of cardiovascular disease. Aortic stiffness was assessed by the carotid-femoral pulse wave velocity (PWV). Thrombin generation and its delimitation by activated protein C (APC) were assessed by thrombography. Free tissue factor pathway inhibitor (TFPI), von Willebrand factor (vWF) and factor VIII were quantified in plasma. Women aged >70 years had higher levels of PWV, vWF, free TFPI and factor VIII than women aged 60-64 years. Rate and maximum level of thrombin increased with age without increase in the endogenous thrombin potential in the absence of APC (ETP0). This can be explained by increase in free TFPI counteracting thrombin formation driven by increased factor VIII and vWF. Neither ETP0 nor APC sensitivity were correlated with PWV. There was a strong correlation between free TFPI or vWF and PWV even after adjustment for age. Multiple regressions identified for the first time PWV as an independent determinant of free TFPI but not vWF concentrations. In conclusion, free TFPI is an active marker of the vascular wall dysfunction which retains its anticoagulant activity to limit age-related prothrombotic phenotype in women free of overt cardiovascular disease.

I004

CARDIOTROPHIN-1 ACTIONS IN VASCULAR SMOOTH MUSCLE CELLS. A ROLE IN ARTERIAL STIFFNESS

N. LOPEZ [1], M.-A. FORTUNO [2], C. LABAT [1], N. SLOBODA [1], J. NUEE-CAPIAUMONT [1], J. DIEZ [2], P. LACOLLEY [1], F. ZANNAD [3], P. ROSSIGNOL [3]

[1] *Risques Cardiovasculaire, rigidité-fibrose et hypercoagulabilité. Inserm U684, Vandoeuvre-lès-Nancy, France*

[2] *Division of Cardiovascular Sciences. Centre for Applied Medical Research, Pampelune, Spain*

[3] *CIC-Inserm CHU de Nancy. Hôpital Jeanne d'Arc., Dommartin-lès-Toul, France*

Background & aims — Cardiotrophin-1 (CT-1) is a cytokine belonging to the interleukin-6 superfamily that exhibits trophic and survival properties in a number of cell types. CT-1 protein expression has recently been identified within the media of atherosclerotic arteries, but its role in the vessel is still unknown. The present study was designed to investigate the effects of CT-1 in vascular smooth muscle cells (VSMC) and its involvement in the arterial phenotype of CT-1-null mice.

Methods — Rat aorta primary cultured VSMC were stimulated with CT-1 (10-11-10-9M) and/or antibodies against CT-1 receptors (LIFR or gp130), and/or chemical inhibitors against CT-1-activated intracellular pathways, for up to 48 hours. Cell proliferation was determined by MTT assay and ki67 immunodetection. The expression of collagen type I and III, elastin and fibronectin was quantified by RT-PCR and Western blot. Matrix metalloproteinases (MMPs) activities were assessed by gelatin and casein zymographies. Arterial mechanical properties were evaluated in 2 years-old WT (n=8) and CT-1-null mice (n=3) by echo-tracking device. Circumferential wall stress, incremental elastic modulus (Einc), media cross-sectional area and collagen and elastin content were recorded.

Results — CT-1 induced VSMC proliferation in a dose-dependent manner ($p<0.01$). CT-1 also increased mRNA and protein expression of collagen type I ($p<0.01$), collagen type III ($p<0.01$), fibronectin ($p<0.05$) and elastin ($p<0.05$), with a parallel and dose-dependent increase in active MMP-2 ($p<0.01$), MMP-3 ($p<0.05$) and MMP-9 ($p<0.01$). All these effects were reversed in the presence of antibodies against CT-1 receptors and intracellular chemical inhibitors. CT-1-null mice presented an increased wall stress ($p<0.05$) and Einc ($p<0.05$) as compared with WT mice. Media cross sectional area and collagen content were reduced ($p<0.05$) in mice lacking CT-1.

Conclusions — In summary, CT-1 induced cell proliferation and a secretory phenotype in VSMC. Moreover, mice lacking CT-1 presented a reduced carotid stiffness accompanied by a reduced media thickness and collagen content. Data here presented suggest that CT-1 actions in VSMC facilitate extracellular matrix deposition and arterial stiffness.

I005

CHEMICAL DENERVATION OF SYMPATHETIC NERVOUS SYSTEM INDUCES ABNORMAL MYOCARDIAL ARCHITECTURE

C. GUILBEAU-FRUGIER [1], B. HONTON [1], F. DESPAS [1,2], G. GENET [1], A. PATHAK [1,2], C. GALES [1], J.-M. SENARD [1,2]

[1] *Inserm U858, I2MR, Toulouse, France*

[2] *Laboratoire de Pharmacologie, Faculté de Médecine, Toulouse, France*

Introduction — The role of autonomic nervous system (ANS) on heart function modulation is well-known. By contrast, ANS role on myocardial tissue architecture has scarcely been investigated. The aim of the present work was to investigate changes in heart tissue architecture after chemical sympathetic denervation by 6OH-Dopamine (6OH-DA) in mice.

Methods — Two months old mice (n=18) received 3 injections of 6OH-DA (200 mg/kg, ip) or saline (n = 6) at 3 days of interval. At 15 and 30 days after first injection, ECG was recorded (PowerLab, DSI) under anaesthesia and heart rate spectral variability (HRV) was performed (FFT) in low frequency (LF: 0.15-1.5 Hz) and high frequency (HF: 1.5-5 Hz) ranges; LH/HF ratio was also calculated. After sacrifice, blood was withdrawn for plasma catecholamine determination (HPLC). Heart tissue was fixed (formaldehyde 10%) for histology or frozen for western blot analysis (tyrosine hydroxylase, TH).

Results — When compared to controls (1410±145 pg/ml) plasma norepinephrine levels were significantly lower at D15 (766±186 pg/ml) and D30 (675±288 pg/ml) after 6OH-DA without significant change in epinephrine levels. TH expression was absent at D15 and present but significantly lower than in controls at D30. When compared to controls (48.5±6.2%), LF HRV was significantly reduced at D15 (31.6±5.4%) but not at D30 (58.2±16.2%) without any change in HF. LF/HF ratio was lower in 6OH-DA treated mice at D15 (0.49±0.13 vs 1.29±0.17 in controls) but was normal at D30 (1.63±0.31). At D15, hearts from 6-OH-DA treated mice exhibited mild structural abnormalities with wavy cardiomyocyte appearance in septum. At D30, histological abnormalities concerned whole myocardium with myocytes intersecting at various angles with bundles wavy appearance. Variability in cell size with anisocaryosis, attenuated myocytes with perinuclear halo and shaped nuclei were observed. No inflammation, interstitial fibrosis or necrosis were noticed.

Conclusion — This study suggests that heart denervation induces myocardial tissue disorganization. Relationship between these pathological changes and sympathetic nerve destruction and/or catecholamine depletion remains to be elucidated. Apart from physiological significance, these results also bring new structural basis to explain increased risk of cardiac disease during human autonomic failure.