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FM1

Autocrine/paracrine signaling via adenosine A1 receptor is arrhythmogenic in the developing heart

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We previously showed that exogenous adenosine (ADO) induces transient arrhythmias in the developing heart via the ADO A1 receptor (A1AR) stimulation through downstream activation of ERK and PLC/PKC pathways. This study aimed to establish a mechanistic basis of how endogenously derived adenosine could modify cardiac activity in an autocrine/paracrine manner.
Experiments were performed using the spontaneously beating heart obtained from 4-day-old chick embryos. Blockade of Equilibrative Nucleoside Transporters (ENTs) by dipyridamole (1 μM), which is known to increase interstitial ADOlevel, induced transient arrhythmias (e.g., atrial dysrhythmias and atrio-ventricular blocks). This inhibitor also increased the phosphorylation of ERK2 during the first 10 minutes of treatment. When the conversion of AMP to ADOby ecto-5'-nucleotidase/CD73 was inhibited by AOPCP (50 μM), the dipyridamole-induced arrhythmias were prevented. The arrhythmias provoked by inhibition of ENTs were also prevented by the specific antagonist of the A1AR (DPCPX) but not by the antagonists of A2AAR (SCH58261), A2BAR (MRS1754) or A3AR (MRS1523). Furthermore, specific inhibition of A1AR prevented the dipyridamole-induced ERK2 phosphorylation.
These findings 1) suggest that an increase of endogenously derived ADO in the microenvironment of the cardiomyocytes can provoke transient pacemaking and conduction disturbances via A1AR and ERK pathway in an autocrine/paracrine manner, and 2) provide new insights into the mechanisms associated with an overproduction of ADO in the developing heart submitted to a hypoxic episode.

FM2

Myocardial protection conferred by high density lipoproteins implicates Connexin43 gap junction channels

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Purpose: High-density lipoprotein (HDL) is known for its cardioprotective properties independent from its cholesterol transport activity, but mediated by activation of protein kinases. Connexin43 (Cx43) is a gap junction protein present in ventricular cardiomyocytes. PKC-dependent phosphorylation modifies Cx43 gap junction channel properties and is involved in cardioprotection. We hypothesized that cardioprotective properties of HDL may be mediated in part by affecting Cx43 gap junction channels.
Methods and Results: Neonatal rat cardiomyocytes were treated with HDL and Cx43 phosphorylation was evaluated by Western blotting and immunofluorescence. We found that HDL promoted phosphorylation of Cx43 with a maximal induction at 5 min, which was inhibited by pre-treatment with various PKC inhibitors. These effects on Cx43 phosphorylation was mediated by S1P2 and S1P3 receptors. HDL significantly reduced diffusion of fluorescent dye between cardiomyocytes (~50%), which could be prevented by PKC inhibition. As observed during optical recordings of transmembrane voltage, HDL depressed impulse conduction only minimally (<5%). Moreover, with the *ex vivo* model of Langendorff perfusion, we have shown that 5 min of HDL

treatment at the onset of reperfusion is sufficient to significantly reduced infarct size in response to 30 min of global no-flow ischemia (23 ± 3% vs 13 ± 2%, p <0.05), and to significantly reduced the duration of fibrillation induced by 15 minutes of left coronary artery ligation (6.4 ± 1.2 min vs 2.3 ± 0.7 min, p <0.05).
Conclusions: Short-term treatment with HDL induces phosphorylation of Cx43 by a PKC-dependent pathway. HDL-induced phosphorylation of Cx43 reduced the diffusion of large tracer molecules between cells, whereas impulse conduction was maintained. Moreover, 5 min treatment with HDL confers cardioprotection against ischemia/reperfusion injury. These results link Cx43 for the first time to the short-term cardioprotective effects of HDL.

FM3

S6K1 promotes vascular endothelial aging and enhances inflammatory adhesion molecule expression through arginase-II

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Aims or Purpose: Both hyperactive S6K1 and arginase have been shown to promote endothelial nitric oxide synthase (eNOS)-uncoupling, i.e., generation of O2.- instead of NO from eNOS in aging. Here we investigate the interplay between S6K1 and arginase-II (Arg-II), the major isoenzyme in human endothelial cells, in vascular aging and inflammation.
Design & Methods: Vascular endothelial aging phenotypes were studied in non-senescent "young" and replicative senescent cells isolated from human umbilical veins and in aortas of young (3 months) and old (20 to 22 months) wild type and Arg-II knockout mice (Arg-II-/-).
Results: We show increased Arg-II expression/activity in senescent endothelial cells. Silencing Arg-II in senescent cells suppresses eNOS-uncoupling, senescence-associated-b-galactosidase activity, and expression of vascular adhesion molecule-1 (VCAM1) and intercellular adhesion molecule-1 (ICAM1). Conversely, overexpressing Arg-II in young cells promotes eNOS-uncoupling, endothelial senescence, and enhances expression of VCAM1/ICAM1 and monocyte adhesion. All these effects of Arg-II are ameliorated by co-expressing superoxide dismutase-1. Moreover, overexpressing S6K1 in young cells increases, whereas silencing S6K1 in senescent cells decreases Arg-II gene expression and enzymatic activity. Furthermore, S6K1 overexpression exerts the same effects as Arg-II on endothelial senescence phenotypes which are prevented by silencing Arg-II. Similarly, increased Arg-II gene expression, VCAM1/ICAM1 levels, eNOS-uncoupling, and aging markers i.e., p53 and p21 levels in the aortas of old mice are blunted in age-matched old Arg-II-/- mice.
Conclusions: S6K1 promotes endothelial aging and inflammation through up-regulation of Arg-II. These results reveal a novel mechanism of endothelial aging and inflammation promoted by S6K1 signaling. Targeting S6K1 and Arg-II may represent a promising therapeutic strategy for age-associated cardiovascular disease.

