HYPERCAPNIA INHIBITS THE HYPOXIA-INDUCED COLLAGEN BREAKDOWN IN THE WALLS OF PERIPHERAL PULMONARY ARTERIES. ***L. Bláhová,*** *[1], J. Novotná [2],J. Herget ,[1]* Charles University, Department of Physiology[1] and Department of Medical Chemistry and Biochemistry[2], 2nd Medical School, Prague, Centre of Experimental Cardiovascular Research, Prague., Czech Republic.

Hypoxia induces the hypoxic pulmonary hypertension (HPH) by remodelling of peripheral pulmonary arteries (PPA). Hypercapnia attenuates the HPH and hypoxia-induced radical injury to pulmonary vessels [1]. We hypothesised that the vascular remodelling is related to expression and/or activation of matrix metalloproteinases which results from radical tissue injury [2]. Collagen composition of peripheral pulmonary arteries (PAP) was studied in male adult rats exposed four days in isobaric hypoxic chamber to hypoxia (F_{iO2} = 0,1, F_{CO2} = 0, N = 7), hypoxia and hypercapnia (F_{iO2} = 0,1, F_{CO2} = 0.045, N = 7) and normoxic controls (N = 7). Hypoxic, hypercapnic and control animals were anestesised and peripheral pulmonary arteries (PPA) were dissected and analysed. Collagenous proteins were extracted from the PPA and analysed by SDS-PAGE electrophoresis [3]. Similarly to previous study [3] we detected in all hypoxic animals 3/4 and 1/4 collagen type I cleavage products. Similar collagen breakdown products were not detected in PPA collagenous extracts of normoxic rats and rats exposed to hypoxia combined with hypercapnia.We conclude that hypercapnia attenuates HPH by inhibition of collage breakdown products in the walls of PPA. The mechanism is probably related to the inhibition of hypoxia induced radical tissue injury by hypercapnia.

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HETEROZYGOUS APOLIPOPROTEIN E-DEFICIENT MICE AS AN EXPERIMENTAL MODEL OF HYPERLIPOPROTEINEMIA. ***D. Bobková,*** *R. Poledne,* Laboratory for Atherosclerosis Research, Centre for Experimental Cardiovascular Research, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Apolipoprotein E (apo E) plays very important role in lipoprotein metabolism. As a surface part of VLDL, IDL and HDL apo E mediates binding to receptors. Loss of apo E synthesis has been shown, in an experimental model homozygous apo E-deficient mice, to increase basal cholesterol concentraction and risk of atheroclerosis. We tested the effects of heterozygous apo E gene loss in mice on lipoprotein concentractions. Heterozygous (+/-) and homozygous (-/-) apo E-deficient mice (C57Bl/6 strain), two-month-old males and females, were used. The animals were fed standard laboratory diet (chow diet) or a 2% cholesterol diet (cholesterol diet) for 21 days. Cholesterol and triglyceride concentrations were measured using enzymatic kits, the lipoprotein fractions were isolated by sequential ultracentrifugation of pooled serum. Basal cholesterolemia of heterozygous mice is lower compared to homozygous mice (2 mmol/L vs. 10 mmol/L). After feeding cholesterol diet, cholesterolemia rose to 4 mmol/L in heterozygous mice and to 39 mmol/L in homozygous mice. While the ratio of cholesterol/triglyceride concentractions in VLDL, IDL and LDL fractions was not increased in heterozygous mice, it was increased in homozygous mice on chow diet. The increase in cholesterolemia on cholesterol diet was due to the increases in all lipoprotein fractions. More pronounced changes were seen in the VLDL fractions in either group. In summary, subnormal expression of the apo E protects from changes in lipoprotein concentrations in heterozygous apo E-deficient mice only on chow diet. After feeding cholesterol diet, one functional allele cannot prevent an increase in cholesterol concentration and susceptibility to atherosclerosis.

SEX HORMONES DETERMINE THE PERMANENT EFFECTS OF PERINATAL HYPOXIA ON THE PULMONARY CIRCULATION. ***J. Bíbová,*** *I. Ošťádalová, J. Herget, V. Hampl,* Department of Physiology, Charles University 2nd Medical School, Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

Unlike hypoxia in adulthood, hypoxia in the perinatal period has permanent effects on the pulmonary circulation (*Am Rev Respir Dis* 142: 619, 1990). Because sensitivity to chronic hypoxia in adults differs between sexes, we tested the hypothesis that the effects of perinatal hypoxia are also gender-dependent. We compared perinatally hypoxic (12 % O_2, 1 week before and 1 week after birth) and normoxic male and female rats. Some of the animals were gonadectomized at the end of the first postnatal week. All measurements were performed when the animals were adult (age > 12 weeks). Systemic and pulmonary arterial blood pressures (SAP and PAP) and cardiac output (CO) were measured in thiopental anesthesia (40mg/kg BW, i.p.). As pulmonary hypertension is associated with increased muscularization of peripheral pulmonary vessels and right ventricular hypertrophy, we determined weight ratio of right ventricle to left ventricle plus septum (RV/LV+S) and the percentage of muscularized small vessels (%DL) in histological lung sections. In males, neither perinatal hypoxia nor neonatal castration had any significant effect on SAP, PAP, or RV/LV+S. Perinatal hypoxia increased %DL regardless of the gonadal status. In females, PAP and %DL were significantly elevated in perinatally hypoxic rats gonadectomized as pups. RV/LV+S was elevated by perinatal hypoxia in both ovariectomized and sham operated females. SAP and CO did not differ among the female groups. Perinatally hypoxic rats of both genders gonadectomized when adult (~6 weeks prior to the measurements) did not differ significantly from the corresponding perinatally hypoxic intact groups in any of the measured parameters. We conclude that the long-lasting effects of perinatal hypoxia on the pulmonary circulation are more pronounced in females than in males. The presence of female gonads during maturation appears essential for this gender difference.

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IMMUNOLOGICAL MECHANISMS IN ATHEROGENESIS: A RETROSPECTIVE ANALYSIS AND AN EXPERIMENTAL STUDY. ***A. Dvořáková,*** *P. Suchánek, P. Stávek, R. Poledne,* Centre for experimental cardiovascular research, IKEM, Prague, Czech Republic.

