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**Results.** Multiple silent cerebral infarcts were more common in the DM group than the non-DM group (50 vs 25%). Baseline ICA and MCA volume flow were comparable between the 2 groups, while CVR in ICAs (25 vs 35%, p=0.03) and MCAs (20 vs 31%, p=0.01) were lower in the DM group than the non-DM group. Cerebral N-acetylaspartate (NAA: an indicator of neuronal cell loss) was decreased in DM group than in non-DM group (8.35 vs 9.58 mmol/kg, p=0.007). These baseline CVR and NAA values of the DM group were lower than those of the normotensive group (CVR: 44% for ICA, 41% for MCA; NAA: 10.5 mmol/kg, all p<0.005). After candesartan therapy, CVR in ICAs and MCAs were significantly increased compared with the baseline (p=0.001), while cerebral NAA level did not change. The CVR increase in ICAs (15 vs 5.7%, p=0.03) and that in MCAs (20 vs 7.3%, p=0.01) were higher in the DM group than in the non-DM group.

**Conclusion.** In hypertensive patients, silent cerebrovascular disease with reduced CVR and neuronal mass occurs when accompanied by diabetes. ARB partly improved this impaired CVR, indicating that the renin-angiotensin-aldosterone system may play a role in regulating cerebrovascular microcirculation.

**Key Words:** Diabetic Hypertension, Cerebral Circulation, Angiotensin Receptor Blocker

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**P-333**

**ARGININE-AMINOPEPTIDASE IN RAT CARDIAC FIBROBLASTS PARTICIPATES IN ANGIOTENSIN TURNOVER**

The aim of this study was to elucidate the presence in rat cardiac fibroblasts of Arginine-aminopeptidase and its involvement in the hydrolysis of angiotensin peptides. Aminopeptidase activity was measured as hydrolysis of synthetic substrates, aryl-p-nitroanilides, and the appearance of peptides in concert with other peptidases present in cardiac fibroblasts. Some of these peptides could probably not be hydrolyzed by Arginine-aminopeptidase. Instead, they could be firstly hydrolyzed by another peptidase present in fibroblasts and the product of this hydrolysis could be a substrate for Arginine-aminopeptidase.

**Key Words:** Transforming Growth Factor-beta1, Cardiac Fibroblasts, Angiotensin Turnover

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**P-334**

**REVERSAL OF ANGIOTENSIN II-STIMULATED COLLAGEN GEL CONTRACTION IN CARDIAC FIBROBLASTS BY AMINOPEPTIDASE-INHIBITION**

The purpose of this investigation was to determine whether aminopeptidase (AP)-inhibition could affect the angiotensin II (Ang II)-stimulated collagen gel contraction in basal (control) and TGF-beta1-treated cardiac fibroblasts (or myofibroblasts). The tested AP-inhibitors were the broad range AP-inhibitor bestatin, the specific inhibitor of Alanine-AP leuhistin and the specific inhibitor of Arginine-AP arphamenine A. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluence and incubated with(out) 400 pmol/L TGF-beta1 in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% foetal bovine serum (FBS). These fibroblasts were further incubated in a floating collagen gel lattice with the tested products 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. Ang II (0.1 microM) reduced the gel volume in control and TGF-beta1-treated fibroblasts. The Ang II-stimulated collagen gel contraction in control and TGF-beta1-treated fibroblasts was completely reversed by leuhistin and arphamenine A (100 microM). Bestatin (100 microM) only partially inhibited the Ang II-stimulated collagen gel contraction in control fibroblasts while it did not affect the Ang II-induced contraction in TGF-beta1-treated fibroblasts. In control and TGF-beta1-treated fibroblasts, 100 microM leuhistin or arphamenine A only partially inhibited Alanine-AP activity, while bestatin (100 microM) completely inhibited the Alanine-AP activity. Anginine-AP activity was only partially inhibited by lehistin and arphamenine A at 100 microM in control and TGF-beta1-treated fibroblasts. Bestatin (100 microM), however, completely blocked the Arginine-AP activity in control fibroblasts and only partially in TGF-beta1-treated fibroblasts.