FM4

Oscillatory shear stress down-regulates the expression of the vasoprotective protein Cx40 in endothelial cells

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Endothelial cells (ECs) of healthy arteries express high Cx40 levels, but this is lost in ECs overlying atherosclerotic plaques. Atherogenesis is accelerated in *apoE-/-*mice with EC-specific deletion of this gap junction protein, which suggests that Cx40 has anti-atherogenic properties. The spatial distribution of atherosclerosis is governed by shear stress. Low/oscillatory shear stress observed in bends and bifurcations of arteries induces inflammatory activation of ECs via the activation of the NF-κB pathway. Here, we investigate the relation between shear stress, Cx40 and NF-κB.
Shear stress-modifying casts were placed around the common carotid artery of mice expressing eGFP under the Cx40 promoter. Cx40 and eGFP expression in response to flow were assessed by *en face* immunofluorescence in heterozygous Cx40/eGFP mice. We found that Cx40 expression is down-regulated in regions of oscillatory flow (0.45 ± 0.13, p = 0.04), but is conserved in regions of high and low laminar flow (0.85 ± 0.11 and 1.45 ± 0.48, n = 8). To identify potential binding partners for the regulatory intracellular C-terminus of Cx40 (Cx40CT) we performed phage display. Thus, 22 different 12-mer peptidic sequences were isolated. Eleven peptides contained the 4-residue motif HS[I,L,V][K,R] starting with a Histidine and a Serine, and followed by a hydrophobic residue (Isoleucine, Leucine or Valine) and a basic residue (Lysine or Arginine). Twelve peptides shared 4 or more residues with one of the retrieved peptides, HSLRPEWRM-PGP. Sequence alignment against the NCBI protein database indicated 58.3% homology of this peptide with IκBa, a member of the family of inhibitory proteins that control the translocation of NF-κB. *In vitro* binding of this peptide or of the homologous IκBα region to Cx40CT was confirmed by crosslinking experiments using the chemical crosslinker BS3.
Our data show a novel functional interaction between IκBa and Cx40 that may be relevant for the control of NF-κB activation by shear stress.

FM5

Proteomic identification of junction plakoglobin as a potential novel biomarker of coronary artery disease

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Purpose: Despite advances, there is still a high medical need for biomarkers that allow or improve 1) early diagnosis of acute vascular events, 2) vascular risk prediction and prognostics, and 3) monitoring of progression and regression of atherosclerosis.
Methods: We applied subtractive antibody phage display and mass spectrometry to identify protein biomarkers that are released from tissue cultures of atherosclerotic lesions, so-called secretomes.
Results: Recombinant antibodies were isolated that recognize proteins that are specifically released by atherosclerotic tissue. Nine of the most promising antibodies were used as baits to isolate their target antigens from secretomes and these targets were identified by mass spectrometry. One of them, junction plakoglobin (JUP), was confirmed by immunoblotting to be present in atherosclerotic secretomes, but not in control secretomes. Furthermore, immunohistochemistry demonstrated that JUP is present in both atherosclerotic plaques and in thrombi that were aspired from the culprit coronary arteries of patients with acute coronary syndrome (ACS). JUP was detected primarily in monocytes and macrophages, as well as extracellularly. The plasma concentration of JUP was then semi-quantitatively analyzed by immunoblotting in 12 ACS patients, 15 patients with stable coronary artery disease (CAD) and 15 angiographically confirmed CAD-free controls. The median plasma concentration of JUP was 2-fold elevated in the stable CAD group as compared with the control group. In addition, the median JUP concentration was 6- and 14-fold elevated in the ACS group as compared with the stable CAD and the control groups, respectively. Finally, the JUP plasma concentration was elevated in mice with atherosclerosis, in comparison with non-atherosclerotic mice.
Conclusions: JUP is a potential novel biomarker of ACS and stable CAD, and will be further evaluated in larger patient cohorts.

FM6

A novel electrospun biograft for heart function stabilisation after myocardial infarction

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Aim and Purpose: Epicardial implantation of cells induces beneficial effect on heart function after a myocardial infarction. Further, tissue engineering provides numerous tools for the creation of an implantable microenvironment for enhanced cell survival. In this

regard, micro-fibrous matrices were functionalised with an oxygen functional hydrocarbon coating and optimised for bone marrow derived mesenchymal stem cell (MSCs) growth. The biograft was then evaluated in a rodent model of myocardial infarction.
Design and Method: Microfibrous PCL non-wovens were produced by electrospinning and surface-coated by an RF plasma process. MSC were cultured for 7 days on the substrate. Two weeks post LAD ligation, Lewis rats with reduced ejection fraction (EF of 49 ± 9%) were randomised into 4 groups: MSC-seeded patch (n = 11), cell-free patch (n = 11), glue only (n = 15) and sham operation (n = 14). Echocardiography and pressure-volume loops were recorded after 28 days.
Results: FACS analysis, MTT staining, SEM imaging and Cy-Quant analysis prior to *in vivo* evaluation confirmed the implantation of 0.6 Mio viable CD90+, CD45-and CD31- MSC, forming a confluent layer on the substrate. Relative to pre-treatment, MSC-seeded patches induced a stabilisation of EF (49 ± 11% vs. 48 ± 8% respectively, p = 0.9), whereas cell-free patches did not (47 ± 10% vs. 37 ± 4%, p = 0.006). Nestin+ and sca-1+ cardiac progenitor cells were localised within the border zone of the infarcted area. Sex mismatched transplantation and chromosome Y tracking confirmed cell presence 4 weeks post implantation.
Conclusion: The herewith presented substrate design is devoid of any immunogenic ECM-proteins and is novel in its stable functionalisation. Epicardial implantation of plasma-coated, MSC-seeded PCL grafts is safe and effective for heart function stabilisation and attenuated remodeling. Ongoing evaluation on hemodynamics and immunohistology will further provide inside into the regenerative capacity of cardiac tissue engineering.

FM7

Fatty acid amide hydrolase deficiency is associated with a vulnerable plaque phenotype in atherosclerotic mice

