**Key Words:** Essential Hypertension, Polymorphism, Synergistic Effects

**Conclusions:**
E-selectin A561C (S128R) polymorphism affect blood pressure significantly.

The aim of this study was to determine whether the aminopeptidase inhibitor arphamenine A could affect the collagen production and expression in control and TGF-beta1-treated cardiac fibroblasts. Cardiac fibroblasts from passage 2 from normal male adult rats (n=5) were cultured to confluence, incubated with (no) 600 nmol/l TGF-beta1 for 2 days in serum-free Dulbecco’s modified Eagles’s medium and then incubated with 100 nmol/l arphamenine A for 1 day in this medium with added ascorbic acid, beta-aminopropionitrile and tetratide proline. Soluble collagen was measured in the conditioned medium and nonsoluble collagen in the cell layer. Aminopeptidase activity was estimated by spectrophotometric determination of the liberation of p-nitroaniline from alanine and arginine-p-nitroanilide. Matrix metalloproteinase (MMP) and lysyl oxidase activity were assayed in the conditioned medium. A semi-quantitative reverse transcription polymerase chain reaction was used to determine the expression of lysyl oxidase and collagen type I and III. Arphamenine A dose-dependently inhibited the basal and TGF-beta1-stimulated aminopeptidase activity. Arphamenine A reduced the soluble and nonsoluble collagen production in control and TGF-beta1-treated cardiac fibroblasts, while it decreased the collagen type I and III expression only in TGF-beta1-fibroblasts. Lysyl oxidase, MMP-1 and MMP-2 activity were inhibited by arphamenine A in the conditioned media of control and TGF-beta1-treated cardiac fibroblasts, while MMP-13 and TIMP-2 were not affected by arphamenine A. Our data show that the specific aminopeptidase inhibitor arphamenine A reduces the collagen production in cardiac fibroblasts and that this reduction is accompanied by a pronounced inhibition of lysyl oxidase.

**Key Words:** Cardiac Fibroblasts, Collagen, Transforming Growth Factor-Beta

**Results:**
The frequencies of T allele and GT+TT genotype of eNOS gene G894T polymorphism in EH were significantly higher compared with that estimated alone from either eNOS 786C allele (2.51) was markedly increased compared with estimated alone from either eNOS 786C allele (0.542) or a allele (0.389).

**Conclusions:** eNOS 894T allele should be a risk factor for EH in Chinese Han nationality, there was a significant synergistic effects of eNOS 786T allele and GNB3 825T allele in EH.

**Key Words:** Essential Hypertension, Polymorphism, Synergistic Effects

**P-432**
**SYNERGISTIC EFFECT BETWEEN ENOS GENE G894T AND GNB3 GENE C825T POLYMORPHISMS IN PATIENTS WITH ESSENTIAL HYPERTENSION**

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**Aim:** To investigate an interaction between the G894T polymorphism of endothelial nitric oxide synthase (eNOS) gene and the C825T polymorphism of GNB3 gene in the risk for essential hypertension (EH).

**Methods:** Polymerase chain reaction combined with restriction enzyme digestion was used to detect the G894T polymorphism of eNOS gene and C825T polymorphism of GNB3 gene in 151 normotensive controls and 310 hypertensive patients.

**Results:** The frequencies of T allele and GT+TT genotype of eNOS gene G894T polymorphism in EH were significantly higher than in control group; There were no significant difference of the GNB3 gene C825T polymorphism CC,CT,TT genotypes and C,T alleles between hypertensive patients and normotensive controls; The odds ratio (OR) estimated by combined analysis with eNOS 894T and GNB3 825T (1.82) was markedly increased compared with that estimated alone from either eNOS 786C (0.542) or GNB3 825T allele (0.954).

**Conclusions:**

**Key Words:** Ambulatory Blood Pressure Monitoring, E-Selectin, Polymorphism

**P-433**
**SYNERGISTIC EFFECT BETWEEN ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE T786C AND 4B/A POLYMORPHISMS IN PATIENTS WITH ESSENTIAL HYPERTENSION**

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**Aim:** To investigate an interaction between eNOS gene T786C and 4b/a polymorphisms in the risk for essential hypertension (EH).

**Methods:** Polymerase chain reaction (PCR) combined with restriction enzyme digestion was used to detect the T786C polymorphism of eNOS gene in 189 normotensive controls and 310 hypertensive patients.

**Results:** The frequencies of a allele, ab,aa genotype and C allele, CC+CT genotype in EH were significantly higher than in control group respectively; The odds ratio (OR) estimated by combined analysis with eNOS 786C and a allele (2.51) was markedly increased compared with that estimated alone from either eNOS 786C (0.542) or a allele (0.389).