Similarly to the older hypothesis from the end of the 19th century, recent opinions appeared claiming infection as one of the important factors in atherogenesis. This hypothesis could help to elucidate the fact, that as much as 40 % of the patients with first myocardial infarction have none of the classical risk factors. We have analysed unusually declining pattern of coronary heart disease mortality in the Czech population in the 1990′s and prevalence of numerous infectious diseases. No relationship appeared. It is in agreement with our opinion that individual immune reactivity is responsible for increased risk of myocardial infarction in individuals with increased concentration of C-reactive protein. Production of CRP in the liver is regulated by inflammatory cytokine IL-6. Macrophages within arterial wall, but also adipocytes, are able to produce this regulating cytokine. The relative importance of IL-6 production in visceral adipocytes was tested in clinical experiment, where 30 obese young women were intervened by a combination of increased physical activity and lower energy intake over 9 weeks. Significant decrease of visceral fat volume (from 1119 to 937 cm3, p<0,001) was combined with highly significant decrease in CRP (from 4,21 to 2,89 g/l, p=0,00034). It is hypothesized that pathophysiologic mechanism of the decrease in CRP concentration could be a decrease in production of IL-6 by diminished volume of visceral fat.

EFFECTS OF BOSENTAN ON THE MALIGNANT PHASE OF HYPERTENSION IN HYPERTENSIVE REN-2 TRANSGENIC RATS. [1]***P. Dvořák***, [1,2]*L. Kopkan,* [1,2] *L. Červenka,,* [1]Center for Experimental Cardiovascular Research, [2]Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

The present study was performed to evaluate whether in transgenic rats harboring the mouse ren-2 renin gene (TGR), a well defined angiotensin II (ANG II)-dependent model of hypertension, nonselective blockade of endothelin (ET) type A and type B (ET_A/ET_B) receptors with bosentan (B) attenuates the course of hypertension and prevents the onset of the malignant phase of hypertension in this model. 23 day old male homozygous TGR and age-matched transgene negative control Hannover Sprague Dawley rats (HanSD) were randomly assigned to control and experimental groups (received a diet containing B). Systolic blood pressure (SBP), proteinuria and endogenous creatinine clearance (C_{Cr}) were measured until the end of experiment (180 days of age). The ratio of heart weight (mg)/body weight (g) was used as an index of cardiac hypertrophy (HW/BW). Untreated and B-treated HanSD exhibited 100% survival rate and B had no effect on SBP, proteinuria, C_{Cr} and HW/BW ratio in HanSD rats. The mortality rate in untreated TGR rats was 59%. Treatment with B markedly reduced the mortality rate in TGR rate in TGR rats to 12%. B administration in TGR reduced proteinuria and attenuated the development of cardiac hypertrophy compared with untreated TGR. However, treatment with B did not influence neither C_{Cr} nor the final levels of SBP compared with those of control TGR rats. Our data show that nonselective blockade of the ET_A/ET_B receptors markedly improve the survival rate and ameliorate end-organ damage in homozygous TGR rats without significant lowering blood pressure.

A PROPOSAL FOR A METHOD CAPABLE OF REVEALING CHANGES IN COLLAGEN LOCATED AT THE INNER SURFACE OF BLOOD VESSELS. ***A. Eckhardt*** [1,2]*, I. Mikšík* [1]*, Z. Deyl* [1] ,[1]Institute of Physiology, AS CR, Centre for Experimental Cardiovascular Research, Prague, [2]Department. of Physiology., 2nd Medical School, Charles University, Prague, Czech Republic.

Pulmonary hypertension is induced by remodelling of the pulmonary vasculature combined with enhanced connective tissue turnover. Nonenzymatic posttranslational modifications by ROS and NO could be involved as well. The basic concept of this work was to split the collagenous core in situ into a set of low molecular peptides which could be compared/evaluated by an appropriate separation method (typically capillary electrophoresis) and reveal posttranslational changes that could have occured in the collagenous stroma of the pulmonary vasculature. Therefore the rat lungs were perfused by buffer containing bacterial collagenase.
The small peptides (mostly tripeptides) that arise after this treatment are relatively easy to separate. Using bacterial collagenase offers the unique possibility of splitting collagen molecules only, as the specificity of this enzyme is unusually high and does not involve cleavage of other proteins.
In the present study we document that the approach outlined above yields reproducible results which makes it possible to materialize pulmonary collagen peptide maps and compare them under different physiological/pathological conditions. *This work was supported by Grant Agency of the Czech Republic (Grants Nos. 203/00/D032, LN00A069).*

THE ROLE OF ENDOTHELIN-1 IN THE DEVELOPMENT OF SALT-SENSITIVE HYPERTENSION AND END-ORGAN DAMAGE IN HETEROZYGOUS HYPERTENSIVE REN-2 TRANSGENIC RATS. [1]***P. Dvořák***, [1,2]*L. Kopkan,* [1,2]*L. Červenka,* [1]Center for Experimental Cardiovascular Research [2]Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

The role of interplay between endothelin (ET) and angiotensin II (ANG II) in the development of salt-dependent hypertension and associated end-organ damage remains unclear. The first aim of the present study was to asses whether high salt intake (HS) would accelerate the development of hypertension in heterozygous transgenic rats harboring the mouse Ren-2 renin gene (TGR) compared to TGR fed by normal-salt diet (NS)). The second aim of the present study was to elucidate whether blockade of ET receptors with bosentan (B) can prevent the onset of salt-sensitive hypertension in this model. 23 days old male TGR and age-matched transgene negative Hannover Sprague rats (HanSD) were allocated to groups given either NS and HS diet alone or rats fed by NS and HS with B. Systolic blood pressure (SBP), proteinuria were measured until the end of experiment (180 days of age). The ratio of heart weight (mg)/body weight (g) was used as an index of cardiac hypertrophy (HW/BW). All rats on NS diet exhibited 100% survival rate and B had no effect on any followed parameters. The mortality rate in HS treated TGR was 56%. Treatment with B reduced the mortality rate to 24%. HS diet caused acceleration of development of hypertension in TGR. B administration in HS fed TGR markedly reduced proteinuria and attenuated the development of cardiac hypertrophy compared with untreated TGR. However, the treatment with B did not influence the course of hypertension in HS treated TGR. Our data show that TGR develop salt-sensitive hypertension and that nonselective ET blockade markedly improve the survival rate and ameliorate end-organ damage in TGR fed by HS without significant lowering blood pressure.