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Elevated endocannabinoid levels are linked with the development of atherosclerotic vascular disease. However, it remains unclear whether endocannabinoid levels might represent a risk factor or diagnostic biomarker for acute atherosclerotic vascular events. Here, we studied the involvement of fatty acid amide hydrolase (FAAH) deficiency, the major enzyme responsible for endocannabinoid anandamide degradation, in atherosclerotic plaque vulnerability.
We generated apolipoprotein E-deficient (ApoE-/-) FAAH-/- mice by interbreeding ApoE-/- with FAAH-/- mice and measured serum levels of anandamide and related FAAH metabolites palmitoyl- and oleoylethanolamide (PEA, OEA). We assessed atherosclerosis in ApoE-/- and ApoE-/-FAAH-/- mice after 5, 10 and 15 weeks on high cholesterol diet (HCD; 1.25% cholesterol) and analyzed weight, serum cholesterol and atherosclerotic plaque composition.
Levels of FAAH metabolites anandamide, PEA and OEA were 1.4 to 2-fold higher in FAAH-/-ApoE-/-mice. FAAH deficiency attenuated atherosclerotic plaque size increase (by ~50% in thoracoabdominal aortas after 15 weeks HCD; n = 7–10; $P = 0.007$), but plaques had significantly lower content of smooth muscle cells (reduced by 36% at 10 weeks HCD in aortic sinuses; n = 10–15; $P = 0.01$) and increased matrix metalloproteinase MMP-9 expression (by 73%; $P = 0.049$). There was no difference in macrophage content, but a 65% increase in neutrophil infiltrates ($P = 0.0007$) in aortic sinus plaques from ApoE-/-FAAH-/- mice compared to ApoE-/- controls. This was accompanied by 1.9-fold increased chemokine CXCL1 mRNA levels ($P = 0.004$) in mouse aortas. CXCL1 expression in plaques was confirmed by immunostaining. MMP-9 mainly colocalized with neutrophils rather than macrophages and positively correlated with their intraplaque infiltration (r = 0.6529; $P = 0.006$).
Increased endocannabinoid anandamide and related FAAH metabolite levels are associated with smaller atherosclerotic plaques with more vulnerable phenotype.

FM8

p38mapk is required for glucosamine-induced endothelial nitric oxide synthase uncoupling and plasminogen-activator inhibitor expression

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Aim: Hexosamine biosynthetic pathway (HBP) is implicated in increased plasminogen activator inhibitor-1 (PAI-1) level and endothelial nitric oxide synthase (eNOS) dysfunction in diabetes. Glucosamine (GlcN) that directly activates HBP is a dietary supplement and clinically used supplement to treat osteoarthritis despite uncertain efficacy and adverse cardiovascular effects observed in animal models. p38mapk has been shown to be involved in HBP-mediated biological processes. We investigated roles of p38mapk in GlcN-induced endothelial PAI-1 expression and eNOS dysfunction.
Design & Methods: For this purpose, human endothelial cells were isolated from umbilical veins. The effects of GlcN on PAI-1 expression, eNOS function, and related signaling mechanisms were investigated in these cells and in isolated mouse aortas.
Results: In cultured human endothelial cells, GlcN time- and concentration- dependently increased PAI-1 protein level that was further enhanced by tumor necrosis factor-a (TNF-a) as analyzed by immunoblotting. The stimulation of PAI-1 by GlcN alone or by GlcN and TNF-a in combination was ameliorated by the specific inhibitor of p38mapk, but not that of JNK or ERK1/2. Moreover, in isolated mouse aortas, GlcN caused eNOS uncoupling resulting in enhanced superoxide and decreased nitric oxide production as well as impaired endothelium-dependent relaxations, which were also fully prevented by the p38mapk inhibitor.
Conclusions: HBP activated by GlcN increases PAI-1 expression and eNOS uncoupling depending on p38mapk, which not only explains hyperglycemic vascular complications, but also questions GlcN utilization as anti-inflammatory supplement in humans. Targeting p38mapk prevents PAI-1 expression and re-couples eNOS function. The results support current ongoing clinical application of p38mapk inhibitor in patients with cardiovascular disease.

FM9

Alpha-linolenic acid increases platelet survival by reducing platelet activation and clearance

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Aim: Our recent studies showed a protective effect of the plant-derived n-3 fatty acid α-linolenic acid (ALA) by reducing experimental atherosclerosis and platelet-dependent thrombosis in mice. Since ALA reduced platelet activatability we hypothesized that this might lead to their prolonged survival in the circulation and to an increased platelet count.
Methods: 8-week-old male ApoE-/- mice were fed a 0.21 g% cholesterol diet containing either a high (7.3 g%) or low (0.03 g%) ALA content for 16 weeks. Platelet counts were assessed monthly. Platelet production was analyzed by reticulated platelet staining and megakaryocyte-CFU. Megakaryocyte number in bone marrow sections was determined by immunofluorescence. Platelet turnover was assessed by plasma glycocalicin (GC) concentration (ELISA), normalized to the platelet count (the GC-Index). Platelet clearance in spleen and liver was determined by immunofluorescence staining on frozen sections.
Results: After 16 weeks platelet counts differed substantially in the high vs low ALA group (1591 ± 650x103/µl vs 10538 ± 323x103/µl, n = 30, p = 0.0002), while tail bleeding times were rather longer in the high ALA group (285 ± 83 vs 221 ± 56 sec, n = 5, p = n.s.). The reticulated platelet fraction was not significantly different (72 ± 34 x103/µl in the low ALA vs 55 ± 47 x103/µl in the high ALA group, n = 15, p = n.s.), nor was the number of megakaryocyte-CFU from bone marrow (12 ± 4 vs 11 ± 4, n = 3). CD41+ cells in bone marrow sections were not different between the two groups (65 ± 13 low ALA vs 69 ± 8 high ALA, n = 3). Plasma GC was significantly lower in the high ALA group (25 ± 15 vs 53 ± 27 µg/ml, n = 17, p = 0.0007), and the GC-Index

was 6.6 ± 3 vs 15 ± 5, n = 17, p <0.0001. Platelet clearance in spleen and liver was significantly reduced in the high ALA group (spleen CD41+ area: 143482 µm² vs 94724 µm², n = 3, p = 0.0049; liver: 54095 mm² vs 33224, n = 3, p = 0.033).
Conclusions: A diet rich in ALA increases the platelet count by reducing platelet clearance. Mechanisms may include the inhibition of MAP kinase p38 phosphorylation and therefore reduced platelet activation and a reduced GPIbα cleavage. The phenomenon might be of clinical importance in transfusion medicine.

FM10

Metabolic consequences of experimental uninephrectomy

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Metabolic syndrome, dyslipidemia-inflammation-oxidative stress, obesity, diabetes and cardiovascular diseases may lead to reduced kidney function. Here we chose to test the converse: does reduced kidney function impact on lipid metabolism? Six-week old male Sprague Dawley rats were submitted to either unilateral nephrectomy (UniNX) or sham surgery. We then compared blood and tissue parameters at 1, 2 and 4 weeks after surgery. UniNX resulted in decreased fat pad weights. This was associated with increased blood glycerol over 4 weeks compared to the Sham control rats. Furthermore, adipose triglyceride lipase and hormone sensitive lipase mRNA were increased in epididymal tissue of UniNX animals. Similarly, lipoprotein lipase and the fatty acid transporter CD36 mRNA were increased in the liver of UniNX group. An increase in lipolysis can explain the decrease in fat mass following UniNX. Blood hormones insulin, ghrelin, corticosterone, T3 and leptin were not different between the two groups. However, UniNX did induce a low grade inflammation as evidenced by an increase in circulating cytokines such as IFNγ, TNFα, IL1α, GM-CSF and EPO; these cytokines are potentially capable of inducing lipolysis. We also observed that UniNX resulted in increased oxidative stress in the liver (increased lipid peroxidation and decrease in the antioxidant glutathione). Our study suggests that the kidney has an important and significant impact on chronic lipid metabolism, activation of inflammation and increased oxidative stress, which are characteristics of metabolic diseases.

