to obtain membrane-specific loss of function (iii) mice lacking the AF2 ligand-dependant-transcriptional function domain of ERα (AF2°) and (iv) wild type (WT) mice. Hypertension was induced using Angiotensin II (Alzet minipumps) and blood pressure measured using tail cuff. After 28 days vascular structure and function were measured in vitro. Angiotensin II-induced hypertension was greater in ERα/-/ and in AF2° mice than in WT and C451A-ERα mice. These data suggest that the protective effect of ERα against elevated blood pressure involved nuclear AF2-mediated transcriptional functions rather than MISS-mediated function. Changes in pressure were associated with proportional changes in endothelium-dependent dilation and wall thickness. Thanks to this work, we hope to better understand how ERα is able to mediate the protective effects of estrogen against hypertension.

0343

Essential role of P2Y6 UDP receptor in Angiotensin-II dependent arterial hypertension

Gilles Kauffenstein (1), Charlotte Roy (1), Linda Grimaud (1), Bertrand Toutain (1), Jean-Marie Boeynaems (2), Bernard Robaye (2), Daniel Henrion (1)

(1) Université d’Angers, UMR CNRS 6214 INSERM U1083, Angers, France – (2) IRIBHIM Université Libre de Bruxelles, Bruxelles, Belgique

Extracellular nucleotides are responsible for pleiotropic effects in the vasculature. Uracil nucleotides are vasoactive and trophic agents and promote inflammation. The participation of specific P2 receptors in these effects remains undefined and their potential contribution in arterial hypertension is unknown. Objective: To evaluate the contribution of the UDP receptor P2Y6 in hypertension in mouse. Methods: Arterial contraction was evaluated using a wire myograph. Blood pressure was measured following nucleotides iv infusion and experimental hypertension was induced either by Angiotensin-II (Ang-II 1mg/kg/j) or DOCA-salt (1%) in uni-nephrectomized mice. Histological approaches, immunofluorescence and RTqPCR were used to evaluate the nature of vascular remodeling. Results: P2Y6 displayed the highest arterial expression level among other P2Y receptors. Contraction of conductance (thoracic aorta) and resistance (mesenteric) arteries was abrogated in P2y6-/- mice in response to UDP and UTP while other vasoconstrictor induced normal responses. P2Y6 receptor triggered a moderate intracellular calcium increase while RhoA (calcium facilitating pathway) activation was abrogated in P2y6-/- mice. Both genetic deletion and pharmacological blockade of P2Y6 receptor abolished Ang-II-induced blood pressure increase (40 mmHg in wild type mice). By contrast, hypertensive response in DOCA-salt was equivalent in both genotypes. Following Ang-II treatment, P2ry6-/- mice developed a reduced arterial hypertrophic remodeling and fibrosis but equivalent immune cell recruitment/infiltration compared to wild type. These changes were corroborated to reduced mRNA expressions of TGFβ and NADPH oxidase subunits. Conclusions: Vascular P2Y6 receptor contributes to exaggerated vascular tone, hypertrophy and fibrosis in the context of Ang-II-dependent hypertension. Its absence or pharmacological blockade limits vascular damages and prevents blood pressure increase associated to hypertension.

0356

Disseminated arterial calcification and enhanced myogenic response are associated with Abcc6 deficiency in a mouse model of pseudo-xanthoma elasticum

Gilles Kauffenstein (1), Anne Pizard (2), Yannick Le Corre (1), Emilie Vessières (1), Linda Grimaud (1), Bertrand Toutain (1), Carlos Labat (2), Yves Mauras (3), Theo G. Gorgels (4), Arthur A. Bergen (4), Olivier Le Saux (5), Patrick Lacolley (2), Georges Lefthériotis (1), Daniel Henrion (1), Ludovic Martin (1)

(1) Université d’Angers, UMR CNRS 6214 INSERM U1083, Angers, France – (2) CHU Angers, Laboratoire de Pharmacologie Toxicologie, Angers, France – (3) CHU Angers, Laboratoire de Pharmacologie Toxicologie, Angers, France – (4) Netherlands Institute for Neuroscience, Molecular Ophthalmogenetics, Amsterdam, Pays-Bas – (5) John A. Burns School of Medicine, Cell and Molecular Biology, Honolulu, Etats-Unis