HYPEROXIA ATTENUATED NITROTYROSINE CONCENTRATION IN THE LUNG TISSUE OF CARRAGEENAN TREATED RATS. ***B. Fišárková****, R. Vytášek, M. Vízek, D. Miková,* Institute of Pathophysiology, Institute of Physiology and Institute of Biochemistry, 2nd Medical Faculty, Charles University, and Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

To test whether hyperoxia affects nitration of lung tissue proteins during lung inflammation, we measured 3-nitrotyrosine (3NTYR) concentrations in lung tissue and serum of rats exposed to hyperoxia during carrageenan-induced pneumonia. 29 Wistar male rats were assigned to one of 4 groups. In groups 1 and 2 0,5 ml of 0,7 % carrageenan was applied intratracheally (carrageenan groups). Groups 1 and 3 were then placed for 7 days into hyperoxia (F_IO_2 0,8) (hyperoxic groups). Rats of groups 2 and 4 breathed for the same time air (normoxic groups). At the end of exposure were all rats anesthetized, intubated, their ventilation measured and 1 ml of venous blood and lower lobe from the left lung taken to examine serum and tissue (3NTYR) concentrations (ELISA). Carrageenan instillation increased 3NTYR concentration in lung tissue (carrageenan-normoxic group 147±7 nmol/g protein, control-normoxic 90±10), but it did not affect it's concentration in serum (carrageenan-normoxic 82±9 nmol/g protein, control-normoxic 91±7). Hyperoxia blocked increase of lung tissue 3NTYR in carrageenan treated rats (carrageenan-hyperoxic 82±13 nmol/g protein) but did not change 3NTYR concentration in control rats (control-hyperoxic 100±14). Serous concentration of 3NTYR was decreased in both hyperoxic groups (carrageenan-hyperoxic 51±5 nmol/g protein, control-hyperoxic 67±7). The results suggest that hyperoxia affects either process of tyrosine residues nitration or process of 3NTYR degradation. *This work was supported by Grant GAČR 305/01/0794 and Research project MSM 111300002.*

PULMONARY VASOCONSTRICTION PREVENTS ARTERIAL HYPOXEMIA IN ISOLATED RAT LUNGS VENTILATED WITH HYPOXIC GAS. ***D. Hodyc**, V. Lachmanová, J. Herget,* Department of Physiology, Charles University, 2nd Medical School, Prague, Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

Regional hypoxic pulmonary vasoconstriction (HPV) is an important local lung mechanism which reduces blood flow through poorly ventilated regions of the lungs and therefore prevents arterial hypoxemia. The role of HPV in conditions of global lung hypoxia, however, is not obvious. We tested the hypothesis that vasoconstriction in global ventilatory hypoxia prevents the arterial hypoxemia and that it is caused by differences in distribution of vascular closing pressures in various parts of the lung. We used isolated lungs of adult male rats (n=12) perfused with salt solution with albumin (4g/100ml), Meclophenamate ($17x10^{-6}$) and L-NAME ($5x10^{-5}$) in constant constant flow (4ml/min/100g b.w.) conditions. The lungs were ventilated with normoxic (21% O_2 + 5% CO_2) or hypoxic (10, 5, 3, 0% O_2 + 5% CO_2) gas mixture. We continually monitored perfusion pressure and P_{O2} in the lung outflow. We observed that there is an indirect relation between perfusion pressure (increased by HPV) and decrease of P_{O2}. Inhibition of HPV by nitroprusside lowers P_{O2} in lung outflow. The decrease of P_{O2} was 32,8 ± 0,7 (SE) before and 58 ± 7,9 (SE) after administration of nitroprusside. After adding 5mM KCl into perfusate, K^+ inducted vasoconstriction, outflow P_{O2} increased. We conclude that in conditions of global hypoxia vasoconstriction prevents arterial hypoxemia. *The study was supported by grant GACR 305/97/S070.*

EFFECT OF ACUTE ISCHAEMIA ON LIPID FATTY ACID PROFILE IN SERUM AND HEART OF RATS ADAPTED TO CHRONIC HYPOXIA. ***J. Ježková**[1,4], O. Nováková[2], F. Kolář[4], E. Tvrzická[3], J. Neckář[4] , F. Novák[1],* [1]Department of Biochemistry and [2]Animal Physiology, Faculty of Science, [3]1th Faculty of Medicine, Charles University, [4]Institute of Physiology ASCR and Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

Long-lasting adaptation to intermittent high altitude (IHA) hypoxia increases natural resistance of heart against ischaemic damage. We examined whether chronic hypoxia affects the profile of fatty acids in lipids of ischaemic and non-ischaemic rat ventricular myocardium. Adult male Wistar rats were exposed to IHA hypoxia of 7000 m in a barochamber for 8 h/day, 5 days/week; total number of exposures was 24-32. Control (normoxic) animals were kept at the altitude of 200 m. Day after the last exposure, the regional ischemia was induced in anesthetized open-chest animals by occlusion of the LAD coronary artery for 9 and 30 min. Phospholipids (PL) and triacylglycerols (TG) were separated by thin layer chromatography and their fatty acid composition was analyzed by gass chromatography. Our results demonstrate that chronic hypoxia increases the ratio of n-3/n-6 polyunsaturated fatty acids (PUFA) in PL and TG in the heart. This is namely due to increase of 22:6n-3 PUFA. The increase in 22:6n-3 PUFA was even potentiated by acute 9 min. ischaemia only in hearts of chronically hypoxic rats. 30 min. ischaemia increased proportion of 20:4n-6 in heart TG in both normoxic as well as hypoxic heart. IHA hypoxia does not influence n-3 PUFA and increased content of 20:4n-6 in serum TG. We conclude that the increased n-3/n-6 PUFA ratio could play a role in protective mechanism of myocardium adapted to IHA hypoxia. *Supported by grants: MSM 1131 00001, MSM 113 00003, GA ČR 305/01/0279.*

LACK OF PERSISTENT IMPROVEMENT OF CARDIOVASCULAR RISK FACTORS IN REN-2 TRANSGENIC HYPERTENSIVE RATS FOLLOWING EARLY SHORT-TERM AT_1 RECEPTOR BLOCKADE. ***L. Kopkan**[1,2], P. Dvořák[1], J. Zicha[1,3], L. Červenka[1,2],* [1]Center for Experimental Cardiovascular Research, [2]Institute for Clinical and Experimental Medicine, [3]Institute of Physiology, Academy of Sciences of the Czech Republic.

The first aim of the present study was to determine the critical period (developmental window) for the development of hypertension in transgenic rats harboring the mouse Ren-2 renin gene (TGR). The second aim was to evaluate whether the treatment with angiotensin II (ANG II) type 1 (AT_1) receptor antagonist (candesartan, 5 $mg.kg^{-1}.day^{-1}$) during the developmental window prevents hypertension development, attenuates cardiac hypertrophy and normalizes proteinuria to same levels as observed in normotensive age-matched transgene-negative Hannover Sprague-Dawley rats (HanSD). We found that the systolic blood pressure (SBP) in TGR suddenly increased between 28 to 31 days of age from normotensive to hypertensive levels (from 133 ± 4 to 164 ± 4 mmHg). Candesartan treatment between 24 to 38 days of age fully normalized SBP in TGR to levels observed in HanSD. However, after candesartan withdrawal the SBP in TGR returned during 10 days to levels as observed in untreated TGR. In addition, short-term candesartan treatment in TGR did not attenuate the development of cardiac hypertrophy and did not lower proteinuria compared with untreated TGR and in both groups were all parameters markedly higher compared with normotensive HanSD (all parameters were measured at 90 days of age). These findings indicate that the developmental window of hypertension in TGR is in contrast to another genetic model of hypertension very narrow (days compared to weeks) and that the short-term transient blockade of AT_1 receptors does not exhibit any persistent beneficial effects on cardiovascular risk factors in this model of hypertension.