FM11

ROS-induced hyperglycaemic memory is abolished by in-vivo silencing of the mitochondrial adaptor p66Shc

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Purpose: Up-regulation of the adaptor p66Shc determines increased ROS production, mitochondrial swelling and cellular death. Expression of p66Shc is increased in human and experimental diabetes and triggers endothelial dysfunction (ED). However, it is not known whether up-regulation of p66Shc persists despite glucose normalization and, hence, contributes to the "hyperglycaemic memory". Such phenomenon is indeed a powerful determinant of morbidity in people with diabetes.
Methods: Diabetes was induced in wild-type 129sv mice (4–6 month old) by a single i.p. dose of streptozocin. Mice were divided into 5 experimental groups: 1) healthy controls; 2) untreated diabetics; 3) diabetics treated with insulin, 4) diabetics receiving insulin plus p66Shc siRNA; 5) diabetics with insulin plus scrambled siRNA (n = 6–7/group). Insulin implants were placed subcutaneously 3 weeks after the induction of diabetes for the following 3 weeks. Silencing of p66Shc was obtained by i.v. administration (once every 5 days) of a mix of 2 siRNAs specifically targeting p66Shc. Protein expression was assessed by Western blot (WB) in aortic lysates and data are shown as percentages of control. Endothelium-dependent relaxation to acetylcoline (Ach, 10-9-10-6 mol/L) was determined by organ chamber experiments.
Results: Lipid profile was comparable in all groups. In contrast to scrambled siRNA, *in vivo* delivery of p66Shc siRNA abolished protein expression (93 ± 46 vs 24 ± 15%, respectively, p <0.01). Endothelium-dependent relaxations to Ach were significantly impaired in diabetic mice compared to control (max relaxations: 40 ± 15 vs 80 ± 8%, respectively, p <0.01). Restoration of glycaemic control with insulin did not improve ED (47 ± 14 vs 40 ± 15% in untreated diabetics, p = NS). Interestingly enough, endothelial function was restored in mice receiving insulin plus p66Shc siRNA (70 ± 16%, p <0.01 vs insulin alone). Scrambled si-RNA did not exert any effect on endothelial function (44 ± 14%).
Conclusions: In vivo silencing of p66Shc allowed us to demonstrate its role in maintaining ROS-mediated ED, despite glucose normalization. p66Shc may represent a potential molecular target in the prevention of hyperglycaemic memory in diabetes.

FM12

Type 2 diabetes and the progression of visualized atherosclerosis to clinical cardiovascular events

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Aim: We aimed at prospectively evaluating to what extent pre-existing coronary artery disease (CAD) accounts for the increased long-term vascular event risk of patients with type 2 diabetes (T2DM).
Design & Methods: Vascular events were recorded over 8 years in 750 consecutive patients whose baseline CAD state was verified angiographically.
Results: The baseline prevalence of CAD (87.8% vs. 80.4%; p = 0.029) and of significant coronary stenosis ≥50% (69.5% vs. 58.4%; p = 0.010) as well as the extent of CAD, defined as the number of significant coronary stenoses (1.7 ± 1.6 vs. 1.4 ± 1.5; p = 0.014) were higher in patients with T2DM (n = 164) than in non-diabetic subjects. During follow-up, T2DM and CAD proved to be mutually independent predictors of vascular events: T2DM predicted vascular events (n = 257) independently from the presence and extent of baseline CAD (hazard ratio (HR) 1.36 [1.03–1.81]; p = 0.032); conversely, the presence and extent of baseline CAD predicted vascular events independently from T2DM (HRs 3.29 [1.93–5.64]; p <0.001 and 1.37 [1.23–1.53]; p <0.001, respectively). However, the overall risk increase conferred by T2DM was driven by an extremely high 53.3% event rate of patients with both T2DM and significant CADat baseline; individuals with T2DM but without significant baseline CAD showed a significantly lower event rate (22.0%; p <0.001).
Conclusion: We conclude that T2DM and angiographically visualized coronary atherosclerosis are mutually independent predictors of vascular events. However, the overall risk increase conferred by T2DM is driven by accelerated progression of pre-existing atherosclerosis to clinical cardiovascular events, whereas vascular risk is much lower in diabetic patients without pre-existing significant CAD.

P1

The brain natriuretic peptide modulates cardiomyocyte maturation and stem cell antigen-1 expression on non-myocyte cells

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The BNP is a cardiac and vascular-derived hormone known for its role in cardiovascular homeostasis. BNP binds to the guanylyl cyclase-linked natriuretic peptide receptor A (NPR-A) or B (NPR-B). NPR-A mediates all the known biological effect of BNP, whereas the exact role of NPR-B is unknown. BNP is mainly secreted by cardiomyocytes in response to hypoxia, pressure or volume overload. However, its effect on cardiac cells is not clearly established. Thus, the aim of our study was to determine the effect of BNP stimulation on cardiac cells *in vitro* but also *in vivo*. In murine hearts, cardiomyocytes, endothelial cells and stem cell antigen-1 (Sca-1) positive cells are able to secrete BNP and express its receptors. Interestingly, higher number of BNP positive cells was detected in neonatal compared to adult mouse

hearts. Cardiomyocytes and non-myocyte cells (NMCs) were isolated from neonatal mouse hearts and cultured with or without BNP (5 µg/ml). Three days BNP treatment accelerated cardiomyocyte maturation as shown by the increase of the alpha/beta Myosin heavy chain ratio. BNP treated NMCs exhibited increased expression of mRNAs coding for Nkx2.5 and Sca-1 (1.8 and 2 fold, respectively) compared to untreated cells. Flow cytometry analysis revealed that the number of Sca-1+ cells increased from 57 ± 3.8% in untreated NMCs to 67 ± 4.6% in 3 days BNP treated cells.
Finally, we evaluated the effect of BNP *in vivo*. BNP (10 µg/20 g) was injected 3 fold a week during 5 weeks into 12 weeks-old C57BL/6 mice. Cardiac mass was decreased in BNP injected hearts (4.38 ± 0.06 versus 4.72 ± 0.08 in control hearts). Immunostainings revealed that the hearts of BNP injected mice displayed a higher number of cardiomyocytes (+14%) than hearts from control mice. However, the cardiomyocytes found in BNP injected mice were smaller than those found in control mice (cardiomyocyte area: 279 ± 6 versus 312 ± 7 µm^2).
In conclusion, BNP induces cardiomyocyte maturation and increases the percentage of Sca-1+ non-myocyte cells *in vitro*. Whether this implies a role in heart regeneration is under investigation.