Disseminated arterial calcification remains unclear. We investigated arterial structure and function in Abcc6-/- mice, a model of the human Pseudo-xanthoma Elasticum (PXE). Arterial calcium accumulation determined by atomic absorption spectrometry was 1.5 – to 2-fold higher in Abcc6-/- than in wild-type mice. Calcium also accumulated locally leading to a specific punctuated pattern. Abcc6-/- mesenteric arteries mounted, on a wire myograph displayed slight increase in arterial vasoconstrictor tone in response to phenylephrine and thromboxane A2. Interestingly, myogenic tone (Bayliss effect) determined using a pressure myograph was significantly elevated in Abcc6-/- compared to wild type arteries. Arterial blood pressure was not significantly modified in Abcc6-/- animals, despite higher variability. These changes were accompanied with deregulated gene expression (RTqPCR) in both liver and resistance arteries. Old Abcc6-/- mouse mesenteric arteries expressed markers of both osteogenic (Runx2, opn) and chondrogenic lineage (Sox9, col2a1). Surprisingly Enp1 and Alpi genes encoding ectonucleotide pyrophosphatase/phosphodiesterase 1 and alkaline phosphatase were deregulated within Abcc6-/- liver and this was corroborated with reduced alkaline phosphatase circulating levels in PXE patients. As a conclusion, scattered calcium deposits result from osteochondrogenic transdifferentiation of vascular cells. The lower elasticity and increased myogenic tone evidenced in aged Abcc6-/- mice suggest a reduced control of local blood flow, which in turn may alter vascular homeostasis. Our findings argue in favor of a deregulated arterial function and may help to decipher consequences of ABC6 deficiency since PXE is a significant risk factor for small vessel disease and particularly ischemic stroke.

0416

Vascular smooth muscle cells are responsible for a prothrombotic phenotype of spontaneously hypertensive rat arteries

Amel Mohamadi, Jérémy Lagrange, Hugueitte Louis, Patrick Lacolley, Véronique Regnault

Faculté de Médecine, Inserm U1116, Vandoeuvre-Lès-Nancy, France

Objective: The hypothesis that hypertension confers a hypercoagulable state arises from the complications associated with hypertension, stroke and myocardial infarction. Our objective was to determine whether spontaneous hypertension causes changes in the thrombin generating capacity of the vascular wall.

Approach and Results: We used spontaneously hypertensive rats (SHR) compared with Wistar rats. The addition of thoracic aorta rings of SHR to a Wistar or SHR plasma pool resulted in a greater increase in thrombin generation compared to the addition of equivalent rings from Wistar. Comparison of 5-week-old and 12-week-old rats indicate that established hypertension is required to induce increased thrombin generation within the vessel wall. Whereas no difference was observed for endothelial cells, thrombin formation was higher at the surface of cultured aortic smooth muscle cells (SMCs) from SHR than from Wistar. Exposure of negatively-charged phospholipids was higher on SHR than on Wistar aortic rings as well as on SMCs. Tissue factor activity was higher in SHR SMCs. Twelve-week-old SHR exhibited accelerated FcγI-induced thrombus formation in carotid arteries and the resulting occlusive thrombi were disaggregated by blockade of glycoprotein Ib-α von Willebrand factor interactions. SHR SMCs were more sensitive to thrombin-induced proliferation than Wistar SMCs. This cellular effect was totally abolished by a protease-activated receptor 1 inhibitor.

Conclusions: The prothrombotic phenotype of the SHR vessel wall was due to the ability of SMCs to support greater thrombin generation and resulted in accelerated occlusive thrombus formation after arterial injury, which is sensitive to glycoprotein Ib-α von Willebrand factor inhibitors.

0194

Angiotensin II type 2 receptor reduces metabolic and vascular effects of type 1 diabetes in the mouse

Marc-Antoine Begorre, Emilie Vessières, Anne-Laure Guihot, Linda Grimaud, Laurent Loufrani, Céline Fassot, Daniel Henrion

Université d’Angers, Laboratoire de Biologie Vasculaire, UMR CNRS 6214 – INSERM 1083, Angers, France

The renin-angiotensin system has a key role in cardiovascular homeostasis, mainly through activation of angiotensin II type 1 (AT1R) and type 2 (AT2R)
receptors. Although AT2R opposes the effects of AT1R, with vasodilator and antitrophic properties, its effect in diabetes remains debated. Thus we determined the role of AT2R in endothelium-mediated dilation in a mouse model of type 1 diabetes. Diabetes was induced in three-month-old mice by streptozotocin (150mg/Kg, ip). After 45 days, mesenteric resistance arteries were isolated and mounted on a wire myograph to measure vascular reactivity. The absence of AT2R reduced glucose tolerance and body weight and increased STZ-induced hyperglycemia, hyperinsulinemia, creatiniemia, blood thromboxaneA2, PGF2alpha and isoprostane. AT2R, COX-2 and NADPH-oxidase subunits (gp91phox and p22phox) gene expression were higher in arteries of diabetic mice than in control animals. COX-2 level and oxidative stress were also higher in mesenteric and renal arteries in AT2R-/- mice.

Endothelium-dependent relaxation, reduced in diabetic mice arteries, was normalized after cyclooxygenase-2 (COX-2) (CAY10404) blockade or ROS reduction with tempol plus catalase in both AT2R-/- and AT2R+/- mice. AT2R-dependent dilation in isolated perfused mesenteric arteries was greater in diabetic mice than in control animals.

Thus, in a model of type 1 diabetes AT2R reduces the severity of diabetes possibly through a reduction of the production of ROS and COX-2-derived vasoconstrictor agents.