**Conclusions:**

**Key Words:** Essential Hypertension, Gene Polymorphism, Nitric Oxide Synthase(eNOS)

**P-434**
**AMINOPEPTIDASE B-INHIBITION ATTENUATES COLLAGEN PRODUCTION IN CARDIAC FIBROBLASTS THROUGH INHIBITION OF LYSYL OXIDASE**

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The aim of this study was to determine whether the aminopeptidase inhibitor arphamenine A could affect the collagen production and expression in control and TGF-beta1-treated cardiac fibroblasts. Cardiac fibroblasts from passage 2 from normal male adult rats (n=5) were cultured to confluence, incubated without (no) 600 nmol/l TGF-beta1 for 2 days in serum-free Dulbecco’s modified Eagles’s medium and then incubated with 100 nmol/l arphamenine A for 1 day in this medium with added ascorbic acid, beta-aminopropionitrile and tetratide proline. Soluble collagen was measured in the conditioned medium and nonsoluble collagen in the cell layer. Aminopeptidase activity was estimated by spectrophotometric determination of the liberation of p-nitroaniline from alanine and arginine-p-nitroanilide. Matrix metalloproteinase (MMP) and lysyl oxidase activity were assayed in the conditioned medium. A semi-quantitative reverse transcription polymerase chain reaction was used to determine the expression of lysyl oxidase and collagen type I and III. Arphamenine A dose-dependently inhibited the basal and TGF-beta1-stimulated aminopeptidase activity. Arphamenine A reduced the soluble and nonsoluble collagen production in control and TGF-beta1-treated cardiac fibroblasts, while it decreased the collagen type I and III expression only in TGF-beta1-fibroblasts. Lysyl oxidase, MMP-1 and MMP-2 activity were inhibited by arphamenine A in the conditioned media of control and TGF-beta1-treated cardiac fibroblasts, while MMP-13 and TIMP-2 were not affected by arphamenine A. Our data show that the specific aminopeptidase inhibitor arphamenine A reduces the collagen production in cardiac fibroblasts and that this reduction is accompanied by a pronounced inhibition of lysyl oxidase.

**Key Words:** Cardiac Fibroblasts, Collagen, Transforming Growth Factor-Beta

**P-435**
**INHIBITION OF COLLAGEN GEL CONTRACTION IN CARDIAC FIBROBLASTS BY IMIPRamine**

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The aim of this study was to determine whether (amino)peptidase inhibition could affect the collagen gel contraction in basal (control) cardiac fibroblasts. Cardiac fibroblasts (from 6 normal male adult rats) were cultured to confluence in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS). These fibroblasts (100,000 cells) were then further incubated in a floating collagen gel lattice with various concentrations (1, 10, 50, 100 or 300 nmol/l) of imipramine, a pumerycin-sensitive aminopeptidase inhibitor, or with des-Tyr-Met-enkephalin (10 or 100 nmol/l), a neutral endopeptidase (NEP)-24.11) or enkephalinase inhibitor, and compared with the NEP inhibitors phosphoramidon or thiorphan (10 nmol/l) for 3 days in DMEM without FBS. The contraction of the collagen gel lattice by cardiac fibroblasts was determined by measuring the gel volume with titrated water. The original gel volume of the hydrated collagen gel in the absence of fibroblasts averaged 240.1 ± 3.2 ml and decreased to 148.1 ± 9.7 ml in control fibroblasts. Imipramine at a concentration of 1 nmol/l did not affect the gel volume of control fibroblasts (146.7 ± 9.6 ml). The gel volume is however increased with higher concentrations of imipramine to respectively 164.7 ± 9.6, 220.0 ± 12.9, 238.3 ± 1.4 and 243.8 ± 5.4 ml with 10, 50, 100 and 300 nmol/l. The latter 2 concentrations of imipramine even completely inhibited the collagen gel contraction in fibroblasts and this...
inhibitors (tiron 100 μmol/l) alone or combined did not affect the gel volume in basal fibroblasts. Thus, Met-enkephalin or Met-enkephalin acetate (1 μmol/l) and naltrexone (1 μmol/l) alone or combined did not affect the gel volume in basal fibroblasts. Thiophan also did not affect the collagen gel contraction (154.3 ± 7.1 versus 149.4 ± 10.8 μl) in basal fibroblasts, while phosphoramidon had a small inhibitory (12%) effect (154.3 ± 7.1 versus 172.5 ± 12.8 μl). Our data indicate that imipramine inhibits the collagen gel contraction in control cardiac fibroblasts while des-Tyr-Met-enkephalin and thiophan have no effect and phosphoramidon a small inhibitory effect.

Key Words: Aminopeptidase Inhibition, Cardiac Fibroblasts, Collagen Gel Contraction

P-436
ANG II ACTIVATES JAK2 IN HUMAN NON FAILING MYOCYTES ONLY IN THE PRESENCE OF HIGH GLUCOSE CONCENTRATION

Purpose: Left ventricular hypertrophy with diastolic dysfunction is often detectable in diabetic patients. Diabetic cardiomyopathy almost invariably transforms into a dilated form with depressed contractile behaviour.

In non failing myocytes a relation between glucose, Ang II and death signalling pathways was reported. In other cell types, Ang II was demonstrated to induce growth promotion by phosphorylation of JAK2, a soluble tyrosine kinase also involved in the inflammatory process. However, no study exists on human ventricular myocytes.