P2

Inflammatory stimulus effect on HDL and apoA-I transport through endothelial cells

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Atherosclerosis is a chronic disease characterised by lipid retention and inflammation in the arterial wall. High density lipoproteins (HDL) and its major apolipoprotein (ApoA-I) transport cholesterol from macrophage foam cells to the liver for disposal. However they must pass the endothelium to get access to the foam cells, a process which is not well understood so far. Our previous data show that endothelial cells transport apoA-I and HDL by distinct specific mechanisms involving the scavenger receptor (SR)-BI, the ATP binding cassette transporters (ABC) A1 and G1 and the endothelial lipase (EL). Therefore what would be the effects of pro-inflammatory cytokines on the interaction between endothelial cells and HDL or apoA-I?
Stimulation of endothelial cells with IL-6 demonstrated an enhancement of the specific HDL cell binding and transport capacity and a reduction on apoA-I cell binding and transport rate through endothelial cells. Analyzing the protein expression of the stimulated cells on western-blots demonstrated an increase in EL and ABCG1 expression levels.Experiments are on the way to localize ABCA1 in the cells and to inhibit EL to prove its role in the effect of IL-6 stimulation.
TNFα stimulation revealed a decrease of HDL transport but no change in HDL cell binding. Based on this finding we analyzed if TNFα stimulation reduced the uptake of HDL by cell surface biotinylation. We could clearly demonstrate that the TNFα stimulated cells show 33% reduced internalization. Analyzing the effects of TNFα stimulation on ApoA-I cell binding demonstrated no change whereas transport rate is reduced similar as demonstrated for HDL. We are currently testing if apoA-I is less internalized. On the protein analysis we obtained enhanced expression levels for ABCG1 and ABCA1 whereas EL was reduced. These finding indicate that it will be essential to analyze the cellular distribution of the HDL binding proteins ABCG1 and EL as well as the apoA-I binding protein ABCA1.

P3

HDL protects pancreatic beta-cells from apoptosis

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In healthy individuals, an increased metabolic load is compensated by an increase in beta-cell mass and function. In type 2 diabetes however, this compensation collapses. Pre-diabetic and diabetic patients often exhibit a shift in their lipoprotein profile towards low plasma levels of high density lipoprotein (HDL), a high triglyceride concentration and an elevated fraction of small dense low density lipoprotein. Previously, it has been described that HDL protects pancreatic beta-cells from human and murine donors from basal and interleukin-1beta (IL-1b)-stimulated apoptosis.
HDL was isolated from human plasma of healthy donors. INS-1E cells (a beta-cell-line) were exposed to HDL. Apoptosis was stimulated with IL-1b and analyzed by using the Cell Death Detection ELISA Plus Kit. RNA was isolated from the cells after 12h and 24h stimulation of apoptosis with IL-1b. Gene expression was measured with the Rat Gene Expression Microarray (Agilent Technologies) in collaboration with the Functional Genomics Center Zürich.
The anti-apoptotic effect of HDL and its protein moiety could be reproduced in the INS-1E beta-cell line. To identify signalling pathways mediating the anti-apoptotic effect of HDL, we performed a genome-wide expression analysis in INS-1E cells in the presence or absence of IL-1b and HDL. The expression data obtained from the microarray analysis highlight specific apoptotic pathways which are up or down regulated by IL-1b and attenuated by HDL. These pathways include the endoplasmatic reticulum stress response pathway, Akt/PKB signalling, TNF receptor 1 signaling, FAS signaling, and p53 mediated apoptosis.
The study of pathways involved in the protective effect of HDL will help us to understand the mechanisms by which HDL exerts its protective effect against the inflammatory cytokine IL-1b in pancreatic beta-cells. This knowledge may help to exploit HDL towards preventing or delaying the manifestation of diabetes mellitus type 2 in pre-diabetic subjects.

P4

Transport of high density lipoproteins through the endothelium

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Atherosclerosis is an ongoing, progressive accumulation of lipids in the arterial intima leading to plaque formation. Epidemiological studies show an inverse association of high density lipoprotein (HDL) cholesterol with atherosclerotic vascular events. HDL and its main apolipoprotein A-I (ApoA-I) have multiple anti-atherogenic functions: Some of these take place in the vessel wall. To get access to the intima and the lipid-laden macrophages, HDL has to pass the endothelial barrier. Previously, we showed that ApoA-I transcytosis is modulated by ATP binding cassette transporter (ABC) A1, and HDL transcytosis is modulated by ABCG1 and scavenger receptor B I (SR-BI).
To elucidate the itinerary of ApoA-I and HDL through endothelial cells (ECs), we investigated localisation and distribution of fluorescently labelled ApoA-I and HDL. ApoA-I and HDL can be detected inside the cells. After 10 min, ApoA-I and HDL co-localises perfectly indicating the same route of trafficking. Further experiments show that HDL is not targeted to lysosomes nor the Golgi or the endoplasmatic reticulum. However they co-localise with the early endosome marker Rab5 and endosome to trans-golgi network marker Rab9 to some extent, but not at all with the recycling endosome marker Rab11a. Pharmacological inhibition of known trafficking routes show the involvement of Phosphatidylinositol Kinases and Dynamin as well as the cytoskeleton in the uptake and trafficking of HDL. Pulse-chase experiments show a change in distribution and stepwise progressing of HDL through distinct vesicular compartments. Routes and receptors by which HDL is taken up by the ECs are currently under investigation using iRNA technology. Live microscopy and electron microscopy experiments as well as biochemical data will help us to understand the itinerary of HDL through aortic ECs. This gives new detailed insight on the involvement of the endothelium in the reverse cholesterol transport and interaction with HDL.

