Conclusion: Alteration of cellular signaling upstream of GSK-3β is responsible for the lack of EPO protective effect in STZ-induced diabetic hearts.

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AngiotensinII induced atrial remodelling is worsened in mice overexpressing aldosterone synthase in cardiomyocyte

Ludovic Bénard [Orateur] (1), Laure Champ-Rigot (2), Sophie Gomes (2), Camille Rodroguez (1), Jane-Lise Samuel (1), Claude Delcayre (1), Paul Milliez (1)
(1) INSERM U942, Paris Cedex 10, France – (2) CHU caen, Caen, France

The aim of this work was to check the hypothesis that increased cardiac aldosterone level combined with arterial hypertension may enhance the deleterious effects at the heart level. Transgenic mice overexpressing Aldo Synthase (AS) in cardiomyocytes and wild type (WT) littermates were submitted to AngII-induced hypertension by osmotic pump (1mg/kg/day) during 3 or 8 weeks.

Results: 1) Physiological analysis indicated that the arterial pressure increased similarly (+50 mmHg) in AngII-perfused groups whatever the genotype. At the ventricular level, the hypertrophy and the fibrosis (x3, $p<0.05$) developed identically in the 2 AngII-groups ($p<0.05$ versus matched groups) independently of the time. In contrast, at the level of the atria, 3 wk AngII perfusion significantly worsened the dilatation +46% for AS mice and +33% for WT ($p<0.05$ versus matched groups) and fibrosis in AS mice (+20% AngII AS versus AngII WT, $p<0.05$). In the 8 wk perfused groups, the atrial diameters and the fibrosis were increased compared to 3 weeks. Besides, we noticed that AngII increased more P wave duration in AS mice than in WT. Interestingly Eplerenone treatment (50mg/kg/day) prevented all these changes.

2) The electrical changes led us to study the atrial expression of connexin (Cx) 40 and 43. In AS mice at basal state, we found a 4-fold increase in functional Cx43 when compared to WT whereas the functional levels of Cx40 were similar in both groups. In AngII-mice we found a decrease of functional Cx40 (–50% and –40% in AS and WT mice, respectively) whereas functional Cx43 increased by 30% whatever the genotype.

In conclusion, we show for the first time that both cardiac aldosterone and AngII regulate the expression of Cx40 and 43 in mouse left atria. In addition our results suggest that cardiac aldosterone worsened the deleterious effects of AngII-induced hypertension on left atria, through increases in dilatation, fibrosis and it increases conduction time.

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Non-invasive assessment of murine pulmonary arterial pressure: validation and application to models of pulmonary hypertension

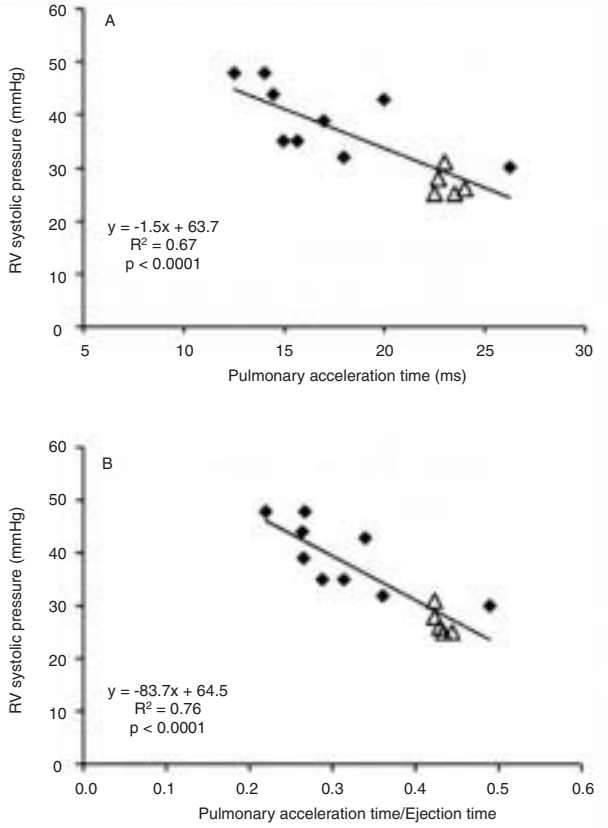
Helene Thibault (1), Baptiste Kurtz [Orateur] (1), Michael Raher (2), Rahamthulla S. Shaik (3), Aaron Waxman (3), Elkan Halpern (4), Kenneth D. Bloch (5), Marielle Scherrer-Crosbie (1)
(1) Massachusetts General Hospital, Ultrasound Laboratory in the Cardiology Division of the Department of Medicine, Boston, Etats-Unis – (2) Massachusetts General Hospital, Cardiovascular Research Center, Boston, Etats-Unis – (3) Massachusetts General Hospital, Pulmonary Critical Care Unit, Boston, Etats-Unis – (4) Massachusetts General Hospital, Institute for Technology Assessment, Boston, Etats-Unis – (5) Massachusetts General Hospital, Anesthesia Center for Critical Care Research, Department of Anesthesia, Boston, Etats-Unis