Therefore we investigated if in human adult myocytes Ang II may induce JAK2 phosphorylation and the effects of high glucose concentrations (HG: 25mM, corresponding to plasma levels of 4.5g/l).

Methods: Non failing myocytes were obtained from five tentative tissue donors (aged 47 ± 5 years), whose hearts could not be transplanted because of non cardiac reason. Myocytes were isolated with enzymatic digestion methods, followed by percoll purification and suspended in MEM with or without Ang II (100nM) and with glucose 5.5mM (NG) or HG. To investigate the role of receptor subtypes and to characterize the ROS production, myocytes were incubated with selective AT1 (valsalan 1μM) or AT2 (PD123319 1μM) receptor antagonists and with ROS inhibitors (tiron 100μM and DPI 100μM), or specific inhibitors for NADPH oxidase (apocinin 10μM) and mitochondrial ROS (rotenone 5μM). JAK2 phosphorylation was detected in Western studies by using specific antibodies for total (Santa Cruz) and phosphorylated JAK2 (Upstate).

Results: Differently from what reported in other cell types, in non failing human ventricular myocytes Ang II was not able to induce JAK2 phosphorylation. Conversely in the presence of high glucose concentration, Western studies revealed a significant Ang II induced activation of JAK2. The response was inhibited by AT1 antagonism, JAK2 activation was prevented by inhibitors of ROS generation (DPI, tiron, rotenone, apocynin).

Conclusions: In non failing myocytes Ang II and HG alone did not induce JAK2 activation. Conversely a synergic action was exerted by Ang II and glucose via an increased ROS production.

Key Words: Angiotensin II, Glucose, Myocyte

P-437
INSULIN-LIKE GROWTH FACTOR-1 ELEVATED IN HYPERTENSIVE PATIENTS
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Introduction: Insulin-like growth factor-1 (IGF-1) is a single-chain peptide, which shares structural homology with pro-insulin. The majority of circulating IGF-1 originates in the liver and is regulated mainly by growth hormone (GH)/(hypophysis-hepatic axis) but also by insulin and nutritional intake. GH and IGF-1 exert opposite effects on glucose metabolism.

In the pathophysiology of essential hypertension there are structural changes that modify the wall-to-lumen ratio, termed vascular remodelling. Once endothelial dysfunction is established, smooth muscle cell proliferation ensues. Vascular hypertrophy associated with hypertension can also lead to the closure of small vessels and could therefore contribute to the increased vascular resistance in long-standing hypertension. Several growth factors such as FGF, TGF-β, and PDGF play an important role in this process. It is yet unknown if IGF-1 contributes in the same way.

Objectives: To evaluate circulating IGF-1 levels in a non selected population of hypertensive patients treated with antihypertensive drugs.

To correlate them with systolic and diastolic blood pressure levels obtained by ambulatory blood pressure monitoring (ABPM).

Material and Methods: Study population: N=30 hypertensive patients, aged 38 to 77 years (60±11), 15 males, 15 females, 14 type 2 diabetics.

Anthropometric parameters: BMI (Kg/m²), waist circumference (cm). Biochemical parameters: Glycaemia, insulinaemia (u/mL) - Immuno-nomeric assay. Immulite. DPC, insulin resistance (IR)(HOMA).

HbA1c by HPLC. IGF-1 (ng/ml) 100 t kit. RIA: Nichols Institute Diagnostic.

Hemodynamic parameters: 24-hour ABPM (90207spacelabs); systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP).

Statistical analysis: Chi-square, linear regression.

Results: IGF-1 levels did not correlate with age, gender, type 2 diabetes mellitus, insulinaemia, IR, or HbA1c.

There was an excellent correlation between IGF-1 and average DBP (r=0.4; p=0.019).

Conclusions: The hypophysis-hepatic axis could contribute to blood pressure control.

This phenomenon is not related to insulin resistance.

Key Words: Growth Hormone, Hypertension, Insulin-Like Growth Factor

P-438
MITOGEN-ACTIVATED PROTEIN (MAP) KINASE SIGNALING THROUGH CALCINEURIN-DEPENDENT PATHWAYS IN CARDIAC FIBROBLASTS
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Calcium/calmodulin-calcineurin dependent signaling has been implicated in cardiac hypertrophy. Downstream targets underlying calcineurin-mediated cardiac actions are unclear, but may involve activation of mitogen-activated protein kinases (MAP) kinases. In the present study we questioned whether calcineurin regulates ERK1/2, p38MAPK and JNK, important signaling molecules contributing to cardiac growth, apoptosis, fibrosis and inflammation. Rat cardiac fibroblasts were studied. Cells were treated with ionomycin (10⁻⁵ mol/L) or Ang II (10⁻⁶- 10⁻⁸ mol/L) in the absence and presence of FK506 (10⁻⁵-10⁻⁶ mol/L) or cyclosporine A (10⁻⁵-10⁻⁶ mol/L) calcineurin...