P5

Lipid parameters in acute coronary syndromes versus stable coronary artery disease

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Aims: Differences in lipid parameters between patients with acute coronary syndromes (ACS) and patients with stable coronary artery disease (CAD) are unclear and are addressed in the present study.

Design & Methods: We enrolled 582 patients with angiographically proven stable CAD, of whom 26.9% had diabetes mellitus type 2 (T2DM) and 182 patients with ACS, of whom 35.8% had T2DM.
Results: When compared to patients with stable CAD, HDL cholesterol (45.8 ± 15.5 mg/dl vs. 50.2 ± 16.1 mg/dl; p <0.001) and apolipoprotein A1 (139.0 ± 30.4 mg/dl vs. 154.6 ± 31.0 mg/dl; p <0.001) were significantly lower in patients with ACS in the total population as well as among subjects with T2DM. Analysis of covariance confirmed an independent impact of the ACS state on these lipid parameters after multivariate adjustment both in the total population and among subjects with T2DM. In contrast, total cholesterol, LDL cholesterol and apolipoprotein B neither in the total population (p = 0.583, p = 0.884 and p = 0.834 respectively) nor among subjects with T2DM (p = 0.133, p = 0.234, and p = 0.371, respectively) differed significantly between ACS and stable CAD patients. Triglycerides were significantly higher in patients with ACS than in patients with stable CAD in total study population (155.8 ± 121.1 mg/dl vs. 140.8 ± 90.6 mg/dl; p = 0.037) but not in patients with T2DM (p = 0.972).
Conclusion: We conclude that HDL cholesterol and apolipoprotein A1 are lower in the ACS state than with stable CAD; this particularly holds true among patients with T2DM.

P6

Angiopoietin-like 4 is elevated in type 2 diabetes but is not associated with angiographically determined coronary artery disease

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Aims: Angiopoietin-like 4 (ANGPTL4, fasting-induced adipose factor), a protein inhibitor of lipoprotein lipase, is synthesized and secreted during fasting in adipose tissue and the liver. Its associations with metabolic syndrome traits are uncertain, and it is not known whether it is associated with type 2 diabetes (T2DM) or coronary artery disease (CAD).
Design & Methods: We therefore measured serum ANGPTL4 in 493patients undergoing coronary angiography for the evaluation of established or suspected stable CAD; significant CAD was diagnosed when coronary stenoses ≥50% were present.
Results: ANGPTL4 was significantly positively correlated with age (r = 0.177; p <0.001) and fasting glucose (r = 0.112; p = 0.013) but was not correlated with waist circumference, triglycerides, HDL cholesterol, systolic blood pressure or diastolic blood pressure. ANGPTL4 was significantly higher in patients with T2DM (n = 115) than in non-diabetic subjects (28 ± 32 vs. 25 ± 38; p = 0.032); however, it was not significantly different between patients with significant CAD (n = 246) and individuals without significant CAD (p = 0.112).
Conclusion: We conclude that ANGPTL4 is positively correlated with fasting glucose and elevated in T2DM but is not significantly associated with angiographically determined CAD.

P7

FABP4, identified by combining proteomics and transcriptomics, is increased in patients with cardiovascular disease

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Purpose: The aim of this study was to identify novel potential biomarkers of cardiovascular disease.
Methods: Transcriptomics analysis of coronary thrombi was combined with proteomics analysis of secretomes derived from atherosclerotic tissue.
Results: four shared proteins were found, namely complement C1s, EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), fatty acid-binding protein 4 (FABP4) and fibronectin. Subsequently, plasma levels of EFEMP1 and FABP4 were analyzed in two small cohorts. EFEMP1 did not differ, but in both the separate and in the combined cohort FABP4 was significantly increased in plasma of patients with acute coronary syndrome compared to patients with stable coronary artery disease (p <0.01 for the combined cohort) and healthy controls (p <0.05 for the combined cohort).
Conclusion: State-of-the-art biomarker discovery approaches led to the identification of FABP4 as a potential biomarker for atherosclerosis, which further strengthens recent data describing FABP4 in cardiovascular disease. Currently, we are measuring FABP4 plasma levels in a larger cohort of patients (n ~500) with cardiovascular disease to validate these initial results.

P8

JAK3 tag single nucleotide polymorphism rs3212780 is significantly associated with diabetes-related metabolic phenotypes

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Aim: Janus kinase (JAK) 3 is involved in cytokine receptor-mediated intracellular signal transduction. Inhibition of JAK3 protects beta-cells from cytokine toxicity and has been shown to delay the onset of diabetes in the mouse model. The influence of JAK3 single nucleotide polymorphisms (SNPs) on diabetes risk or on diabetes-related metabolic traits is unknown.
Design & Methods: We therefore investigated the association of JAK3 tagging SNP rs3212780 (C>T) with metabolic phenotypes and type 2 diabetes (T2DM) in a cohort of coronary patients including 1220 non-diabetic subjects and 375 patients with T2DM, totally comprising 1595 individuals.
Results: Among non-diabetic subjects SNP rs3212780 was significantly associated with HbA1c (CC: 5.8 ± 0.4, CT: 5.7 ± 0.4, TT: 5.6 ± 0.4%; p = 0.001), fasting glucose (CC: 5.4 ± 0.7, CT: 5.3 ± 0.7, TT: 5.5 ± 1.1 mmol/L; p = 0.010), and HDL-cholesterol (CC: 55 ± 17, CT: 55 ± 16, TT: 51 ± 16 mg/dL; p = 0.009), as well as with total cholesterol (CC: 212 ± 44, CT: 206 ± 46, TT: 196 ± 48 mg/dL; p = 0.002) and LDL-cholesterol (CC: 134 ± 37, CT: 131 ± 40, TT: 124 ± 42 mg/dL; p = 0.013). In patients with T2DM, the JAK3 variant was significantly associated with fasting glucose (CC: 8.3 ± 2.7, CT: 8.7 ± 2.8, TT: 7.4 ± 1.9 mmol/L; p = 0.036). The association between SNP rs3212780 and T2DM did not reach statistical significance (allelic odds ratio = 1.18 [0.98–1.40]; p = 0.076).
Conclusion: We conclude that JAK3 tagging SNP rs3212780 is significantly associated with phenotypes conferring an increased cardiometabolic risk, at least in non-diabetic coronary patients. The association between rs3212780 and the risk of T2DM warrants further investigation.

