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SYNTHETIC GRGDSG-BEARING POLYMERS SUPPORT SPECIFIC RECEPTOR-MEDIATED ADHESION OF VASCULAR SMOOTH MUSCLE CELLS

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Biodegradable synthetic polymers endowed with ligands for cell adhesion receptors are considered as advanced materials for tissue engineering, e.g. for construction of bioartificial vascular implants. We studied adhesion of rat vascular smooth muscle cells (VSMC) in cultures on glass coverslips coated with thin polymeric films consisting of a bottom layer of poly(L-lactic acid), PLLA, and a top layer of poly(DL-lactic acid), PDLLA, mixed with a copolymer of PDLLA and poly (ethylene oxide), PEO. The content of PEO in the surface layer was 33%. A fraction of PEO chains (5% or 20%) was functionalized with an integrin-binding peptide Gly-Arg-Gly-Asp-Ser-Gly (GRGDSG). On both PLLA and PDLLA, the cell spreading and formation of vinculin-containing focal adhesion plaques were similar as on conventional tissue culture supports, such as polystyrene or glass. The cell adhesion on both polylactides was mediated by adsorption of extracellular matrix molecules (ECM) from the serum of the culture medium. In contrast, on the PDLLA-PEO copolymer, the cells were not able to spread and form focal adhesion plaques, because highly hydrophilic PEO prevented adsorption of cell adhesion-mediating ECM. However, when the PDLLA-PEO was functionalized with the oligopeptide Gly-Arg-Gly-Asp-Ser-Gly (GRGDSG), i.e., a ligand for integrin adhesion receptors on cells, the cell spreading and formation of vinculin-containing focal adhesion plaques markedly improved, especially on samples with 5% GRGDSG. The cell adhesion on the GRGDSG-bearing material was improved even in serum-free media, which do not contain cell-adhesion-mediating ECM, and was followed by DNA synthesis and cell proliferation. These results suggest that the adhesion of VSMC to the GRGDSG-PDLLA-PEO construct is mediated specifically by integrin receptors, and its extent could be controlled by the type, concentration and spatial distribution of the attached adhesion ligand.

CVT-313 INHIBITS VASCULAR SMOOTH MUSCLE CELL (VSMC) PROLIFERATION BY LOWERING CDK2 ACTIVITY AND CHANGES IN ITS ENDOGENOUS REGULATORS

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Control of cell proliferation is essential in engineering of bioartificial vascular grafts, as excessive VSMC proliferation can cause obstruction of these implants. We studied effects of CVT-313, a selective cyclin-dependent kinase 2 (CDK2) inhibitor [1], on VSMC in cultures derived from the thoracic aorta of adult or newborn Wistar rats. The cells were exposed to CVT-313 concentrations from 63 to 10000 ng/ml in Dulbecco`s modified Eagle`s medium with 10% of fetal bovine serum. As previously described [2], newborn VSMC were much more inhibited by CVT-313 compared to those from adult rat. In the present study, measurements of phosphorylation of histon H1 using [32P]ATP showed that CVT-313 decreased phosphorylation activity of CDK2 in newborn VSMC, whereas in adult VSMC, this compound did not influence the CDK2 activity at lower passages (5) or even increased it at higher passages (21 to 27). As revealed by Western blotting, the total content of CDK2 as well as its stimulator cyclin A was higher in cells from newborn than adult rats, and it remained unchanged after administration of CVT-313. The total content of another CDK2 stimulator, cyclin E, was even increased in CVT-313-treated cells. However, CVT-313 increased the total content of CDK2 inhibitors p21 and p27 [3] in VSMC from newborn rats. Moreover, immunoprecipitation revealed a higher binding of p27 on CDK2 kinase after CVT-313 treatment and a lower binding of cyclin A in comparison with VSMC from adult rats. These results suggest that VSMC from adult donors are more resistant to stimuli controlling their proliferation than their counterparts from newborns. *This work was supported by the Grant Agency of the Acad. Sci. CR (grant No. A4050202)*

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HEME OXYGENASE 1 AND 2 EXPRESSION IN THE RAT HEART AFTER AORTIC STENOSIS