THE ROLE OF *CD36* GENE IN METABOLIC SYNDROME. ***M. Kadlecová**, J. Čejka, J. Zicha, J. Kuneš,* Institute of Physiology AS CR and Center for Experimental Research of Cardiovascular Diseases, Prague, Czech Republic.

Objective: The link among essential hypertension, obesity, hyperlipidemia, insulin resistance syndrome and diabetes was demonstrated in human and experimental animals. Genetic basis of these disorders is not clear. The mutation in *CD36* gene was proposed as the underlying cause of insulin resistance in SHR. Nevertheless, this is only true for NIH-derived SHR because *CD36* mutation is absent in the original SHR strain, maintained since their development in Japan. Here we examined the role of *CD36* gene polymorphism in Prague hereditary hypertriglyceridemic hypertensive and spontaneously hypertensive rats with defective fatty acid and glucose metabolism. Material and Methods: Age-matched 3-month-old hereditary hypertriglyceridemic (HTG), spontaneously hypertensive (SHR), Wistar-Kyoto (WKY) and Lewis (LEW) male rats were used. Blood pressure was measured under light ether anesthesia by direct puncture of the left carotid artery. Total serum triglycerides, cholesterol, oral glucose tolerance test and non-esterified fatty acids were determined in basal conditions or after receiving 10 % fructose (instead of water) as a drinking fluid for three weeks. Genomic DNA was analyzed by PCR with two allele-specific primers: CD36-2F 5´-CAG AGA ATG ACA ACT TCA CAG-3´ and CD36-1R 5´-GGA ACA TAG AAG ACT TGG AC-3´. Results: Blood pressure and plasma levels of triglycerides in both SHR and HTG were significantly increased compared to WKY and LEW controls. Basal level of plasma glucose was the same in all four strains studied. Oral glucose tolerance test curves were significantly higher in SHR and HTG 30, 60 and 120 min after glucose load, suggesting the existence of insulin resistance in both hypertensive strains. Drinking of 10 % fructose solution for three weeks increased basal level of fasted glucose and shifted oral glucose tolerance test curves to a higher level. Non-esterified fatty acids were significantly higher in SHR in comparison with WKY only. Genotype analysis of PCR products has shown two copies of *CD 36* in HTG and in both control strains (LEW and WKY), whereas only one copy was found in SHR. Conclusion: We have revealed that the *CD36* mutation seems to be dominant only for NIH-derived SHR substrain and not for other strains with hypertension and insulin resistance. Therefore other genes might play a role in insulin resistance and other symptoms of metabolic syndrome. *Partially supported by grant 305/00/1638 (Grant Agency of Czech Republic).*

LACK OF PERSISTENT IMPROVEMENT OF CARDIOVASCULAR RISK FACTORS IN REN-2 TRANSGENIC HYPERTENSIVE RATS FOLLOWING EARLY SHORT-TERM AT_1 RECEPTOR BLOCKADE. ***L. Kopkan***[1,2]*, P. Dvořák*[1]*, J. Zicha*[1,3]*, L. Červenka*[1,2]*,* [1]Center for Experimental Cardiovascular Research, [2]Institute for Clinical and Experimental Medicine, [3]Institute of Physiology, Academy of Sciences of the Czech Republic.

The first aim of the present study was to determine the critical period (developmental window) for the development of hypertension in transgenic rats harboring the mouse Ren-2 renin gene (TGR). The second aim was to evaluate whether the treatment with angiotensin II (ANG II) type 1 (AT_1) receptor antagonist (candesartan, 5 $mg.kg^{-1}.day^{-1}$) during the developmental window prevents hypertension development, attenuates cardiac hypertrophy and normalizes proteinuria to same levels as observed in normotensive age-matched transgene-negative Hannover Sprague-Dawley rats (HanSD). We found that the systolic blood pressure (SBP) in TGR suddenly increased between 28 to 31 days of age from normotensive to hypertensive levels (from 133 ± 4 to 164 ± 4 mmHg). Candesartan treatment between 24 to 38 days of age fully normalized SBP in TGR to levels observed in HanSD. However, after candesartan withdrawal the SBP in TGR returned during 10 days to levels as observed in untreated TGR. In addition, short-term candesartan treatment in TGR did not attenuate the development of cardiac hypertrophy and did not lower proteinuria compared with untreated TGR and in both groups were all parameters markedly higher compared with normotensive HanSD (all parameters were measured at 90 days of age). These findings indicate that the developmental window of hypertension in TGR is in contrast to another genetic model of hypertension very narrow (days compared to weeks) and that the short-term transient blockade of AT_1 receptors does not exhibit any persistent beneficial effects on cardiovascular risk factors in this model of hypertension.

RENAL FUNCTIONAL RESPONSES TO INTRARENAL AT_1 RECEPTOR BLOCKADE IN HYPERTENSIVE REN-2 TRANSGENIC HYPERTENSIVE RATS. ***L. Kopkan***[1,2]*, L. Červenka*[1,2]*,* [1]Center for Experimental Cardiovascular Research. [2]Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Previous studies have shown that hypertensive transgenic rats harboring the mouse Ren-2 renin gene (TGR) exhibit enhanced renal vascular responsiveness to angiotensin II (ANG II) compared with normotensive age-matched transgene-negative Hannover Sprague-Dawley rats (HanSD). The present study was performed to assess and compare renal functional responses to intrarenal ANG II blockade in TGR and HanSD rats. To avoid the confounding effects of decreases in mean arterial pressure (MAP), we administrated AT_1 receptor antagonist (candesartan, 750 ng) directly into renal artery. This dose of candesartan did not significantly decrease MAP either in TGR or HanSD. Intrarenal administration of candesartan elicited significant increases in glomerular filtration rate (GFR), renal plasma flow (RPF) and sodium excretion in both TGR and HanSD. However, the magnitude of increases of in GFR, RPF and sodium excretion was markedly greater in TGR than in HanSD. The present findings indicate that augmented renal vasoconstrictor sensitivity to endogenous ANG II might contribute to a compromised ability of the kidney to respond to blood pressure elevations by appropriate increases in sodium excretion and thus contribute to the maintenance of hypertension in this model.