Background: Genetically modified mice offer the unique opportunity to gain insights into the pathophysiology of pulmonary arterial hypertension (PAH). In mice, right heart catheterization is the only available technique to measure right ventricular systolic pressure (RVSP). However, it is a terminal procedure and does not allow serial follow-up. Our objective was to validate a non-invasive technique to assess RVSP in mice.

Methods: Right ventricle catheterization and echocardiography were simultaneously performed in mice with pulmonary hypertension induced acutely by infusion of a thromboxane analogue, U-46619 or chronically by lung-specific over-expression of interleukin 6 (IL-6). In a subgroup of mice, echocardiography was performed using light anesthesia before catheterization. Pulsed-Doppler of pulmonary artery flow was recorded in the parasternal short axis view. Pulmonary acceleration time (PAT), and ejection time (ET) were measured.

Results: Infusion of U-46619 acutely increased RVSP, shortened PAT and decreased PAT/ET. The pulmonary flow pattern changed from symmetric at baseline to asymmetric at higher RVSPs. Transgenic IL-6 mice had high RVSP measured by catheter (39±7 mmHg), short PAT (17±4 ms) and low PAT/ET ratio (31±8%). The PAT correlated linearly with RVSP (r^2=–0.67; $p<0.0001$), as did PAT/ET (r^2=–0.76, $p<0.0001$). Sensitivity and specificity for detecting high RVSP (>32 mmHg) were 100% (7/7) and 86% (6/7), respectively, for both indexes (cutoff values: PAT <21 ms and PAT/ET <39%). PAT/ET measured during light anesthesia correlated with PAT/ET obtained during invasive catheterization (r^2=0.87, $p<0.0001$). Intra-observer and inter-observer variability of PAT and PAT/ET were less than 6%.

Conclusion: Pulmonary artery systolic pressure can be estimated noninvasively in mice. Echocardiography allows to monitor acute changes of RVSP and to detect pulmonary hypertension. This technique enables to follow PAH evolution easily and repeatedly in mice.



Correlation between RVSP (catheter) and echo

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Cardiac β3-adrenoceptors as a new therapeutic target in dilated cardiomyopathy

Nolwenn Merlet [Orateur] (1), Nicolas Piriou (2), Emmanuelle Lavazais-Blancou (1), Sabine Erbibou (1), Jean-Luc Balligand (3), Bertrand Rozec (1), Chantal Gauthier (1)
(1) Institut du Thorax, INSERM U915, Nantes, France – (2) CHU Nantes, Institut du Thorax – Clinique cardiologique et maladies vasculaires, Nantes, France – (3) Université Catholique Louvain, Louvain, Belgique

Introduction: β-adrenergic system is altered in heart failure (HF) due to non-ischemic dilated cardiomyopathy (DCM). There are few data concerning the relative contribution of β_1- and β_3-adrenoceptor subtypes (β_1- and β_3-AR) during DCM development. We evaluated the expression and the role of each β-AR subtype in this pathology.

