



## Artery Research

ISSN (Online): 1876-4401

ISSN (Print): 1872-9312

Journal Home Page: <https://www.atlantis-press.com/journals/artres>

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### **3.3: PRESSURE-INDEPENDENT AORTIC STIFFENING EVALUATION IN VARIOUS HYPERTENSIVE RAT MODELS IN VIVO, VIA PULSATILE DIAMETER RECORDING**

C. Vayssettes-Courchay, C. Ragonnet, M. Isabelle, J.P. Vilaine

**To cite this article:** C. Vayssettes-Courchay, C. Ragonnet, M. Isabelle, J.P. Vilaine (2012) 3.3: PRESSURE-INDEPENDENT AORTIC STIFFENING EVALUATION IN VARIOUS HYPERTENSIVE RAT MODELS IN VIVO, VIA PULSATILE DIAMETER RECORDING, Artery Research 6:4, 145–146, DOI: <https://doi.org/10.1016/j.artres.2012.09.021>

**To link to this article:** <https://doi.org/10.1016/j.artres.2012.09.021>

Published online: 21 December 2019

### Oral session 3 Free Communication Oral Presentations

#### 3.1

##### THE EFFECTS OF ANTICONTRACTILE PROPERTY OF PERIVASCULAR ADIPOSE TISSUE IN OBESE MICE

C. Agabiti Rosei <sup>1</sup>, C. De Ciuceis <sup>1</sup>, C. Rossini <sup>1</sup>, E. Porteri <sup>1</sup>, R. Rezzani <sup>2</sup>, L. F. Rodella <sup>2</sup>, A. M. Heagerty <sup>3</sup>, D. Rizzoni <sup>1</sup>, E. Agabiti Rosei <sup>1</sup>

<sup>1</sup>Clinica Medica, Department of Medical and Surgical Sciences, University of Brescia, Italy

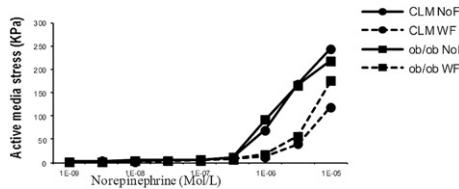
<sup>2</sup>Chair of Human Anatomy, University of Brescia, Italy

<sup>3</sup>Cardiovascular Research Group, Core Technology Facility, University of Manchester, United Kingdom

It's known that perivascular adipose tissue(PVAT)has an anticontractile effect maybe mediated by adiponectin, a physiological modulator of local vascular tone with mechanism still unknown. The PVAT function is lost in obese patients with the development of adipocyte hypertrophy, hypoxia, inflammation and oxidative stress(Circulation 2009; 119(12):1661-1670).The aim of the study was to investigate functional responses of small mesenteric arteries in a animal model of genetic obesity.

**Materials and Methods:** we investigated 8 obese mice(B6.V-Lep ob/OlaHsd, ob/ob) and 8 control lean mice (CLM). Mesenteric small resistance arteries(internal diameter about 200 µm) were dissected and mounted on a wire myograph. A concentration-response curve to norepinephrine(NE, from  $10^{-9}$  to  $10^{-5}$  Mol/L) was evaluated in vessels with intact perivascular fat tissue(WF) and in vessels in which perivascular fat tissue was removed(NoF) in basal conditions and during vascular hypoxia (30', 95%N<sub>2</sub>/5%CO<sub>2</sub>).Concentration response to NE was repeated in the presence of iberiotoxin(100 nm/L for 30'), a selective blocker of calcium-dependent potassium channels (BK<sub>CA</sub>), that have been previously suggested to be involved in vascular tone regulation.

**Results:** are summarized in the figure(active media stress:KPa).The presence of PVAT reduced the contractile response to NE in both ob/ob(ANOVA p= 0.002 vs. noF)and CLM(ANOVA p= 0.001 vs. NoF),however, the effect was reduced in ob/ob compared with CLM. The anticontractile effect of PVAT completely disappear with iberiotoxin preincubation(Maximum contraction to NE.ob/ob:WF:176±47,68, NoF:218±66,02\*, WF+iberiotoxin:289±98,06\*;CLM:W-F:118±53,92, NoF:245±51,8\*, WF+iberiotoxin:211±31\*, \*p<0.05 at least vs. WF) In conclusion, the anticontractile effect of perivascular fat is partially maintained in an animal model of genetic obesity. This effect may be related to the activity of BK<sub>CA</sub> channels, since it is blocked by iberiotoxin, a scorpion toxin that inhibits calcium-dependent potassium channels