N-ACETYLCYSTEINE (NAC) BUT NOT L-NAME INHIBITS PULMONARY VASCULAR EFFECTS OF 5 DAYS HYPOXIA IN RATS. ***V. Lachmannová****, J. Herget,* Department of Physiology, Charles University, 2nd Medical School, Prague, Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

We hypothesize that structural remodelling of pulmonary vasculature in hypoxic pulmonary hypertension results from vascularinjury in early phases of exposure to hypoxia mediated by oxygen- and/or nitric oxide (NO)-related radical species (1, 2). Hemodynamic correlates of the hypoxic injury to peripheral pulmonary blood vessels include blunted hypoxic pulmonary vasoconstriction and increased resistance of pulmonary vasculature to blood flow. To dissect the role of oxygen and NO related radicals in the pathogenesis of chronic hypoxic pulmonary hypertension, we studied adult male rats exposed for 5 days to isobaric hypoxia (F_{iO2} = 01) and treated with an antioxidant (NAC 20 g/l of drinking water) or NO synthase inhibitor (L-NAME, 500 mg/l of drinking water). NAC or L-NAME were given preventively (for last 5 days before the onset of hypoxia), therapeutically (during the hypoxic exposure) or in combination (before and during exposure). Corresponding control groups were kept in normoxia. In hypoxic rats, NAC treatment inhibited a hypoxia-induced increase of serum nitrotyrosine (marker of NO and superoxide interaction; 131 ± 12 nM in treated and 250 ± 36 nM in not-treated hypoxic rats, $P<0.001$). L-NAME treatment inhibited NO production (0.257 ± 0.041 ppb/min in exhaled breath of not-treated and 0.119 ± 0.011 ppb/min in treated normoxic rats, $P<0.05$). Lung hemodynamics was studied in isolated saline (+ 4% albumin) perfused ventilated lungs. Reactivity to acute hypoxic challenges (10, 5, 3 and 0 % O_2) was depressed by chronic hypoxia and this hyporeactivity was prevented and corrected by NAC, but not by L-NAME, treatment. Perfusion pressure-flow relationship (measure of resistive properties) was shifted towards higher pressures by chronic hypoxia, and this shift was significantly smaller in groups treated with NAC, but not L-NAME. We conclude that increase of oxygen radicals is involved in the pathogenesis of hypoxic pulmonary hyperension. Inhibition of hypoxia-induced increase of NO production does not alter the hemodynamic changes in early phases of exposure to hypoxia.

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NIFEDIPINE ABOLISHES GENDER-DEPENDENT DIFFERENCE IN Ca^{2+}; RESPONSE TO ANGIOTENSIN II IN VSMC ISOLATED FROM SHR. ***J. Loukotová****, J. Tichá, J. Kuneš, J. Zicha,* Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Center for Experimental Research of Cardiovascular Diseases, Prague, Czech Republic. Objective**:** We have previously demonstrated substantially greater response of free cytosolic calcium ($[Ca^{2+}]_i$) to angiotensin II (Ang II) in aortic vascular smooth muscle cells (VSMC) isolated from male spontaneously hypertensive rats (SHR) compared to those from females. To further evaluate this sex-dependent difference we have investigated the role of calcium influx and the effects of calcium channel blocker nifedipine. Design and Methods: Male (n=10) and female (n=10) SHR aged 10 weeks were used in this study. $[Ca^{2+}]_i$ was measured by image analysis of single myocytes (n=289) loaded with Fura-2. Confluent primary cultures were used. $[Ca^{2+}]_i$ response of VSMC to Ang II (10^{-7} M) was measured in the presence and absence of extracelullar Ca^{2+}. The effect of calcium channel blocker nifedipine (10^{-7}M, 10^{-6}M, 10^{-5}M) on resting $[Ca^{2+}]_i$ and on $[Ca^{2+}]_i$ response to angiotensin II has been evaluated in VSMC of both genders. Results**:** Gender-dependent difference in $[Ca^{2+}]_i$ response to angiotensin II was abolished in the absence of extracellular calcium. Nifedipine caused a decrease in resting $[Ca^{2+}]_i$, which was significantly greater in VSMC isolated from male SHR compared with VSMC isolated from females (46.9±3.4 % vs. 24.7±2.6 %). Furthermore, nifedipine attenuated angiotensin II-stimulated $[Ca^{2+}]_i$ response more in VSMC isolated from male SHR than from female SHR. Conclusions**:** These results suggest that in SHR gender-dependent difference in $[Ca^{2+}]_i$ response to angiotensin II is dependent on Ca^{2+} influx, which seems to be greater in males. Our findings also suggest that Ca^{2+} influx via L-type voltage-dependent calcium channels is increased in VSMC isolated from male SHR compared with those isolated from female. *Supported by research grant 305/02/P066 from the Grant Agency of CR.*

HYPOXIA INCREASES SYNTHESIS OF METALLOPROTEINASES IN ISOLATED RAT LUNG MAST CELLS. ***H. Maxová***[1], *J. Novotná*[3], *L. Vajner*[4], *M. Vízek*[1], *R. Vytášek*[3], *J. Herget*[2], [1]Department of Pathological Physiology, [2]Department of Physiology, [3]Department of Medical Chemistry and Biochemistry, [4]Department of Histology and Embryology, Second Faculty of Medicine, Charles University, Centre for experimental Cardiovascular Research, Prague, Czech Republic.

Exposure to chronic hypoxia increases collagenolytic activity in the walls of small pulmonary arteries due to activation of matrix metalloproteinases (MMPs). One possible source of these enzymes are lung mast cells (LMC). Present study was designed to determine whether hypoxia increases MMPs formation in LMC. LMC were isolated from lungs of 8 adult male rats by enzymatic digestion of the lung tissue and purified on the continuous Percoll gradient in two separate experiments. LMC were divided after one-day of recovery into two groups and placed into desiccators. First group was exposed to 24 h of *in vitro* hypoxia (10 % O_2, 5 % $C0_2$), the control group was kept in normoxia (20 % 0_2, 5 % $C0_2$). At the end of given exposure cells of each group were divided into a part for histological examination and a part for zymography. In histological tests, Toluidine Blue was used to detect the mast cells and monoclonal anti MMP-13 antibody to demonstrate presence of MMP-13. Set of 100 cells from each group was examined; in the first experiment MMP13 was found in 4 LMC from the normoxic group and in 25 LMC in hypoxic group, in the second experiment it was 1 and 20 LMC respectively. Zymography revealed increased formation and activity of MMP-2 and MMP-9 in LMC exposed to hypoxia. The results suggest that short exposure to *in vitro* hypoxia increases formation of MMPs in LMC. *This work was supported by grant GAČR 304/02/1348 and Research project MSM 111300002.*

HYPERCAPNIA ATTENUATES CARDIOPROTECTIVE EFFECT OF ADAPTATION TO CHRONIC HYPOXIA. ***Jan Neckář***[1,3], *Ondrej Szárszoi*[1,3], *Jan Herget*[2,3], *František Papoušek*[1,3], *Bohuslav Ošťádal*[1,3], *František Kolář*[1,3]. [1]. Inst. of Physiology Acad. Sci. CR, [2]Dept. of Physiology, 2nd Medical Fac., Charles Univ., and [3]Center for Exp. Cardiovasc. Res., Prague, Czech Republic.