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Gender has a pronounced influence on the type and severity of cardiovascular diseases, including adaptation to overload and consequently heart failure (HF); possible sex differences in endogenous signaling pathways remained to be clarified. Heme oxygenase (HO) as a heme degrading enzyme is suggested to be involved in the regulation of the development of left ventricular hypertrophy. The aim of the present study was, therefore, (i) to characterize gender differences in weight and hemodynamic parameters in rats subjected to aortic stenosis and (ii) to analyze whether pressure overload-induced HF alter the expression of HO-1 (inducible) and HO-2 (constitutive) isoforms in male and female left ventricular myocardium. For this purpose, aortic stenosis was induced in 25-day-old male and female Wistar rats. Analyses (weight and hemodynamic parameters, immunofluorescent labeling, Western blots) were performed 2, 24 and 20 weeks after operation (PO). Pressure overload increased left and right ventricular weights both in males and females; the degree of left ventricular hypertrophy was, however, more expressed in females 20 weeks PO. On the other hand, end-diastolic pressure increased significantly more in males. Immunoreactivity of HO-1 and HO-2 was detected in cardiomyocytes at the level of intercalated discs, nuclear membranes, and vascular compartment. Expression of HO-1 increased with age; aortic stenosis accelerated this increase in females only, expression of HO-2 remained unchanged. It may be concluded that gender-dependent modulation of HO-1 expression may be involved in cardiac adaptation to pressure overload.
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NITRIC OXIDE PRODUCTION INTO THE EXHALED AIR IS ELEVATED BY 4 DAYS OF HYPOXIA IN THE LOWER, BUT NOT THE UPPER, RESPIRATORY TRACT

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The exact role of nitric oxide (NO) in hypoxic pulmonary hypertension is incompletely understood. Exhaled NO provides a non-invasive means of assessing the NO signaling in the respiratory system. Exhaled NO quickly increases during chronic hypoxia. While under normal circumstances the exhaled NO comes mostly from the upper airways, the source of its elevation in hypoxia is unknown. Its determination was the objective of the present study. Adult male Wistar rats were kept in hypoxic chamber (10% O_2; n = 6) or room air (n = 7). After 4 days of hypoxia, all animals were intubated via a tracheostomy under thiopental anaesthesia (50 mg/kg i.p.). To assess total exhaled NO, they were placed into a sealed pletysmograph (2.1 l) and NO accumulation after 10 min was measured with chemiluminescence NO analyzer (CLD 77 AM, EcoPhysis, Switzerland). NO production in the nose and paranasal sinuses is large enough to support discernible diffusion efflux of NO even in the absence of nasal ventilation. To measure NO production into the exhaled air from the lung tissue and lower airways, the exhalate for NO measurement was then collected directly from the tracheal tube into a latex bag (250 ml). The exhaled NO measured directly from the tracheal tube doubled in hypoxia (1.1 ±0.1 ppb) compared to the normoxic group (0.6±0.7 ppb, p=0.001). The difference between the tracheal tube and plethysmograph measurements, reflecting the contribution of the upper airways, did not differ between the hypoxic (1.0±0.5 ppb) and normoxic (0.9±0.3 ppb) groups (p=0.6).
Thus, the source of the elevated NO exhalation in hypoxia is the lower respiratory tract. While the contribution of the upper airways remains remarkable, it does not rise in hypoxia.

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THE TREATMENT OF ATRIAL FIBRILLATION USING RADIOFREQUENCY MAZE PROCEDURE – HEART RHYTHM DURING FOLLOW UP

