1029-178 Potential Involvement of Novel Scavenger Receptor in Oxidized Low-Density Lipoproteins Promoted Atherogenic Effects in Human Aortic Smooth Muscle Cells

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Oxidized low density lipoproteins (OxLDL) play a key role in atherogenesis and induce a wide range of biological effects on smooth muscle cells including the induction of scavenger receptor expression. Recently we identified expression of mRNA for a novel scavenger receptor, SRECII, in human aortic smooth muscle cells (HASMC) and showed that OxLDL upregulated SRECII mRNA level.

To determine whether HASMC express SRECII protein and to characterize this novel scavenger receptor in HASMC we synthesized and purified a 15 amino acids unique SRECII peptide and raised rabbit polyclonal antiserum against this peptide. High antibody titer (>100,000) was assessed by ELISA analysis. Using immune antiserum we found in HASMC extracts a major antigenic protein at around 75 kDa, equivalent to the predicted molecular weight of SRECII. Only one protein band (72±7kD, n=6, p<0.05) was detected using affinity purified SRECII antibody. Band density was significantly reduced by antibody preadsorbtion (60min, 37C) with 10-fold excess of SRECII peptide.

It has been established that the CD36 scavenger receptor is involved in OxLDL-promoted atherogenesis. We tested a blocking antibody to CD36 and our antibody to SRECII for their ability to prevent OxLDL-induced (60 ug/ml for 15h) rise in superoxide production (CDC-H_2F hydroethidine fluorescence assay) and apoptosis (FACS analysis with annexin V staining). Pretreatment of HASMC by anti-SRECII antibodies or blocking antibodies to CD36 (20 ug/ml for 1h) reduced OxLDL-promoted apoptosis by 49% and significantly reduced OxLDL-induced rise in superoxides. Pretreatment with normal immunoglobulin or SRECII antibody preadsorbed by SRECII peptide did not alter OxLDL-induced apoptosis and rise in superoxides.

We conclude that the SRECII scavenger receptor mediates at least two OxLDL-induced pro-atherogenic effects: rise in superoxides and induction of smooth muscle cell apoptosis. This is the first report of SRECII expression in vascular smooth muscle cells and our finding strongly suggests its involvement in atherogenesis.

1029-179 Left Ventricular Mass Index and the Common, Functional, X- Linked Angiotensin II Type 2-Receptor Gene Polymorphism (1332 G/A) in Patients With Systemic Hypertension

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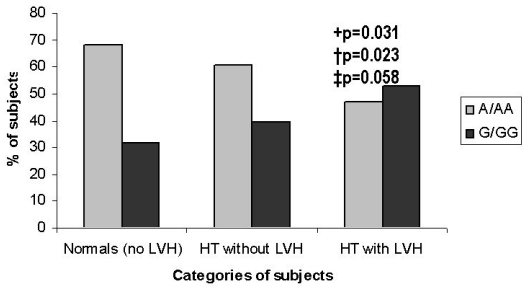
Background: A common intronic polymorphism, (-1332 G/A) of the angiotensin type 2 (AT_2) receptor gene, located on the X-chromosome, has been reported to be biochemically functional. The aim of this study was to evaluate this polymorphism for an association with left ventricular hypertrophy (LVH).

Methods: LV mass was measured in 197 patients with systemic hypertension and 60 normal volunteers, using a 1.5-Tesla Philips MRI system. Genotyping was performed using a restriction enzyme digestion of an initial 310 bp PCR product that included the AT_2 (-1332 G/A) locus.

Results: The mean LV mass index for the male patients was 94.3±19.6 g/m² (n =125) and for the female patients was 71.2±12.0g/m² (n =72). Seventy three (37.1%) of all patients had an elevated LV mass index, defined as the mean LV mass index for normal volunteers plus 2 S.D (males 77.8 ± 9.1 g/m², n = 30; females 61.5 ± 7.5g/m², n = 30). Comparison of LV mass index, of the A/AA genotype (mean LV mass index = 82.4±21.1 g/m²; n = 123) against that of the G/GG genotype (mean LV mass index = 88.1±19.0 g/m²; n = 89), as a continuous variable was significant by analysis of variance (p=0.044). Chi-square comparison revealed an excess of the G/GG genotype among hypertensives with LVH when compared with, all subjects without LVH (p=0.031), normal subjects (p=0.023) and when compared with hypertensives without LVH (p=0.058).

Conclusion: We observed an association between the AT_2 receptor (-1332 G) allele and the presence of LVH in hypertensive subjects.



+Significance for comparison of HT with LVH vs. all subjects without LVH
† Significance for comparison of HT with LVH vs. Normals
‡ Significance for comparison of HT with LVH vs. HT without LVH

1029-180 Impact of Protein Kinase C-Epsilon for Myocardial Hypertrophy After Chronic Pressure Overload: In-Vivo Study in Protein Kinase C-Epsilon Knockout Mice

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Background: Protein kinase C (PKC) is involved in the signal transduction of myocardial hypertrophy. Overexpression and increased activation of PKCε leads to myocardial hypertrophy in mice. However, if PKCε is required for signaling in myocardial hypertrophy, the lack of PKCε is expected to result in either reduced or even no myocardial hypertrophy after chronic pressure overload.

Methods: Hearts of wild type (WT; n=17) and PKCε -KO-mice (KO; n=20) were examined by echocardiography before and 4 weeks after transverse aortic constriction (TAC). Hearts were excised and preserved in N_2 or formalin.