Chronic hypoxia is associated with increased production of reactive oxygen species (ROS), which contribute to the development of tissue damage. It has been shown that the extent of hypoxic injury can be reduced by hypercapnia because CO2 interacts with radical intermediates1,2. On the other hand, chronic hypoxia increases cardiac tolerance to subsequent acute ischemic injury and ROS signaling may be implicated in this process. Therefore, the effect of chronic hypercapnia on cardioprotection afforded by chronic hypoxia was investigated. Adult male Wistar rats were exposed to chronic isobaric hypoxia (10 % O2) for three weeks. In the first experimental group, CO2 in the chamber was fully absorbed; in the second group, its level was increased to 4.1 % and continuously monitored. Normoxic controls were kept in atmospheric air. One day after the last exposure, anesthetized open-chest animals were subjected to 20-min regional ischemia (LAD coronary artery occlusion) and 3-h reperfusion for infarct size determination (TTC staining). Chronic hypoxia reduced body weight and increased weight of the right ventricle, pulmonary artery blood pressure and hematocrit; these effects were significantly diminished by concomitant hypercapnia. The infarct size was reduced from 61.9 ± 2.2 % of the area at risk in the normoxic controls to 44.5 ± 3.3 % in the hypoxic group. Hypercapnia blunted the infarct size-limiting effect of hypoxia (54.8 ± 2.4 %). In conclusion, increased production of ROS during long-term exposure of rats to chronic hypoxia may contribute to the development of increased ischemic tolerance of their hearts. Concomitant hypercapnia attenuates this protective effect, possibly by reduction of oxidative stress.

[1]Herget et al: Physiol Res 50:P7,2001; [2]Veselá et al: Physiol Res 50:P32,2001.

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CARDIOPROTECTION BY CHRONIC HYPOXIA: ROLE OF NITRIC OXIDE. ***O. Szárszoi***, *G. Asemu, B. Ošťádal, F. Kolář.* Institute of Physiology, Academy of Sciences of the Czech Republic and Centre for Experimental Cardiovascular Research, Prague.

Adaptation to intermittent high altitude (IHA) hypoxia increases cardiac tolerance to acute ischemia/reperfusion injury and mitochondrial ATP-sensitive potassium channels (mito K_{ATP}) are involved in this protective mechanism (1). We examined whether nitric oxide (NO) may play a role as a potential mediator of protection by IHA hypoxia, upstream to mito K_{ATP} channels. Adult male Wistar rats were exposed to IHA hypoxia of 5000 m for 4 h/day (arrhythmias study) or 7000 m for 8h/day (contractile dysfunction study) in a barochamber for 5 days a week; the total number of exposures was 24-32. A control group was kept under normoxic conditions (200 m) for the same period of time. Reperfusion arrhythmias were assessed on isolated heart perfused according to Langendorff under constant flow after 15-min occlusion of LAD coronary artery. Recovery of the contractile function was evaluated on isolated heart perfused under constant pressure after 20-min global no–flow ischemia followed by 40-min reperfusion. NO synthase (NOS) inhibitor L-NAME (200 μmol/l) and NO donor GSNO (10 μmol/l, used only in arrhythmias study) were added to the perfusion solution either 5 min (arrhythmias) or 10 min (contractility) before ischemia and were present throughout reperfusion. In the normoxic group, the incidence and severity of reperfusion arrhythmias was significantly higher (score: 3.67 ± 0.25) as compared with chronically hypoxic hearts (score: 1.58 ± 0.38). Postischemic recovery of contractile function (d*P*/dt) reached 62.5 ± 3.7 % of a preischemic value in controls and it was improved to 83.0 ± 3.8 % by adaptation to hypoxia. L-NAME markedly reduced the severity of reperfusion arrhythmias in controls but had no additional protective effect in the hypoxic group. On the contrary, GSNO did not influence arrhythmias in controls but significantly increased arrhythmia score in hypoxic animals. NOS inhibition had no influence on the recovery of the contractile function either in controls or in the hearts of IHA rats. Our results suggest that NO may play distinct roles in pathogenesis of various manifestations of ischemia/reperfusion injury but it is unlikely to mediate increased ischemic tolerance of the chronically hypoxic rat hearts.

(1) Asemu et al.: J. Mol. Cell Cardiol., 31:1821-1831, 1999.

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ONTOGENETIC DEVELOPMENT OF MITOCHONDRIAL FUNCTION IN THE RAT HEART. ***L. Škárka***[1], *K. Bardová*[2], *P. Brauner*[2], *P. Flachs*[2], *D. Jarkovská*[3], *J. Kopecký*[2] , *B . Ošťádal*[1];[1]Centre for Experimental Cardiovascular Research , [2]Centre for Integrated Genomics, Institute of Physiology, Academy of Sciences of the Czech Republic, [3]Institute of Histology and Embryology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic.

Postnatal maturation of the heart depends on the switch from glycolytic to oxidative metabolism and it is associated with decreasing tolerance to oxygen deprivation.The role of mitochondria in these changes is, however, not yet clear. The aim of our study was to analyse developmental changes of mitochondrial energy state. Left-ventricular myocardium of prenatal, and 1, 2, 5, 10, 28, 50, 60, and 90-day-old male Wistar rats was studied. The expression of uncoupling proteins (UCPs), adenine nucleotide translocase (ANT), and PPARα genes was characterized by Northern blotting (UCP2), real-time quantitative RT-PCR (UCP2, UCP3, ANT1, ANT2, and PPARα), and by immunoblotting (UCP3). In isolated mitochondria, cytochromes $a + a_3$ were quantified by a spectrophotometry, and membrane potential (MMP) was measured using Rhodamine 123 (by spectrofluorimetry and flow cytometry). The specific content of cytochromes in mitochondria increased 2-fold between birth and day 30, similarly as the expression of ANT1 and PPAR genes. Postnatal development of UCP2, UCP3, ANT1 and PPAR genes resulted in the expression maxima between days 20 – 30. The content/expression declined following day 20 (UCP2, UCP3, and PPAR) or 30 (cytochromes and ANT1), while expression of ANT2 deccreased continuosly during the first month of life. Significant ontogenetic differences appeared in MMP: in one-day-old animals a single population of mitochondria with a relative high MMP was observed. With increasing age, a second population of mitochondria with a significantly lower MMP appeared; the average value of MMP thus decreased during development. The results support the view that efficiency of mitochondrial energy conversion in the heart changes during ontogeny and suggest involvement of UCP3 and/or ANT1 in the control mechanism.