Methods: DCM rat model is performed by doxorubicin injections (cumulative dose: 15 mg.kg^{-1}) and validated by ***in vivo*** measurements with echocardiography-doppler. The variations of β_1- and β_3-AR transcript expression in left ventricle (LV) are evaluated by real-time RT-PCR. The ***ex vivo*** cardiac responses induced by selective β_3-AR or non-selective β-AR stimulations are evaluated on isolated perfused heart.

Results: DCM rats present LV dilation, systolic and diastolic dysfunction (see table). Compared to controls, β_1-AR transcripts and β_3-AR transcripts are increased in DCM LV (+36%, n=8, p<0.05 and +358 %, n=8, p<0.05). ***Ex vivo*** parameters are summarized in the table.

Table: Basal parameters and maximum values obtained by non-selective β_1-AR stimulation (isoproterenol) or selective β_3-AR stimulation (SR58611A). Results are expressed by mean ± SEM.*: *p*<0.001 *vs* Control, £: *p*<0.001 *vs* Basal.

		Control rats (n=9-31)	DCM rats (n=10-31)
Basal ***in vivo***	LV end-diastolic diameter (mm)	8.82±0.33	7.61±0.17 *
	LV ejection fraction (%)	83.0±1.9	71.2±2.8*
	LV Isovolumic relaxation time (ms)	21.41±1.13	32.39±1.10*
Basal ***ex vivo***	DP/dt max (mmHg.s^{-1})	2035±365	2669±504*
	DP/dt min (mmHg.s^{-1})	–1258±226	–1847±349*
Isoproterenol ***ex vivo*** (1 µM)	DP/dt max (mmHg.s^{-1})	5263±1754	4373±1383*
	DP/dt min (mmHg.s^{-1})	–3815±1271	–3227±1020*
SR58611A ***ex vivo*** (1 µM)	DP/dt max (mmHg.s^{-1})	1722±497	1930±611£
	DP/dt min (mmHg.s^{-1})	–1027±297	–1138±360£

Conclusion: DCM induces a β_3-AR gene over-expression, associated to an increase of β_3AR-induced negative inotropic and lusitropic effects. Those results could partly explain the alteration of isoproterenol response in our model, suggesting that β_3-AR could be a new therapeutic target in DCM.

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Endothelial Estrogen Receptor α mediates the atheroprotective action of 17β-Eestradiol in LDLr deficient mice

Audrey Billon, Coralie Fontaine, Pierre Gourdy, Jean Francois Arnal [Orateur]
INSERM U858 – I2MR, Départ Biologie Vasculaire, Toulouse Cedex 4, France

Background: Although estrogen administration to hysterectomized menopausal women did not prevent the occurence of myocardial infarction in a randomized controlled trial (WHI 2004), epidemiological studies suggest and experimental results clearly demonstrate a major atheroprotective action of estrogens. The goal of the present study was to identify the cellular target(s) accounting for the estradiol (E2) beneficial action on fatty streak development.

Methods and Results: We first confirmed the key role of estrogen receptor α (ERα) in atheroprotective effect of E2 as this action was completely abolished in mice deficient both in Low Density Lipoprotein receptor (LDLr) and in ERα. Comparison of LDLr–/– mice transplanted with either ERα+/+ or ERα–/– bone marrow showed that functional ERα in the hematopoietic lineage is not required for E2 atheroprotection. We then showed that ERα floxed mice (ER_flox/flox) bred with the Tie2-Cre mice on the LDLr–/–- background had a complete inactivation of ERα both in bone marrow and in endothelial cells. Remarkably, in this mouse model, the E2 atheroprotective action was completely abolished.

Conclusions: Altogether, this is the first in vivo demonstration that endothelial ERα represents a key target of the atheroprotective effect of E2, whereas the hematopoietic ERα isdispensable for the protective action. Selective estrogen receptor modulators that mimic this endothelial action of E2 should now be considered in hormonal treatment as well as in atheroprotection.

