arteriolar dilator). Cerebral metabolites in white matter were measured by proton MR spectroscopy. We also studied the 12 age- and gender-matched normotensive mice.

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 functional neuronal mass) 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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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. Peptidase activity was measured as hydrolysis of synthetic substrates, aryl-p-nitroanilides, and the appearance of fluorescein. Immunoblotting was performed with antibodies to aminopeptidase B. Arg-aminopeptidase found in cardiac fibroblasts was arginine- and lysine-specific, sensitive to various aminopeptidase inhibitors and to the inhibitor of metalloproteases, 1,10-phenanthroline. Experiments with arphamenine A, a specific inhibitor of Arginine-AP, have shown the presence of two Argaminopeptidase activities: arphamenine-sensitive: chloride-stimulated aminopeptidase and arphamenine-insensitive: chloride-insensitive aminopeptidase. TGF-beta1 (400 pmol/L) stimulatd both Arg-aminopeptidase activities by 3-fold. Immunoblot with an antibody specific to rat aminopeptidase B has revealed that arphamenine-sensitive: chloride-stimulated aminopeptidase is an aminopeptidase B. Arphamenine-p-nitroanilide hydrolysis was significantly inhibited by angiotensin peptides such as angiotensin (1–10), (1–8), (1–7), (1–4), (5–8), (4–8), (3–8) and (2–8) at a concentration of 50 micromol/L, which was 4-fold less than the Arginine-p-nitroanilide concentration. Our data suggest that chloride-insensitive Arginine-aminopeptidase could contribute to the hydrolysis of all studied angiotensin peptides in concert with other peptidases present in fibroblasts. Some of the 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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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. Arginine-AP activity was only partially inhibited by leuhistin 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. Our data suggest that both Alanine- and Arginine-AP are involved in the reversal of the Ang II-stimulated collagen gel contraction in control and TGF-beta1-treated cardiac fibroblasts or myofibroblasts.

Key Words: Angiotensin II, Cardiac Fibroblasts, Aminopeptidases

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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 5 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-aminoproprionitrile and titrated proteine. 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.