POLYMORPHISMS IN ABCG5 AND ABCG8 TRANSPORTERS AND PLASMA LIPIDS. ***J. Štefková**, J. Piťha, R.. Poledne, Z. Škodová, J.A. Hubáček,* Institute for Clinical and Experimental Medicine, Atherosclerosis Research Department, Prague and Center for Experimental Cardiovascular Research, Prague, Czech Republic.

Introduction: Cardiovascular disease is the most common cause of death in all industrialized countries, and high plasma lipid levels are one of the most important risk factors of cardiovascular disease. Two ABC transporters, ABCG5 and ABCG8, with a remarkable homology in gene structure, cDNA and protein sequences have been described which mutations caused a rare form of lipid disease β-sitosterolemia. We have analyzed the missense polymorphisms in ABCG5 (Gln604→Glu) and ABCG8 (Asp19→His, Tyr54→Cys, Thr400→Lys and Ala632→Val) and associated them with plasma lipid levels.
Participants and methods: Using PCR and restriction analysis, we have measured all polymorphisms in two groups of children selected from the opposite ends of the cholesterol distribution curve of 2,000 children. Eighty-two children in high- (HCG) and eighty-six children in low- (LCG) cholesterolemic groups participated in the study. A second group (131 unrelated men and 154 women) represented an 8-year cohort of a 1% representative Czech population sample aged 25-64 years and selected in 1988 as part of the MONICA study.
Results: The Asp19His polymorphism influenced the decrease in total cholesterol ($p<0.001$) in HCG children. The Gln604Glu polymorphism induced the decrease in LDL cholesterol ($p<0.05$) in the same group. In the MONICA part of the study, Tyr54Cys polymorphism influenced the decrease in plasma ($p<0.04$) and LDL cholesterol ($p<0.03$) levels between 1988 and 1996 in females, but not in males. Male Thr400 homozygotes showed a higher decrease in total ($p<0.02$) and LDL cholesterol ($p<0.04$) than in Lys400 carriers. No such association was observed in females.
Conclusions: The associations between the analyzed polymorphisms and lipids are not consistent. Age and sex can contribute, together with ABCG5 and ABCG8 polymorphisms, to genetic/environmental determination of plasma lipids.

BODY WEIGHT REDUCTION IN YOUNG OBESE WOMEN BY A COMBINATION OF INCREASED PHYSICAL ACTIVITY AND DECREASED ENERGY INTAKE. ***P. Suchánek**, J.Tintěra, P.Stávek, J. Kovář, A. Dvořáková, R. Poledne*, Experimental Cardiovascular Disease Research Center, IKEM, Prague, Czech Republic

Group of patients: The group included 40 women aged 25-35 years, BMI ≥ 28 (range 28- 44.5) with abdominal obesity. The probands had their lipid parameters and blood pressure determined and, using magnetic resonance imaging, also their visceral fat.
Method: The probands volunteering to participate in the study underwent 9-week intervention comprising controlled physical activity (6 units/week) and a continuous individualized dietary regimen (3-day food consumption recalls). The physical activity unit was defined as at least 55 minutes at 65% of peak heart rate ((220 – age)*0,65). On two weekdays, physical activity took the form of a bout of body-building training in a fitness center. Alternative physical activity with the same exercise characteristics was required for the remaining weekdays (walking, jogging, stationary cycling, cycling). The diet with a balanced proportion of nutrients (fats <30%, carbohydrates <55%) was calculated to have an energy content of up to 8000 kJ/day. The baseline and final values are shown in the table below:

	Weight (kg)	BMI (kg/m^2)	LDL (mmol/l)	HDL (mmol/l)	NEFA (μmol/l)	Insulin (μIU/ml)	Visc. Fat (cm^3)
Before	32.0±4.6	32.0±4.6	3.1±0.7	1.5±0.3	0.9±0.2	10.2±2.7	1560±734
After	29.4±4.4	29.4±4.4	3.0±0.8	1.6±0.4	0.6±0.1	9.0±2.2	1240±657
T-test	**<0.0001**	**<0.0001**	0.56	**0.05**	**<0.0001**	**0.02**	**<0.0001**

Conclusion: Significant reductions in BMI, NEFA levels, HDL-cholesterol, insulinemia and visceral fat were obtained while the other lipid parameters did not change significantly. The change in visceral fat volume thus increased insulin sensitivity without a change in atherogenic lipoprotein levels. *Supported by grant No. 6361-3 awarded by IGA MZ ČR.*

CONTRACTILITY OF MESENTERIC ARTERIES IN PRAGUE HEREDITARY HYPERTENSIVE RATS. ***M. Vaňková**[2], J. Kuneš[1,2], J. Zicha[1,2], J. Herget[2],* Department of Physiology, Second Medical Faculty, Charles University, [1]Institute of Physiology AS CR, [2]Center for Experimental Cardiovascular Research, Prague, Czech Republic.

Objective: The human metabolic syndrome includes insulin resistance, diabetes, obesity, hyperlipidemia and essential hypertension. Prague hereditary hypertriglyceridemic (HTG) rats constitute a genetic model of hypertension associated with hyperlipidemia and insulin resistance. The aim of our study was to examine: 1) the contraction characteristics of mesenteric arteries isolated from HTG rats in comparison with those isolated from normotensive Lewis (LEW) controls 2) collagenous proteins extracted from pulmonary and systhemic arteries isolated from HTG and LEW rats . Material and methods: Peripheral branches of mesenteric arteries were isolated under dissecting microscope from HTG rats (HTG, n=9) and from normotensive Lewis controls (LEW, n=8). Vessels were tested on small vessel myograph (M 500A, Linton, Norfolk, GB) filled with buffered physiologic salt solution (PSS) and gassed with 95 % O_2 + 5 % CO_2. The diameter after automatic normalisation was 110,6±5,9□m at LEW and 125,4±13,5 □m at HTG at transmural pressure 130 torr. After stabilisation we challenged the vessels twice with KCl (70 mM) and once with prostaglandin $F_{2□}$PGF$_{2□}$, 0,1 mM). The collagenous peptides from pulmonary and systhemic peripheral vessels were obtained using pepsine digestion. The extract was analyzed by PAGE electrophoresis.Results: 1) There was no significant difference in KCl induced contractions between the groups (LEW 4,827±0,472 mN, HTG 4,999±0,427 mN). The contractions induced by PGF$_{2□}$ were significantly bigger in group HTG (5,794±0,758 mN) than in the group LEW (3,636±0,561 mN, $P<0.05$). 2) There was no significant qualitative or quantitative difference between the groups in SDS gel electrophoresis profile of the collagenous fractions.
Conclusions: These results demonstrated that genetic hypertension of HTG rats might be partially dependent on functional changes of resistant arteries and has no effect at collagenous peptides in pulmonary vessels. This is in good agreement with our previous results that structural changes could even precede hypertension development. The exaggerated response to PGF will need other analysis with special attention to calcium metabolism. *The work was partly supported by the grant GAČR 305/97/S070.*

RED CELL ION TRANSPORT AND ABNORMAL LIPID METABOLISM IN SALT HYPERTENSION. ***M. Vokurková**, Z. Dobešová, O. Nováková[1], J. Kuneš, J. Zicha,* Institute of Physiology AS CR, Prague, [1]Department of Animal Physiology and Developmental Biology, Faculty of Sciences, Charles University, Prague, Czech Republic.