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Creation of linear lesions in the left atrium has been suggested as a therapeutic option for patients (pts) indicated for cardiac surgery for valvular heart disease or coronary artery disease (CAD) and concomitant atrial fibrillation (AF).
The aim of the study was to analyze heart rhythm during FU in the group of pts treated by radiofrequency (RF) maze procedure used in our institution. Since August 2000 till August 2004, 248 pts (105 female, mean age 67,5 years, range 25 – 84 yrs) with chronic or paroxysmal AF were indicated for heart surgery with RF maze procedure. Contiguous left atrial lesion lines between the mitral annulus and the pulmonary veins were created by RF energy (MAZEPEN, Medtronic Inc) before valve surgery or coronary artery bypass grafting. Pts were divided according to underlying heart disease: group I (mitral valve disease) - 74 pts, group II (CAD in combination with aortic valve disease or ischemic mitral valve disease) - 127 pts and group III (CAD) - 47 pts.Eight pts died in the early postoperative period, no complications related to the ablation procedure were observed. Three pts had transitory cerebral attack, cardiac pacemaker was implanted in another eleven cases. RF catheter ablation because of symptomatic atrial tachycardia was performed in five pts during FU. Sinus rhythm was present at discharge in 66%, 64%, 69%, at 3 months of FU 54%, 63%, 76%, at 6 months of FU 71%, 62%, 72%, at 12 months of FU 66 %, 68%, 70% pts and at 24months of FU 65%, 75%, -% in groups I, II, III, respectively. In conclusions, intraoperative RF ablation in the left atrium seems to be an effective and safe method for the treatment of AF that results in long-term restoration of sinus rhythm in at least 65% pts with previous chronic or persistent AF.

DOES AMP ACTIVATED PROTEIN KINASE AFFECT CREATINE KINASE IN THE ISCHEMIC, CHEMICALLY SKINNED MOUSE CARDIAC MUSCLE?

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AMP activated protein kinase (AMPK) has emerged as a key enzyme in stress signalling and is implicated in maintaining energy balance of the eucaryote cells. It has been implicated in both the stimulation of energy producing pathways and the down regulation of energy utilizing pathways. Creatine kinase (CK) is the important enzyme that catalyses the transfer of a phosphoryl group between creatine and ADP and has been proposed to be one of the targets for AMPK in the heart. The aim of the study was to investigate whether AMPK is implicated in the function of MM-CK bound to myofilaments in the ischemic myocardium. Muscle fibres were dissected (treated by Triton X-100 and phosphatese inhibitor) from left ventricles of the isolated Langendorff perfused normoxic and ischemic (10 min of global ischemia) hearts. Right ventricles were freeze-clamped at the end of the perfusion and used for Western blot analysis for detection of AMPK phosphorylation in the hearts. Our results showed that this type of ischemia was sufficient to activate AMPK; this enzyme was phosphorylated only in the ischemic hearts. No significant differences were observed in the calcium sensitivity (pCa_{50} = 5.63 ± 0.02 and 5.59 ± 0.04 in the normoxic and hypoxic fibres, respectively) and in the effect of phosphocreatine (PCr) on relaxation of rigor tension ($pMgATP_{50}$ + PCr = 4.99 ±0.05 and 4.99 ± 0.07 in the normoxic and hypoxic fibres, respectively) in the skinned fibres. In conclusion, our results suggest that the AMPK is activated during global ischemia in the isolated perfused mouse heart, is localized in the myofibrillar compartment but its influence on MM-CK bound to myofilaments seems to be unlikely.

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THE EFFECT OF PERINATAL HYPOXIA ON THE TOLERANCE OF THE ADULT MYOCARDIUM TO OXYGEN DEFICIENCY IN THE MALE AND FEMALE RATS

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Chronic hypoxia, the main pathophysiological feature of the congenital cyanotic heart defects, can significantly affect cardiovascular system in the adulthood. The aim of present study was to examine the effect of perinatal hypoxia on the tolerance of the adult myocardium to ischemia/reperfusion injury. Male and female Wistar rats were exposed to intermittent high altitude hypoxia (5000 m, 8 h/day), for 5 prenatal (born in the normoxic condition) and 10 postnatal days. After hypoxic exposure all animals were kept for 3 month in the normoxic condition The control groups were born and then kept for the same time in normoxic condition. All experiments were performed after 90 postnatal days. The ventricular ischemic arrhythmias were assessed on the isolated hearts perfused under constant flow (10 ml/min/g) during 30-min regional ischemia (occlusion of LAD coronary artery). Infarct size (tetrazolium staining) was measured on isolated hearts perfused under constant pressure (75 mmHg) after 40-min regional ischemia (occlusion of LAD coronary artery) and 120-min reperfusion. The number of singles, salvos, ventricular tachycardia and total number of arrhythmias was increased in the perinatal hypoxic male hearts compared with normoxic male hearts. On the other hand ischemic arrhythmias in the female groups were significantly reduced by adaptation to perinatal hypoxia. Perinatal adaptation to hypoxia changed tolerance to ischemia expressed as the infarct size neither in the male nor in the female group. Our results show that hearts exposed to perinatal hypoxia exhibit different tolerance to ischemic arrhythmias; adaptation of female rats induced protective effect, on the contrary perinatal hypoxia in the male group acted proarrhythmicaly. The infarct size was not affected by perinatal hypoxia.