P9

Effect of eplerenone on endothelial function in patients with stable coronary artery disease

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Background: clinical study showed as eplerenone significantly reduced all cause and cardiovascular mortality in patients with left ventricular dysfunction after a recent myocardial infarction. The aim of the present study was to investigate whether endothelial dysfunction associated with stable coronary heart disease (CHD) is altered by selective aldosterone antagonism with eplerenone, as potential anti-inflammatory drug, versus placebo.
Methods and Results: 42 patients with history of CHD (age 63.5 ± 9.1, 37 males) and stable cardiovascular medication for at least 4 months were included in the study. The patients were randomized, in a double blind fashion, to receive eplerenone 25 mg or placebo for 1 month. Endothelial function, non invasive assessed as flow mediated dilation, 24-hour blood pressure (BP), endothelial progenitor cells, platelet adhesion as well as laboratory parameter for safety and evaluation of oxidative stress and inflammation were evaluated in baseline condition and after 2 and 4 weeks of treatment.
In this prospective study adding eplerenone 25 mg on top of standard therapy did not induce significant modification in endothelial function (eplerenone 25 mg: from 4.63% ± 2.35 to 4.65%

± 2.04; placebo: from 5.06% ± 2.18 to 4.86% ± 2.06) as well as in oxidative stress and inflammation parameters and in endothelial progenitor cells or platelet adhesion.
Moreover, in this study population both eplerenone 25 mg and placebo induces a slight and not significant reduction in 24-hour BP (eplerenone 25 mg: systolic BP from 128.1 ± 13.97 to 126.9 ± 17.3 mm Hg, and diastolic BP from 75.3 ± 9.64 to 73.3 ± 12.9 mm Hg; placebo: systolic BP from 124.1 ± 11.5 to 122.3 ± 9.7 mm Hg, and diastolic BP from 72.1 ± 7.6 to 71.7 ± 7.5 mm Hg; all p = ns) when added on top of standard therapy.
Conclusion: 4-week therapy with eplerenone 25 mg on top of a standard therapy did not induce any significant changes in endothelial function and 24-hour blood pressure in patients with coronary artery disease.

P10

Dietary salt (NaCl) intake in free living individuals in the Canton of Zurich

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Introduction: An increased dietary salt (NaCl) intake has a high pathophysiological potential. Nevertheless there are hardly any NaCl intake data available from Switzerland. Accordingly there is the urgent need to know the dietary NaCl intake in the general population and especially in specific groups (such as individuals with hypertension or renal insufficiency).
Methods: In a random sample of free living individuals (n = 166, m/f = 87/79) of all age groups living in the Canton of Zurich 24h-urine was collected and the 24-h-salt excretion measured. At the occasion of a visit to the study centre blood pressure, body composition by bioimpedance, and different biochemical parameters in blood and urine, drug record etc. were assessed. Hypertension status was assessed by BP measurements and recording of the prescribed drugs.
Results: The characteristics (mean ± SD) of the population were: 48 ± 19 years, body mass index (BMI) 25 ± 4 kg/m^2, % fat mass 25 ± 8, systolic blood pressure (sysBP) 126 ± 17 mm Hg, diastolic BP 75 ± 10 mm Hg. The mean ± SD daily salt intake was 8.5 ± 3.8 g/d (median 7.9 g/d); for women 7.6 ± 3.3 g/d and for men 9.3 ± 4.1 g/d (p for difference 0.005). There was a non-significant relation between the sysBD (r = 0.15, p = 0.05) or diastolic BP and the daily salt intake. Individuals with hypertensive office sysBP consumed similar amounts of salt as normotensives. Individuals on antihypertensive drugs consumed 8.5 ± 4.71 g/d as compared to individuals without antihypertensive drugs (8.5 ± 3.7 g/d) (p = 0.9). All age groups had a similar salt intake.
Conclusion: The mean daily salt intake in this free living population seems to be much lower than earlier believed. From the public health perspective it is noteworthy that hypertensive individuals – which are most likely salt sensitive individuals – consumed the same amount of salt as non-hypertensives. Future public health campaigns to reduce salt intake should preferentially focus on salt-sensitive individuals such as hypertensives.

P11

Plasma 1-deoxysphingolipidis as novel biomarkers in the metabolic syndrome

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Metabolic syndrome (MetS) represents a cluster of cardiovascular and metabolic risk factors including; type 2 diabetes mellitus (T2DM), insulin resistance, hypertension, dyslipidemia and obesity. Increasing evidence suggests that sphingolipids play a role in the pathogenesis of both insulin resistance and T2DM. Sphingolipid synthesis is typically initiated by the conjugation of L-serine and palmitoyl-CoA – a reaction catalyzed by the serine-palmitoyltransferase (SPT). Besides the conjugation of other acyl-CoAs (C12 to C18), SPT metabolizes other amino acids such as L-alanine or glycine. This gives rise to 1-deoxysphingolipdis (DSLs), a novel class of sphingolipids. Here we compared in a series of case-control and interventional studies the sphingoid base composition of plasma sphingolipids in healthy humans and patients with an impaired glucose tolerance. Plasma sphingolipids were exposed to sequential acid and base hydrolysis to liberate the sphingoid base backbones which were then measured using LC/MS. DSLs were found to be significantly elevated in the MetS patients independent of the presence or the absence of T2DM. In contrast, plasma C16-sphingosine levels were significantly lower in patients with T2DM but not when T2DM was absent. Multivariate modeling revealed that DSLs are important contributors to the MetS state model, just next to triglycerides and above many of the conventional indicators of MetS like, hypertension, waist circumference, glucose and HDL. In the correlation analysis, it was evident that deoxysphingolipids correlate strongly with triglycerides. ROC curve analysis showed the potential use of DSLs in diagnosing metabolic syndrome versus healthy controls (AUC = 0.875, p = 5.37E-6). Taken together, our findings strongly argue for the potential use of 1- deoxysphingolipids as a new class of biomarkers in the metabolic syndrome.