Salt hypertension in Dahl rats is associated with red cell in transport abnormalities and elevations of plasma triglyceride and cholesterol levels. The severity of salt hypertension, transport abnormalities and plasma lipid alterations is dependent on the age at which Dahl rats are exposed to high salt intake. The aim of our present study was to evaluate membrane lipid composition (cholesterol, total phospholipids and their subclasses) in erythrocytes of young and adult Dahl rats and to determine their relationship to red cell ion transport. It was demonstrated that erythrocyte membrane cholesterol content tended to be decreased in young salt hypertensive Dahl rats but increased in the adult ones. This was not paralleled by the changes of phospholipid content so that cholesterol-to-phospholipid ratio was elevated in adult animals only. Membrane cholesterol content seems to be a major determinant of Rb^+ uptake mediated by Na^+-K^+-$2Cl^-$ cotransport and passive membrane permeability (Rb^+ leak) as it was indicated by positive correlations in both age groups. On the contrary, erythrocyte Na^+ content and Na^+-K^+ pump activity were positively related to membrane cholesterol content in young but not in adult rats. No major changes in phospholipid subclasses (except of moderate reduction of membrane sphingomyelin content) were disclosed in erythrocyte membrane of young salt hypertensive Dahl rats. There was inverse correlation between Na^+ leak and sphingomyelin membrane content in young Dahl rats. Our forthcoming analysis on membrane lipid content indicate the importance of abnormal lipid metabolism in transport alterations observed in age-dependent salt hypertension. *Partially supported by grant 305/00/1638(Grant Agency of Czech Republic)*

INDUCIBLE NITRIC OXIDE SYNTHASE PARTICIPATES IN THE MECHANISM OF CHRONIC HYPOXIC PULMONARY HYPERTENSION *J. Bíbová, D. Miková, A. Bañasová, V. Hampl, J. Herget,* Department of Physiology, Charles University, Second Medical School, Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

Expression of the inducible isoform of nitric oxide synthase (iNOS) is minimal in normal pulmonary vessels but increased in chronic hypoxic pulmonary hypertension. The functional significance of this iNOS induction is unknown. We hypothesize that NO produced by iNOS may contribute to pulmonary vascular wall injury characteristic of the initial stages of hypoxia and thus contribute to the development of pulmonary hypertension. To test this hypothesis, adult male rats were exposed to hypoxia (10 % O_2) for 1 week. During the exposure and for the preceding 3 days, they were treated with a selective iNOS inhibitor L-N^6-(1-iminoethyl)lysine (L-NIL, 8mg/l in drinking water) (NO Biol Chem 6: 398, 2002). Upon removal from the hypoxic chamber, each rat was placed awake in a body pletysmograph for 20 min to measure NO exhalation (CLD 77 AM chemiluminescence analyzer, EcoPhysis, Duernten, Switerland). Subsequently, the rats were anesthetized (thiopental 40 mg/kg BW i.p.) and their systemic and pulmonary arterial blood pressures were measured during spontaneous breathing. To estimate cardiac output, the chest was then opened during mechanical ventilation and ascending aorta blood flow was measured ultrasonically. Exhaled NO was higher in hypoxic, untreated rats (17 ± 3 ppb) than in normoxic controls (5 ± 1 ppb). The hypoxic increase in exhaled NO was markedly reduced by L-NIL (8 ± 3 ppb). The groups did not differ in systemic arterial blood pressure, implying that endothelial isoform of nitric oxide synthase was not inhibited by L-NIL. Pulmonary arterial blood pressure, elevated by the hypoxic exposure (from 16 ± 1 mmHg in normoxic controls to 23 ± 1 mmHg in the hypoxic untreated group), was significantly ($P < 0.03$) reduced by L-NIL treatment (20 ± 1 mmHg). Cardiac output was not significantly affected by L-NIL. We conclude that NO produced by iNOS at the initial phase of chronic hypoxia participates in the pathogenesis of pulmonary hypertension. *Supported in part by GACR 305/97/S070, 305/00/1432, and MSM research project 111300002.*

GENDER DIFFERENCES IN THE ROLE OF CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM IN THE PULMONARY VASCULAR TONE REGULATION. *A.Bañasová, J. Bíbová, V. Hampl,* Department of Physiology, Charles University, Second Medical School, Centre for Experimental Cardiovascular Research, Prague, Czech Republic.

Susceptibility to the pulmonary vascular disease is known to differ between men and women, but the reason for this difference is unknown. In a recent study, while testing the role of calcium (Ca^{2+}) release from sarcoplasmatic reticulum (SR) in the mechanism of hypoxic pulmonary vasoconstriction, we noticed an apparent discrepancy between male and female rats. In the present study we therefore systematically tested the hypothesis that the participation of Ca^{2+} release from SR in pulmonary vasoconstrictor reactivity is greater in females than in males. Vasoconstrictor reactivity to angiotensin II (0.2µg bolus) and hypoxia (0% O_2) was measured in isolated rat lungs in the presence of thapsigargin (10^{-8} M) or its vehicle alone (DMSO). Thapsigargin quickly depletes SR of Ca^{2+} by inhibiting SR Ca ATP-ase. Lungs were isolated from anesthetized (thiopental 40 mg/kg IP) rats, ventilated (21% O_2 + 5% CO_2 + 74% N_2), and perfused with Krebs′-albumin (4%) solution at constant flow rate (so that changes in perfusion pressure directly reflect changes in vascular tone). The potentially confounding effects of vasoactive endothelial mediators, nitric oxide and prostaglandins, were eliminated by including their inhibitors (N^G–nitro-L-arginine methyl ester 50µM and meclofenamate 17µM) in the perfusion medium. In females, thapsigargin significantly reduced pulmonary vasoconstrictor responses to angiotensin II (2.2±0.8 vs. 9.9±2.5 mmHg in DMSO controls, $p< 0.05$) and to hypoxic challenges (4.6±1.2 vs. 21.2 ±2.0 mmHg, $p<0,001$). In males, response to both angiotensin II and hypoxia were not significantly affected by thapsigargin. These data show that Ca^{2+} release from SR significantly contributes to pulmonary vasoconstrictor reactivity, but only in females. *Supported in part by GACR 305/00/1432 and 305/97/S070 and by MSM research project 111300002.*