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EFFECTS OF PERINATAL AND EARLY POSTNATAL HYPOXIA ON RAT PULMONARY VESSELS

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Perinatal hypoxia induces in rats irreversible changes of lung circulation (Hampl and Herget 1990). The pulmonary vessels in adult rats born in hypoxia are more compliant; the hypoxic pulmonary vasoconstriction is increased during the recovery from sojourn in chronic hypoxia. The goal of present study was to detect whether described irreversible changes develop immediately after the birth or later on. Three groups of rats were studied: 1. Control group (C) (n=8, studied at the age 18-20 days). 2. Perinatal hypoxia (PH) (n=8, exposed to hypoxia of 12% O_2 10 days before and 4 days after the birth). 3. Postnatal hypoxia (H) (n=8, exposed to hypoxia of 10 % O_2 from 4th day to 18th day of life. The groups PH and H were studied after 2 weeks of recovery in air. The pulmonary vasculature was studied using the preparation of isolated, ventilated perfused lungs. The lungs were perfused by physiologic salt solution with 4% albumin and 17□M of meclophenamate by constant flow (0.04 ml/min/g b.w.) and ventilated by positive pressure with air + 5% CO_2. Pulmonary vasoconstriction was induced by challenges with ventilation hypoxia (0% N_2 + 5% CO_2) or by bolus injections of 0.05□g of angiotensin II. Perfusion pressure-perfusion flow (P/Q) relation was measured by stepwise increases of perfusion flow. Basal perfusion pressure, reactivity to acute hypoxia and angiotensin II injection and the slope of P/Q relation were significantly higher in group H than in groups C and PH. Groups C and PH did not differ significantly. In conclusion, the vascular hyperreactivity of perinatally hypoxic rats does not develop immediately after the birth. The lower slope of P/Q relation in PH (comparing to H) is consistent with the similar finding in adult rats (Herget and Kuklik 1995).

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AGE-DEPENDENT ION TRANSPORT IN RAT ERYTHROCYTES

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It is generally accepted that immature erythrocytes exhibit a lower mean cellular hemoglobin content (MCHC) than mature erythrocytes (ery). In our previous study significant correlations were found between MCHC and ion transport parameters. Therefore the aim of this study was to find a relationship between the activity of ion transport and erythrocyte maturity in rats subjected to repeated hemorrhage (blood loss 2 ml/day for 6 days). Immature erythrocytes compared with mature erythrocytes exhibited lower MCHC (4.62±0.15 *vs* 5.07±0.14 mmol/l ery, $p<0.01$), decreased erythrocyte Na^+ content (3.747±0.361 *vs* 4.410±0.363 mmol/l ery, $p<0.01$), elevated activity of the Na^+-K^+ pump (10.135±1.383 *vs* 7.162±0.841 mmol/l ery/h, $p<0.05$), reduced activity of the Na^+-K^+ cotransport (0.322±0.095 *vs* 0.688±0.194 mmol/l ery/h, $p<0.05$) and increased Na^+ leak (10.520±2.257 *vs* 5.526±0.860 mmol/l ery/h, $p<0.05$). The dependence of the Na^+-K^+ pump activity on erythrocyte Na^+ content was steeper in the immature erythrocytes than in the mature erythrocytes. It can be concluded that enhanced activity of the Na^+-K^+ pump results from its increased affinity to intracellular Na^+ and augmented maximal velocity in the immature erythrocytes.

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