#### 3.2

##### THE ENDOGENOUS NA,K-ATPASE LIGAND, MARINOBUFAGENIN, INDUCES VASCULAR FIBROSIS VIA A PRESSURE-INDEPENDENT MECHANISM IN NaCl-LOADED DIABETIC RATS

Y. N. Grigorova <sup>1</sup>, A. V. Fadeev <sup>1</sup>, K. A. Bagrov <sup>1</sup>, E. V. Frolova <sup>1</sup>, O. V. Fedorova <sup>2</sup>, A. Y. Bagrov <sup>2</sup>

<sup>1</sup>Amlazov Federal Heart Blood and Endocrinology Centre, St. Petersburg, Russian Federation

<sup>2</sup>National Institute on Aging, NIH, Baltimore, United States

An endogenous steroid marinobufagenin (MBG) induces fibrosis via inhibition of Flt1, a nuclear transcription factor and a negative regulator of collagen synthesis. Because immunization against MBG reduced cardiac fibrosis but minimally affected blood pressure (BP) in uremic rats (Hypertension, 2007;49:215-24), we hypothesized that MBG induces cardiovascular fibrosis via BP-independent mechanism.

We measured BP, urinary MBG, aortic collagen-1, and vascular function in NaCl-loaded male Wistar rats with type 2 diabetes mellitus (DM-NaCl) and in non-diabetic control rats (Ctrl). DM was induced by administration of 65 mg/kg streptozotocin to neonatal animals. At three months, 24 diabetic rats were dietary supplemented with 1.8% NaCl added to the drinking water for four weeks following which rats were twice administered monoclonal anti-MBG antibody (mAb) (n=12), or vehicle (n=12). Isolated rings of thoracic aortae were tested for their responsiveness to endothelin-1 and to sodium nitroprusside (SNP) following endothelin-1-induced constriction.

DM-NaCl rats exhibited a 3.5-fold increase in MBG excretion ( $25.4\pm4.5$  vs.  $7.7\pm1.5$  nmoles in Ctrl; P<0.001) and 2.5-fold increase in collagen-1 in thoracic aortae in the absence of changes of BP or renal function. Compared to Ctrl, in aortic rings from DM-NaCl the responsiveness to endothelin-1 was unaltered ( $EC_{50}=2.2$  and  $3.2$  nmol/L, respectively), but the response to the relaxant effect of SNP was impaired ( $EC_{50}=29$  vs.  $EC_{50}=7$  nmol/L P<0.001). In vivo administration of mAb to DM-NaCl rats did not affect BP, but reduced aortic collagen-1, and restored sensitivity of aortic rings to SNP ( $EC_{50}=9$  nmol/L). Thus, MBG induces vascular fibrosis and increases vascular stiffness without affecting BP.

#### 3.3

##### PRESSURE-INDEPENDENT AORTIC STIFFENING EVALUATION IN VARIOUS HYPERTENSIVE RAT MODELS IN VIVO, VIA PULSATILE DIAMETER RECORDING

C. Vaysettes-Courchay, C. Ragonnet, M. Isabelle, J. P. Vilaine  
Servier Research Institute, Suresnes, France

We have shown that in addition to compliance and distensibility, the analysis of the arterial distension (pulsatile diameter) via echotracking (ArtLab) provides information to discriminate the pressure-dependent and independent arterial stiffening. In the present study we evaluated these parameters in different hypertensive rat models. Mean, systolic, diastolic and pulse arterial pressure (AP) and the aortic diameter were recorded in 6 groups of pentobarbital anaesthetized rats: 20 week old spontaneously hypertensive rat (SHR), SHR treated with the NOS inhibitor L-NAME 6 mg/kg/day in drinking water (SHRLN), 50-60 week old SHR, DOCA-salt (uni-nephrectomized, deoxycorticosterone treated rats) and VDN (VitamineD+Nicotine treated rats); all were compared to their respective controls: Wistar-Kyoto (WKY) and Wistar normotensive rats. The diameter was comparable in all groups. Except in the DOCA-salt, increased stiffness index and decreased compliance, distensibility and distension were observed, indicating an aortic stiffening. When in each rat AP was decreased acutely by clonidine i.v., reaching the control rat values of AP, stiffness index, compliance and distensibility were more or less restored in SHR>old SHR=SHRLN>VDN; however the diameter distension wave (area under the curve adjusted to heart rate) was restored in SHR, partially in old SHR, but not in SHRLN and VDN (table). These data show, via the analysis of the aortic pulse diameter distension, that in SHRLN and VDN the increase in aortic stiffness is in part pressure-independent and due to arterial wall remodeling. The results are in line with previous structural vascular wall studies.