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Estrogen Receptor α expression in both endothelium and hematopoietic cells is required for the accelerative effect of estradiol on reendothelialization

Jean Francois Arnal [Orateur], Celine Toutain, Cedric Filipe, Coralie Fontaine, Pierre Gourdy, Françoise Lenfant
INSERM U858 – I2MR, Départ Biologie Vasculaire, Toulouse Cedex 4, France

Objectives: Although reducing rates of restenosis, drug-eluting stents also impair endothelial healing, resulting in increased risk of thrombosis. Alternatively, inhibition of neointimal hyperplasia is favoured by acceleration of reendothelialization. We previously showed that E2 accelerates reendothelialization through Estrogen Receptor α (ERα) and we now aimed at defining the cellular targets of this action.

Methods and Results: The respective roles of endothelial and hematopoietic cellular targets of E2 were investigated in a mouse carotid injury model, using "en face" confocal microscopy, to follow endothelium repair. Grafting ERα –/– mice with ERα +/+ bone marrow (BM) did not restore the accelerative effect of E2 on reendothelialization, demonstrating the necessary role of extrahematopoietic ERα. Using a cell-specific inactivation of ERα (Cre-lox recombination system), we showed that endothelial ER_ plays a pivotal role in the E2 action. Finally, in wild type mice grafted with ERα –/–, the regenerative effect of E2 was abolished, demonstrating that ERα-expressing hematopoietic cells are also concomitantly required.

Conclusions: We demonstrate that endothelial ERα plays a pivotal role in E2-mediated reendothelialization. However, endothelial targeting alone is not sufficient and the concomitant stimulation of BM ERα is absolutely required. This cooperation should be now taken into account in strategies aimed at optimizing in-stent reendothelialization.

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Zac1, jointly down-regulated by preconditioning and postconditioning in a mouse model of myocardial ischemia/reperfusion: a transcriptomic approach

Gérald Gahide [Orateur] (1), François Roubille (2), Jérome Adda (2), Anne Vincent (2), Christelle Dantec (3), Catherine Sportouch-Dukhan (2), Aurélie Covinhes (2), Dany Severac (3), Laurent Journot (2), Christophe Piot (2), Joël Nargeot (2), Stéphanie Barrère-Lemaire (1)
(1) INSTITUT DE GENOMIQUE FONCTIONNELLE, Inserm U661 – CNRS UMR5203-UMSF-Département de physiologie, Montpellier, France – (2) INSTITUT DE GENOMIQUE FONCTIONNELLE, Inserm U661 – CNRS UMR 5203-UMSF-Département de physiologie, Montpellier, France – (3) INSTITUT DE GENOMIQUE FONCTIONNELLE, Montpellier GenomiX Facilities, Montpellier, France

Ischemic preconditioning and postconditioning are two effective therapeutic strategies for reducing infarct size in animal models and humans. The aim of our study was to compare the early regulated genes of preconditioning and postconditioning using a transcriptomic approach.

Methods: C57Bl6, Zac1+/– KO (n=7) and WT littermates (n=4) mice underwent an IR (40 min. ischemia/60 min.reperfusion) protocol. C57Bl6 mice were randomly assigned to different groups: IR (n=22); postconditioning (PostC, n=21): a protocol of 3 cycles of 1-minute reperfusion and 1-minute reocclusion was applied at the onset of reperfusion; preconditioning (PreC, n=15; same algorithm but applied before ischemia. At the end of surgery, left ventricles were assigned to RNA extraction or infarct size assessment. Homemade mouse oligo microarrays were used for gene expression profiling (Montpellier GenomiX Facilities). Determination of area at risk (AR) and infarct size was assessed by TTC staining and planimetry.

Results: Our study revealed that despite a similar cardioprotection offered by PreC and PostC on infarct size, PostC regulates a larger number of genes compared to PreC (242 versus 40). Only 8 genes were jointly regulated by PreC and PostC and considered as putative cardioprotective key regulators. Among these candidates, Zac1 was down regulated at the transcriptional levels upon PreC and PostC. Moreover, infarct size/AR was 29%-decreased in Zac1 +/– KO mice subjected to a surgical protocol of myocardial IR.

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