**Key Words:** Angiotensin II, Cardiac Fibroblasts, Aminopeptidases

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**P-335**

**COLLAGEN PRODUCTION IN CARDIAC FIBROBLASTS DURING INHIBITION OF ANGIOTENSIN CONVERTING ENZYME AND AMINOPEPTIDASES**

The objective of this study was to determine whether the angiotensin converting enzyme (ACE) inhibitor lisinopril and the aminopeptidase inhibitor with a broad specificity bestatin could affect the collagen production in basal and TGF-beta1-treated cardiac fibroblasts. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluence, incubated with(out) 600 pmol/L TGF-beta1 for 2 days in serum-free Dulbecco’s Modified Eagle’s Medium and further incubated with the tested products (lisinopril or bestatin) for 1 day in this serum-free medium with ascorbic acid, beta-aminopropionitrile and titrinated proline. Soluble collagen was measured in the conditioned medium and nonsoluble collagen in the cell layer. Lisinopril dose-dependently reduced ACE-activity in basal and TGF-beta1-treated cardiac fibroblasts. Bestatin inhibited the basal and TGF-beta1-stimulated aminopeptidase activity in a concentration dependent manner. Lisinopril (10 microM) decreased (p<0.05) the soluble collagen production from 882±160 (mean±SEM) to 658±142 dpm/ng DNA and the nonsoluble collagen production from 267±65 to 193±45 dpm/ng DNA in basal cardiac...
fibroblasts. TGF-β1 increased (p<0.05) the soluble and nonsoluble collagen production to 1346±129 and 639±54 dpnmg DNA, respectively. Lisinopril decreased the TGF-beta-stimulated soluble and nonsoluble collagen production to 1001±118 (p<0.05) and 461±46 (p<0.01) dpnmg DNA, respectively. Bestatin (100 microM) reduced the soluble collagen production in basal and TGF-beta-treated cardiac fibroblasts by 35±6% (p<0.05) and 15±1% (p<0.01) respectively, while it did not affect the nonsoluble collagen production in basal and TGF-beta-treated fibroblasts. Our data suggest that ACE and aminopeptidases are involved in the basal and TGF-beta-stimulated soluble collagen production in cardiac fibroblasts.

Key Words: ACE-Inhibition, Aminopeptidase-Inhibition, Cardiac Fibroblasts

P-336
EFFECT OF LISINOPRIL ON ANGIOTENSIN I- AND TRANSFORMING GROWTH FACTOR-BETA1-STIMULATED COLLAGEN PRODUCTION IN CARDIAC FIBROBLASTS

The aim of this study was to investigate whether the angiotensin converting enzyme inhibitor lisinopril could affect the angiotensin I (Ang I)-stimulated collagen production in cardiac fibroblasts. The effect of lisinopril was compared to that of the chymase inhibitor chymostatin, the calpain (serine protease) inhibitor E64-d and the dipeptidyl peptidase IV inhibitor diprotin A. Cardiac fibroblasts (from 6 normal male adult rats) were cultured to confluence, incubated with (0) 600 nmol/L TGF-beta1 for 2 days in serum-free Dulbecco’s Modified Eagle’s Medium and further incubated with the tested products (lisinopril, Ang I, chymostatin, diprotin A and E64-d) for 1 day in this serum-free medium with ascorbic acid, beta-aminoproprionitrile and tritiated proline. Soluble collagen was measured in the conditioned medium. Ang I (1 microM) stimulated (p<0.001) soluble collagen production in cardiac fibroblasts from 680±72 (mean±SEM) to 972±82 dpnmg DNA and lisinopril (10 microM) suppressed (p<0.05) it further to 811±74 dpnmg DNA Chymostatin, diprotin A and E64-d (100 microM) also reduced the basal and Ang I-stimulated collagen production by 26±3% (p<0.05) and 27±5% (p<0.01), 35±2% (p<0.01) and 40±5% (p<0.05), 21±2% (p<0.01) and 24±3% (p<0.01), respectively. TGF-beta1-stimulated (p<0.01) soluble collagen production from 684±59 to 1267±148 dpnmg DNA and Ang I plus TGF-beta1 increased soluble collagen production to 1354±93 dpnmg DNA compared to the basal values. Lisinopril reduced (p<0.01) Ang I-plus TGF-beta1-stimulated soluble collagen production to 1009±71 dpnmg DNA. Our data suggest that angiotensin converting enzyme together with other peptidases are involved in the basal and Ang I- and TGF-beta1-stimulated soluble collagen production in cardiac fibroblasts.