	WKY	SHR	SHRLN	Old SHR	Wistar	DOCAsalt	VDN
n	6	6	8	5	7	7	9
Mean AP mmHg	basal	148±3	197±9*	209±6*	198±7*	144±3	168±6*
	clonidine		136±7§	133±8§	125±9§		123±8§
AUC aortic distension	basal	2.7±0.3	1.6±0.2*	1.2±0.2*	1.1±0.2*	2.0±0.1	1.9±0.4
	clonidine		2.6±0.2§	1.2±0.1*	1.6±0.2*		2.3±0.2

\* : p<0.05 versus control normotensive rats, § : p<0.05 versus basal value before clonidine.

## 3.4

## CHARACTERISATION OF THE TWO-LAYERED MEDIA IN THE MAMMALIAN CAROTID ARTERY

C. Evagora <sup>1</sup>, C. Hillery <sup>1</sup>, L. H. Timmins <sup>2</sup>, E. Das <sup>1</sup>, S. Badye <sup>1</sup>, J. E. Moore <sup>3</sup>, J. E. Martin <sup>1</sup>, S. E. Greenwald <sup>1</sup>

<sup>1</sup>Blizard Institute, Barts & The London School of Medicine & Dentistry, London, United Kingdom

<sup>2</sup>Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, United States

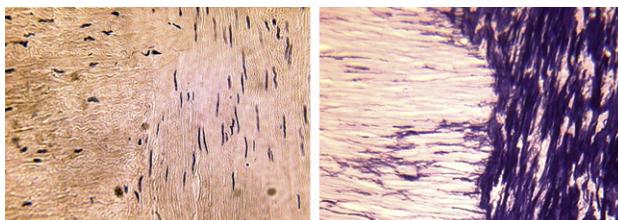
<sup>3</sup>Department of Biomedical Engineering, Texas A&M University, College Station, United States

**Background:** We have recently observed that in some animals the media of the carotid artery consists of two distinct layers. The inner 2/3 has a circumferential orientation of components whereas the outer 1/3 has an axial orientation. The aims of this study were to characterise the differences between the two layers.

**Materials and Methods:** We studied carotid arteries from the mammalian orders: artiodactyla, carnivora, cetacea, erinaceomorpha, lagomorpha, perissodactyla, pilosa, primates, rodentia and soricomorpha. Transmission electron microscopy confirmed the morphology and thickness of the layers. Histological staining was performed to characterise the cells and their phenotypes.

**Results:** We observed the two-layered media in the artiodactyla, perissodactyla and cetacea but not in any of the other orders. Immunohistochemistry showed that the distribution of fibrillin 1, NAV 1.8, tenascin C, fibronectin and collagen I & III were the same in the two layers. Alpha actin, desmin and collagen IV were seen only in the inner layer, suggesting a contractile phenotype for the cells therein, giving no indication of the function of the cells in the outer layer.

**Conclusions:** The inner media had a circumferential orientation of components and contractile smooth muscle cells; whereas the components of the outer layer were orientated axially with fibroblast-like cells of unknown function and more densely distributed scleroprotein. All the animals having the two-layered media are located downstream from a particular point on the mammalian supertree, corresponding to the superorder cetartiodactyla, suggesting that this structure evolved rapidly, approximately 80 million years ago for as yet unknown reasons.



## 3.5

## EFFECT OF SHORT-TERM TREATMENT WITH LERCANIDIPINE ON CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND STRUCTURAL ALTERATIONS IN RETINAL ARTERIOLES

C. De Ciuceis <sup>1</sup>, C. Rossini <sup>1</sup>, E. La Boria <sup>1</sup>, S. Duse <sup>2</sup>, F. Semeraro <sup>2</sup>, C. Agabiti Rosei <sup>1</sup>, P. Airò <sup>3</sup>, M. Scarsi <sup>3</sup>, A. Tincani <sup>3</sup>, M. L. Muijsen <sup>1</sup>, A. Paini <sup>1</sup>, M. Salvetti <sup>1</sup>, E. Porteri <sup>1</sup>, E. Agabiti Rosei <sup>1</sup>, D. Rizzoni <sup>1</sup>

<sup>1</sup>Clinica Medica, Department of Medical and Surgical Sciences, University of Brescia, Brescia, Italy

<sup>2</sup>Chair of Ophthalmology, University of Brescia, Brescia, Italy

<sup>3</sup>Chair of Rheumatology, University of Brescia, Brescia, Italy

**Background:** It has been previously demonstrated that dihydropyridine calcium channel blockers may possess antioxidant properties and might improve microvascular structure. The aim of the present study was therefore to investigate the effects of a short-term treatment with lercanidine on structural alterations in retinal arterioles and on circulating endothelial progenitor cells (EPCs), which are bone marrow-derived cells possibly participating in neovascularization and endothelial protection and repair.