Results: 1. In 12- weeks-old WT- and KO-mice there were no differences in left ventricular dimensions, function and structure. 2. 4 weeks after TAC both groups, WT and KO, developed myocardial hypertrophy to the same extent. 3. Left ventricular systolic function was preserved in both groups after pressure overload. 4. Northern blots of typical markers of hypertrophy, i.e. ß-MHC, ANP and α–SkM-Actin increased comparably in both groups after TAC. 5. Sirius-red staining showed a significant increase of fibrosis in hypertrophied hearts of KO compared with WT.

Conclusions: 1. Until the age of 12 -weeks the lack of PKCε does not lead to alterations in myocardial development in mice. 2. PKCε is not essential for the development of myocardial hypertrophy in mice. 3. The lack of PKCε leads to increased myocardial fibrosis after chronic pressure overload. 4.Further investigations have to elucidate the molecuar mechanism leading to increased myocardial fibrosis in PKCε -KO after TAC and its functional consequences.

1029-181 Enhanced Cardiac Beta$_3$-Adrenergic Functional Response in Alcoholic Monkeys

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Background. Chronic alcohol intake is associated with attenuated cardiac β-adrenergic receptor (AR)-mediated positive inotropic response. Altered cardiac functional β_3-AR expression may contribute to this effect. However, the effect of chronic alcohol on cardiac β_3-AR has not been examined. Moreover, although β_3-AR has been documented in human, rat, and canine myocardium, its existence and functional role in monkey hearts remains undetermined.

Methods. We compared β_3-AR mRNA levels and myocyte contractile and calcium current ($I_{Ca,L}$) responses to β_3-AR agonist, BRL-37344 (BRL, 10^{-8} M), in freshly isolated left ventricle (LV) cardiomyocytes obtained from 8 normal control cynomolgus monkeys and 6 monkeys with self-administered oral alcohol for 9 months (4 moderate and 2 heavy drinkers with mean daily intake of alcohol of 1.9 and 3.4 g/kg, respectively).

Results. Using RT-PCR, β_3-AR mRNA (a single band about 317 bp) was detected in both normal and alcoholic myocytes. Compared with normal myocytes, the signal ratio of β_3-AR mRNA in moderate and heavy drinkers was significantly increased from 7.2% to 28.8% and 53.2%, respectively. These changes were associated with altered β_3-AR-mediated inotropic actions. Compared with normal myocytes, in alcoholic cardiomyocytes, cell contraction (dL/dt_{max}, -32%, 61.3±10.3 vs 89.9±12.2 mm/s) and relaxation (dR/dt_{max}, -22%, 59.4±14.0 vs 76.2±10.5 mm/s) and calcium current ($I_{Ca,L}$, -22%, 6.4 vs 5.0 pA/pF) were significantly reduced. In addition, superfusion of ISO (10^{-8} M) caused a much less increase in dL/dt_{max} (52% vs 79%) and dR/dt_{max}. In contrast, in alcoholic cardiomyocytes, BRL produced a much greater decrease in the percent shortening (17.8% vs 6.9%), dL/dt_{max} (16.6% vs 7.1%), and $I_{Ca,L}$ (26.4 vs 19.6%). These responses were prevented by bupranolol or L-748,337 (β_3-AR antagonists).

Conclusion. In monkeys, chronic alcohol intake (moderate and heavy) increases cardiac β_3-AR mRNA expression and enhances cardiac β_3-AR-mediated negative inotropic response.

1029-182 Biventricular Autocrine/Paracrine Systems in Monocrotaline-Induced Pulmonary Hypertension

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Increasing evidence suggests occurrence of LV contractile dysfunction in pulmonary hypertension (PH) in the absence of LV overload. The role of autocrine/paracrine mechanisms on the development of such dysfunction remains largely unknown.

RV and LV hemodynamic and morphometric measurements along with evaluation of mRNA expression (RT real time PCR, normalized for GAPDH) of angiotensinogen (Agtg), ACE, aldosterone synthase (A-synt), chymase, ET-1, IGF-1 and BNP were carried in Wistar rats 4 (M_4, n=7) and 6 (M_6, n=7) weeks after monocrotaline injection (MCT, 60mg/Kg, sc) and compared with sham (S, n=7), Results presented as mean±SEM; p<0.05: * *vs* S, † *vs* M_4, with mRNA data reported in Arbitrary Units of ratios.

MCT increased systolic RV pressure (S=21±1; M_4=39±2*; M_6=51±4*† mmHg) and RV/LV weight ratio (S=0.23±0.02; M_4=0.37±0.03*; M_6=0.58±0.03*†), whilst end-diastolic LV dimensions decreased (S=8.2±0.6; M_4=6.9±0.7; M_6=5.4±0.9* mm). LV function was impaired only in the M_6 group: dP/dtmax (S=4953±550; M_4=5263±393; M_6=2205±272*† mmHg/s), time constant τ (S=22±2; M_4=19±2; M_6=27±2*† ms). MCT significantly changed gene expression of RV-ACE (S=1.0±0.1; M_4=1.7±0.3; M_6=9.8±1.4*†), LV-ACE (S=1.0±0.1; M_4=1.9±0.1; M_6=4.5±1.1*), RV-ET-1 (S=1.0±0.1; M_4=0.7±0.1*; M_6=3.2±0.8*†), LV-ET-1 (S=1.0±0.2; M_4=0.9±0.1; M_6=6.8±2.0*†), RV-BNP (S=1.0±0.3; M_4=8.2±2.3*; M_6=11.6±1.9*†), but not of LV-BNP or RV and LV Agtg, A-synt, chymase