Key Words: Collagen Production, Angiotensin I, Transforming Growth Factor-beta1

P-337
CAPTOPRIL TEST IDENTIFIES SEVERITY OF RESIDUAL PRESSURE GRADIENT IN REOCARTATION

Blood pressure abnormalities after correction for aortic coarctation are very well described. How much these abnormalities are renin-dependent, and how much captopril test is important to identify recoarctation is the aim of our present study.

Twenty three young patients (6 female/17 male) with a mean age of 17 years old and blood pressure equal or lower than P95 were prospectively submitted to a captopril test as it is currently performed in our department. Arm-leg systolic blood pressure gradient was also measured at that time. Catheterization with aortic angiography, and visualization of renal arteries, was performed in those patients whose captopril test was positive.

In all the patients, except two, active renin rise significantly after captopril (P<0.001). From these, in 11/21 (52%) the test was positive but aortic angiography excluded renal artery stenos. Otherwise a linear correlation could be established between the multiple of renin rise and the arm-leg systolic pressure gradient for all patients (R²=0.485).

These results indicate that blood pressure abnormalities in post-coarctation patients might be related to residual pressure gradient. However, a renin-dependent mechanism is also important to stratify severity of the condition and could explain why hypertension is often a late complication of the aortic disease. Recoarctation can also be identified by means of the captopril test.

Key Words: Coarctation, Renin, Captopril

P-338
EFFECTS OF ADRENOMEDULLIN (AM) ON RENIN-ANGIOTENSIN-ALDOSTERONE (RAA) SYSTEM AND OXIDATIVE STRESS IN RATS WITH ACUTE MYOCARDIAL INFARCTION (MI)
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Previous studies have suggested that AM counteracts the systemic or local RAA system and oxidative stress in vitro and in vivo. AM is expressed in cardiac tissue, and plasma AM levels increase in patients with acute MI. This study was performed to examine the effects of AM on the RAA system and oxidative stress in rats with MI. Rats with MI induced by left coronary ligation were intraperitoneally infused with 1.0 µg/h of recombinant human AM or saline by osmotic mini-pump. Then, the MI rats were examined during and at the end of the AM infusion period for 7 days. Tissue angiotensin-converting enzyme (ACE) mRNA levels were measured by a Real-Time-quantitative PCR method, and urinary isoprostane, a marker of oxidative stress, by an enzyme immunoassay. When compared with the saline infusion in the control, the AM infusion had no effect on organ weights, body weight gain, and renal function (LV) end-diastolic pressure, plasma renin activity and aldosterone concentration, but significantly reduced ACE mRNA level in non-infarct LV (−51%, P<0.05) and urinary excretion of isoprostane (−61%, P<0.01).

To investigate the effects of these AM actions on long-term outcome of MI, another series of rats infused with AM for one week immediately after MI induction were sacrificed at 9 weeks. When compared with the control, the AM infusion significantly improved the survival (59/72 vs. 81%, P<0.05) and body weight gain (2.8±0.2 vs 3.7±0.1 g/day, mean±SEM, P<0.01), and reduced heart weight (4.7±0.2 vs 3.4±0.1 g/kg BW, P<0.01), lung weight (4.7±0.5 vs 4.8±0.5 g/kg BW, P<0.01), LV end-diastolic pressure (11.4±2.0 vs 4.0±0.6 mmHg, P<0.05), collagen volume fraction of non-infarct LV (−39%, P<0.05) and plasma level of endogenous rat AM (−38%, P<0.05), without affecting the infarct size. AM administration during the early period of MI reduced left ventricular ACE expression and oxidative stress in rats. These AM actions may...