**Patients and Methods:** Fourteen essential hypertensive patients were included in the study and treated for 4 weeks with lercanidine 10-20 mg per day orally. Investigations were performed in basal condition, after appropriate wash out of previous treatments, and after 4 week lercanidine treatment. EPCs were evaluated by flow cytometry as CD34+/CD133+/KDR+ cells. Non-invasive measurements of internal diameter (ID), external diameter (ED), wall thickness, wall to lumen ratio (W/L) and wall cross-sectional area (WCSA) of retinal arterioles using scanning laser doppler flowmetry (SLDF) were performed (Heidelberg Retina Flowmeter, Heidelberg Engineering), according to Harazny J et al, Hypertension 2007; 50:623-629.

**Results** are summarized in the Table (\*P<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Basal).

A significant increase in circulating EPC count was observed after treatment with lercanidine, associated with a reduction in W/L and an improvement of other indices of retinal artery structure, which, in the absence of a significant increase in ID, does not seem to be ascribed just to the vasodilator effect of the drug.

**Conclusions:** For the first time, in this study, favourable effects on the EPC-dependent endothelium-repair system and on alterations of retinal arterioles have been reported in men after treatment with a dihydropyridine calcium channel antagonist, lercanidine, possibly related to hemodynamic and antioxidant properties.

Table	Gender (M/F)	Blood pressure (mm Hg)	EPCs	W/L
Basal	11/3	154/90± 9.6/11.9	49.1±40	0.52±0.14
Treatment	-	145/78.7± 10.7/10.5*/*	102±71*	0.26±0.08**
	ID (μm)	ED (μm)	Wall thickness (μm)	WCSA (μm <sup>2</sup> )
Basal	57.3±12.9	85.6±15.6	14.1±3.37	3232±1214
Treatment	61.1±8.05	77.1±11.7*	7.99±2.78***	1784±842**

## 3.6

## CENTRAL PULSE PRESSURE IS AN INDEPENDENT DETERMINANT OF VASCULAR REMODELLING IN THE RETINAL CIRCULATION

C. Ott <sup>1</sup>, U. Raff <sup>1</sup>, J. Harazny <sup>1</sup>, G. Michelson <sup>2</sup>, R. E. Schmieder <sup>1</sup>

<sup>1</sup>Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany

<sup>2</sup>Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen, Germany

**Objective:** Pulse pressure has been recognized as a risk factor for stroke. Moreover, it was shown that central pulse pressure (PP) relates more strongly to vascular disease and outcome than (peripheral) brachial PP. Hence, we analyzed the impact of central PP on retinal vascular structure, mirroring cerebral circulation.

**Design and methods:** The study cohort comprised 135 patients across a wide range of BP, but without evidence of diabetes. Parameters of retinal capillary structure (wall-to-lumen (WLR) and function (change of retinal capillary flow (RCF) to nitric oxide synthase inhibitor N-nomethyl-L-arginine (L-NMMA), reflecting basal NO activity of the retinal vasculature) was assessed non-invasively and in vivo by scanning laser Doppler flowmetry. Central hemodynamics and augmentation index (Alx) were assessed by pulse wave analysis.

**Results:** WLR was correlated with central PP ( $r = 0.302$ ,  $p < 0.001$ ). In accordance, PP amplification (peripheral PP/central PP) was negatively correlated with WLR ( $r = -0.223$ ,  $p = 0.009$ ). In contrast, MAP was not correlated with WLR ( $r = 0.110$ ,  $p = 0.203$ ). Moreover, Alx@75 correlated with WLR ( $r = 0.190$ ,  $p = 0.028$ ). The percent change of RCF to L-NMMA was correlated to WLR ( $r = 0.197$ ,  $r = 0.022$ ).

Regression analysis revealed an independent relationship between WLR and both central PP ( $\beta = 0.277$ ,  $p = 0.009$ ) and percent change of RCF to L-NMMA ( $\beta = 0.170$ ,  $p = 0.046$ ).

**Conclusion:** Thus, central PP is an independent determinant of remodelling in small retinal arterioles indicating a coupling between the micro- and macrovascular changes to hypertension.