P12

PKA phosphorylation of cardiac ryanodine receptor modulates SR luminal Ca2+ sensitivity

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During physical exercise and stress, the sympathetic system stimulates cardiac contractility via b-adrenergic receptor activation, resulting in protein kinase A (PKA)-mediated phosphorylation of the cardiac ryanodine receptor, RyR2, at Ser2808. Hyperphosphorylation of RyR2-S2808 has been proposed as a mechanism contributing to arrhythmogenesis and heart failure. However, the role of RyR2 phosphorylation during b-adrenergic stimulation remains controversial. We examined the contribution of RyR2-S2808 phosphorylation to altered excitation-contraction coupling and Ca2+ signaling using an experimental approach at the interface of molecular and cellular levels and a transgenic mouse with ablation of the RyR2-S2808 phosphorylation site (RyR2-S2808A). Experimentally challenging the communication between L-type Ca2+ channels and RyR2 led to a spatiotemporal de-synchronization of RyR2 openings, as visualized using confocal Ca2+ imaging. b-Adrenergic stimulation re-synchronized RyR2s, but less efficiently in RyR2-S2808A than in control cardiomyocytes, as indicated by comprehensive analysis of RyR2 activation. In addition, spontaneous Ca2+ waves in RyR2-S2808A myocytes showed significantly slowed propagation and complete absence of acceleration during ß-adrenergic stress, unlike wild type cells. This indicates that phosphorylation of RyR2-S2808 is involved in RyR2 modulation by luminal (intra-SR) Ca2+ ([Ca2+]SR). Single channel recordings revealed an attenuation of luminal Ca2+ sensitivity in RyR2-S2808A channels upon addition of PKA. We show here by three independent experimental approaches that PKA-dependent RyR2-S2808 phosphorylation plays significant functional roles at the subcellular level, namely, Ca2+ release synchronization, luminal Ca2+ sensing and functional adaptation of RyR2 to variable [Ca2+]SR. These results indicate a direct mechanistic link between RyR2 phosphorylation and luminal, but not cytosolic, Ca2+ sensing.

P13

Calcium release events evoked by photorelease of InsP3 in mouse atrial myocytes

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In the heart the dominant mechanism that leads to cellular contraction is Ca2+ release from ryanodine receptors (RyRs) that are found in the membrane of internal Ca2+ stores called sarcomplasmic reticulum (SR). In the SR membrane there exists a further Ca2+ release channel called inositol 1,4,5- trisphosphate receptor (InsP3R) that is activated by the intracellular signaling molecule InsP3.
Recent data has shown that the RyR and InsP3R are colocalized at the dyadic cleft and that colocalization is increased in myocytes isolated from hypertrophic hearts. Furthermore, the expression of InsP3R is increased in animal models of heart failure and atrial fibrillation. However, the significance and contribution of the InsP3R to physiological as well as pathophysiological Ca2+ signaling is still unknown.

Our working hypothesis suggests that under pathophysiological conditions, where functional InsP3R expression is increased, InsP3 induced Ca2+ release significantly contributes to Ca2+-induced Ca2+ release, a prerequisite for global Ca2+ transients and subsequent contractions. The aim of our project is to examine the contribution of InsP3R on local and global Ca2+ release events in atrial myocytes where the InsP3R is expressed six to ten times more than in ventricle myocytes.
Freshly isolated atrial myocytes from wild type and transgenic animals overexpressing the InsP3R were used combined with confocal laser scanning microscopy and whole cell patch clamp technique. Pharmacological interventions were used to separate RyR Ca2+ release from InsP3 induced subcellular Ca2+ release events.
We found that rapid InsP3 release, using UV-flash photolysis of caged InsP3, in wild type mice causes a significant increase in local Ca2+ release events, which is not seen in the presence of InsP3R or RyR blockers.
In ongoing experiments performed on transgenic animals overexpressing InsP3R, we predict a significant increase in the number of InsP3- induced Ca2+ release events.

P14

Metabotropic regulation of RhoA/Rho-associated kinase by L-type Ca2+ channels new mechanism for depolarization-evoked mammalian arterial contraction

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Sustained vascular smooth muscle contraction is mediated by extracellular Ca2+ influx through L-type voltage-gated Ca2+ channels (VGCC) and RhoA/Rho-associated kinase (ROCK)-dependent Ca2+ sensitization of the contractile machinery. VGCC activation can also trigger an ion-independent metabotropic pathway that involves G-protein/phospholipase C activation, inositol 1,4,5-trisphosphate synthesis, and Ca2+ release from the sarcoplasmic reticulum (calcium channel-induced Ca2+ release). We have studied the functional role of calcium channel-induced Ca2+ release and the inter-relations between Ca2+ channel and RhoA/ROCK activation.
We have used normal and genetically modified animals to study single myocyte electrophysiology and fluorimetry as well as cytosolic Ca2+ and diameter in intact arteries. These analyses were complemented with measurement of tension and RhoA activity in normal and reversibly permeabilized arterial rings. We have found that, unexpectedly, L-type Ca2+ channel activation and subsequent metabotropic Ca2+ release from sarcoplasmic reticulum participate in depolarization-evoked RhoA/ROCK activity and sustained arterial contraction. We show that these phenomena do not depend on the change in the membrane potential itself, or the mere release of Ca2+ from the sarcoplasmic reticulum, but they require the simultaneous activation of VGCC and the downstream metabotropic pathway with concomitant Ca2+ release. During protracted depolarizations, refilling of the stores by a residual extracellular Ca2+ influx through VGCC helps maintaining RhoA activity and sustained arterial contraction.
These findings reveal that calcium channel-induced Ca2+ release has a major role in tonic vascular smooth muscle contractility because it links membrane depolarization and Ca2+ channel activation with metabotropic Ca2+ release and sensitization (RhoA/ROCK stimulation).

P15

EC coupling elements alterations in mdx cardiomyocytes

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Duchenne Muscular Dystrophy (DMD) is a sex-linked recessive disease which results from the loss of the protein dystrophin, and leads to the degeneration of skeletal and cardiac muscles. Here we describe membrane structural damages and disorganisations of cardiomyocytes from the mdx mouse model of DMD. Experiments have been performed with Scanning Ion Conductance Microscopy (SICM) to characterize the loss of integrity of cardiomyocytes surface in dystrophin deficiency. Furthermore, with SICM nanopipette, we were able to apply ‘‘smart microperfusions’’ of depolarizing solutions, stimulating single T-Tubules. This experimental approach revealed modification of Excitation-Induced Calcium Release involved in Excitation–Contraction (EC) coupling in mdx cardiomyocytes. Finally, 2D Fourier analysis of labelled internal networks suggested local reorganizations of EC coupling proteins as observed in Heart Failure. We propose that, in the heart, dystrophin would play a ‘‘staking’’ role that may be involved in maintaining the membrane integrity not only at the cell surface but also in preserving T-Tubules structure in the depth of cardiomyocytes.