old obese rats). Endothelial NO-synthase (eNOS) blockade (L-NAME) was reduced in old obese rats although eNOS expression level was unaffected. However, indomethacin improved AMR in old obese rats only, suggesting that vasoconstrictor prostanoids were involved. Similarly, COX2 inhibition (NS398) and TxA2/PGH2 receptor blockade (SQ29548) increased AMR in arteries from old obese rats only. Old obese rats presented the highest levels of blood TxB2 (TxA2 metabolite) associated with an increased COX2 immunostaining and expression level. Chronic inhibition of COX2 with NS398 (3 weeks) restored AMR in old obese rats (78% versus 57% in control obese rats) to the level observed in solventtreated old lean rats (84%). Taken together, these data show that obesity in old rats is associated with a reduced endothelium-mediated relaxation due to an excessive production of COX-2 derivatives, most probably TxA2.

C005

SEROTONINERGIC 5-HT2B RECEPTOR BLOCKADE PREVENTS SUPEROXIDE ANION MEDIATED CARDIAC HYPERTROPHY INDUCED BY ANGIOTENSIN II

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Objective — To study the role of the serotoninergic 5-HT2B receptor in the development of cardiac hypertrophy and its link with left ventricular superoxide anion generation in a mouse model of angiotensin II-induced hypertension.

Methods — Wild-type and 5-HT2B receptor knock-out (KO) mice were perfused with angiotensin II (0.2 mg.kg-1.d-1) for 14 days with or without SB215505 (1mg.kg-1.d-1), an antagonist of the 5-HT2B receptor. Heart rate and blood pressure were measured by tail-cuff plethysmography. Cardiac hypertrophy was evaluated by echocardiography and direct measurement of heart weight. Superoxide anion production and maximal NAD(P)H oxidase activity were measured by a chemiluminescence method using lucigenin. Superoxide anion production was also measured in primary left ventricular fibroblasts cell cultures.

Results — Angiotensin II increased superoxide anion production (+32%), the maximal activity of NAD(P)H oxidase (+84%) in left ventricle of wild-type mice concomitantly with the arterial blood pressure (+37mmHg) and the heart/body weight ratio (+17%). A pharmacological blockade (SB215505) or a genetic suppression of the 5-HT2B receptor prevented the increased superoxide anion production and cardiac hypertrophy but had no effect on cardiac hemodynamics or blood pressure. Angiotensin II also increased NAD(P)H oxidase activity in cultured cardiac fibroblasts and this increase was prevented by SB215505.

Conclusion — The 5-HT2B receptor is a new potential target for the prevention of cardiac hypertrophy and its associated superoxide anion production. Cells of the extracellular matrix could possibly be involved in this mechanism.

C006

NADPH-OXIDASES AND UNCOUPLED ENDOTHELIAL NO-SYNTHASE IN PULMONARY ARTERIAL HYPERTENSION INDUCED BY CHRONIC HYPOXIA

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Nitric oxide (NO) production by endothelial NO-synthase (eNOS) is critically dependent on the cofactor, tetrahydrobiopterin (BH4). Depletion in BH4 consecutive to an increase of reactive oxygen species (ROS) production by NADPH-oxidases and/or eNOS over-expression, favour eNOS uncoupling. This study investigates the potential role of NADPH-oxidases and uncoupled eNOS in pulmonary arterial hypertension induced by chronic hypoxia.

Male C57BL/6 and eNOS knockout (eNOS-/-) mice were exposed or not to hypobaric hypoxia (0.5 atm) for 21 days. Fulton index (right ventricular / left ventricular + septum weight ratio) was determined. Lungs were used for measurement of BH4 (by HPLC), for expression of eNOS (by western-blotting) and of the NADPH-oxidases subunits Nox1, Nox2 and Nox4 (by RT-PCR). Pulmonary arteries were also mounted in a wire myograph for evaluation of vasomotor responses.

Chronic hypoxia induced a marked up-regulation of Nox1, Nox2 and Nox4 mRNAs in lungs, and an increase of ROS levels in pulmonary arteries. BH4 levels, as well as eNOS expression, were enhanced in lungs from hypoxic WT mice (1.25 and 4 fold increase compared to normoxic WT mice, respectively). In pulmonary arteries from hypoxic WT mice, the contractile response to phenylephrine was about 1.8 greater than in those from normoxic WT mice. The use of ROS scavengers (PEG-SOD or catalase) and NOS inhibitor (L-NAME) revealed the involvement of ROS in hypoxia-induced hyper-reactivity to phenylephrine, and a loss of NO-dependent relaxation. Chronic treatment of hypoxic WT mice with the BH4 precursor sepiapterin preserved the vasorelaxant effect of NO. This treatment and the deletion of eNOS gene abolished the inhibitory effect of catalase on phenylephrine-induced contraction, and also attenuated hypoxia-induced right ventricular hypertrophy.

These data show that chronic hypoxia induced an up-regulation of Nox isoforms and eNOS in lungs. They suggest that uncoupled eNOS participates to right ventricular hypertrophy and to alterations of vasomotor responses in pulmonary arteries in hypoxia-induced pulmonary hypertension. The weak increase in BH4 and the large over-expression of eNOS suggest the existence of compensatory mechanisms on BH4 synthesis, which may moderate eNOS dysfunction.

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