 atrialcardiomyocytes.
Conclusion: AF results in activation of ER stress and subsequent induction of autophagy resulting in structural remodeling and functional loss of cardiomyocytes. The findings suggest a beneficial role for ER stress and autophagy inhibitors to conserve cell proteostasis in clinical AF.

A shift in the relative importance of EDRFs induced via Dietary Restriction on vasodilator dysfunction due to genomic instability

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Introduction: We explored if dietary restriction (DR) alters the vascular signalling of prostaglandins (PG), nitric oxide (NO) or endothelium-derived hyperpolarizing factors (EDHF) in Ercc1$^{d/-}$ and wild type (WT) littermates that were fed ad lib (AL) or were on DR.
Materials and methods: Male and female mice Ercc1$^{d/-}$ and WT mice were on DR or fed ad libitum for 9 weeks from the age of 7 weeks (Ercc1$^{d/-}$) or 11 weeks (WT). Thoracic aortas were collected for organ bath experiments to investigate endothelial dilator function by exposure to acetylcholine in the absence or presence of L-NMMA, cyclo-oxygenase inhibitor indomethacin or both inhibitors. Inhibitors were added to the organ bath 10 minutes prior to U46619, which was used to precontract the aortic segments. Ach was given after a stabile precontraction was reached. Maximal dilator responses to Ach (mean+/-SEM) are shown between brackets, and were calculated as % decrease of recontraction
Results: In Ercc1$^{d/-}$ mice, fed DR diet group Ach responses were improved In WT no changes occurred. In AL-fed Ercc1$^{d/-}$ L-NMMA decreased the Ach responses , and INDO had no effect. In AL-fed WT, L-NMMA inhibited Ach responses. Also INDO inhibited the vasodilations. The combination of L-NMMA and INDO did not further inhibit dilations as compared to L-NMMA pretreated segments. In DR-fed Ercc1$^{d/-}$ L-NMMA decreased the Ach responses, and INDO inhibited the vasodilations. In DR-fed WT, L-NMMA inhibited Ach responses and INDO had no effect. The combination of L-NMMA and INDO did further inhibit dilations as compared to L-NMMA pretreated segments in Ercc1$^{d/-}$mice.
Conclusions: WT mice do not show an age-related decrease of endothelium-dependent vasorelaxation, NO as the major EDRF in aorta, and DR shows no effect to WT mice. In Ercc1$^{d/-}$ mice, show an accelerated age-dependent decrease of endothelial function, mice respond to DR by increasing prostaglandin-mediated vasorelaxations.

MTOR PLAYS AN IMPORTANT ROLE IN COW'S MILK ALLERGY-INDUCED BEHAVIORAL AND IMMUNOLOGICAL DEFICITS

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Background: Autism spectrum disorder (ASD) is developed by both genetic and environmental factors. Immunological disturbances in autistic individuals have been reported and a role of cow milk allergy (CMA) has been suggested in ASD. Previous studies showed that CMA mice display behavioural and neuroimmunoligical deficits.In ASD, several genetic mutations are found that are strongly linked to an enhanced mammalian target of rapamycin (mTOR) signaling and enhanced mTOR also plays a central role in directing immune responses towards allergy. Therefore, mTOR pathway may be the link between the immune disturbances and behavioral deficits observed in ASD.
The aim of this study was to investigate the role of the mTOR pathway in CMA-induced behavioral and immunological deficits. Method:To induce CMA, male C3H mice were orally sensitized with whey protein and cholera toxin (CT) or CT alone for 5 weeks and 1 week later orally challenged with whey protein once. Rapamycin treatment was performed i.p. 5 days per week for 6 weeks. One day after challenge, social interaction and grooming tests were performed. After sacrificing,blood was collected for analysis of whey-specific immunoglobulins and mouse mast cell protease 1(mMCP-1) measurements.Ileum and brain samples were collected for mTOR activity via Western Blotting.
Results: The validity of the CMA model was confirmed by increased whey-specific IgE, IgG1 and IgG2 levels and increased mMCP-1 levels in serum.In the behavioral tests, CMA mice showed less social interaction and enhanced grooming duration and number.We demonstrated that the CMA-induced immune response and behavioral deficits were associated with enhanced mTOR activity. Inhibition of mTOR with rapamycin showed a dose dependent reduction of the immune and behavioral disturbances in CMA mice.
Conclusion: CMA animals showed less social interaction and increased repetitive behavior and had enhanced mTOR activity in brain and gut. Inhibition of mTOR with rapamycin ameliorated both social and repetitive behaviors and attenuated the allergic response.