triphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate, causing intracellular calcium release. Studies from several different cell types have shown that AII induces tyrosine phosphorylation of multiple proteins including p44/p56LHC, the Jak family proteins Jak2 and Tyk2 and Focal Adhesion Kinase (FAK125) and Jun N-terminal Kinases (JNKs). Transcription factors implicated in proliferative signaling by AII include the STAT proteins and members of the Activator Protein -1 (AP-1 complex). Delayed early genes induced by AII in adrenal cortical cells involved in cellular proliferation and survival include the cyclin D1 gene product, a labile sensor of mitogenic stimulation which plays a critical role in proliferative signaling and cell-cycle control. In recent studies, AII induced expression of the human homologue of Xenopus XPMC2 (HX-PMC2), implicating its role in the AII proliferating/survival response. In order to understand the complex coordination of genomic response to AII we have used gridded cDNA microarray to identify differential expression profiles induced by AII in the H295R human adrenocortical cell line, which expresses AII receptors predominantly of the AT1 subclass. Of the 5,000 human genes interrogated, 1086 revealed transcriptional induction or repression profiles, 27% were ESTs, 5% novel sequences and 68% known genes. Of the known identified genes, 16% are transcriptional regulators and 7.5% are involved in cell cycle control and DNA synthesis. Correlation of expression profile patterns with functional roles demonstrates coordinated interactions between genes governing cellular bio-genesis, metabolism and intracellular transport. Identification of coordinated patterns of functionally related target genes provides a platform for understanding the mechanisms by which AII governs growth survival and cellular proliferation.

Key Words: cDNA Microarrays, Cyclin D1, Angiotensin II

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ANGIOTENSIN II STIMULATES NADPH-OXIDASE AND PHOSPHOLIPASE A2 ACTIVITIES IN PHAGOCYTIC CELLS. A POSSIBLE ROLE OF PYK2 AND MAP-KINASES, ERK AND P38, IN ANGIOTENSIN II-SIGNALING

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It has been suggested that the binding of angiotensin II to AT1 receptors activates a cascade of events that leads to stimulation of oxidative stress, the products of which possess remarkable vasoconstrictive qualities. The major enzyme-producing oxidative radical is the enzymatic complex NADPH-oxidase. The effect of angiotensin II on activation of NADPH-oxidase in monocyte and granulocyte from normotensive volunteers and VSMC. First, we characterized the 12LO protein in accordance with our previous studies demonstrating the essential requirement of cytosolic phospholipase A2 for activation of NADPH-oxidase. Potential upstream components of phospholipase A2 activation were investigated. Angiotensin II stimulated a dose and time-dependent activation of the cytosolic tyrosine kinase, Pyk2, and the MAP-kinases, ERK 1/2 and p38, in differentiated PLB-985 cells. Our results demonstrate the ability of angiotensin II to stimulate phagocytic cells and suggest a possible role of these cells in the pathology of hypertensive disease via the formation of inflammatory mediators.

Key Words: NADPH-oxidase, Phospholipase A2, Angiotensin II
corresponding to exon 4 region of the expressed 12LO gene. Third, upon Western analysis of protein extracts derived from vascular human placental artery smooth muscle cells (VSMC), bands of 74 (major), 62 (major), 110 (minor) and 34 kDa were visualized. Fourth, immunohistochemical analysis of these same cells in culture or within whole placental artery or cultured human mammary artery smooth muscle cells showed a clear cytoplasmic localization. Fifth, on electron microscopy using the affinity-purified exon 4, 12-LO antibody with immunogold, 12-LO was found primarily localized between the cytoplasmic muscle fibrils of VSMC, but not in the body of the nucleus, mitochondria and endoplasmic reticulum. Finally, upon angiotensin-II treatment of cultured VSMC, 12-LO was found by immunoprecipitation, to bind to α-actin, a component of the cytoplasmic muscle fibrils, which was blocked by pretreatment of with the 12-LO inhibitors, baiacilin or esculetine. Angiotensin-II also decreased the association of 15-LO (type 2) with α-actin, albeit with different kinetics. Moreover, the dissociation of 15-LO, but not the binding of 12-LO to α-actin coincided with the tyrosine phosphorylation of this protein. In summary, cytoplasmic juxtafibrillar LO proteins associate with α-actin in response to angiotensin-II which can be blocked by LO inhibitors. These observations suggest a previously unrecognized association between angiotensin-II dependent vasoconstriction, LO protein- α-actin interactions and fatty acid oxidation in human VSMC.

Key Words: Angiotensin-II, Lipooxygenase, Vascular Smooth Muscle Cells

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ANTIOXIDANT-INHIBITABLE ANGIOTENSIN II EFFECTS ON HUMAN VASCULAR ENDOTHELIAL CELL MIGRATION
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As is known, vascular smooth muscle cell migration is stimulated by angiotensin II. To evaluate the possible peptide effect on human vascular endothelial cell (HUVEC) migration, HUVECs were obtained from umbilical cord veins and cultured in Dulbecco’s HAT medium. After 4-5 passages HUVECs were seeded at high density in Boyden chambers modified by the insertion of a polyethylene terphthalate membrane (porosity = 8 μm). HUVEC migration was then quantified as the mean number of migrated cells observed in five high-power fields. In further experiments, “endothelial wound repair” was also assessed, i.e. “wounds” were inflicted by dragging a sterile pipette tip across cell monolayers and the number of cells migrating into the “wounded area” after various time up to 12 hours was quantified by light microscopy. Results; the addition of angiotensin II at various concentrations reduced HUVEC migration by at least 50%. Such effect was abolished by the AT1 receptor inhibitor losartan and the antioxidant N-acetyl-L-cysteine (NAC). In contrast, AT2 receptor inhibition was completely ineffective. Angiotensin II also reduced the so-called “wound repair”, i.e. markedly reduced HUVEC ability to migrate into wound areas and delayed wound closure. Also in this case, the angiotensin II effect was blocked by losartan and NAC but not AT2 receptor inhibition. In conclusion, angiotensin II strongly reduces HUVEC migration in vitro. This effect of angiotensin II is inhibited by losartan and NAC, suggesting oxidative mechanisms are activated by the peptide in the vascular endothelium and might markedly affect endothelial response to injuries.

Key Words: endothelial cell, angiotensin, AT1 receptor

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AUTONOMIC FUNCTION AND BAROREFLEX SENSITIVITY DURING ENALAPRIL OR LOSARTAN TREATMENT IN ESSENTIAL HYPERTENSIVE PATIENTS: A CROSS-OVER STUDY
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To investigate the potential role of angiotensin II and/or the bradykinin system on the autonomic function and baroreflex sensitivity, heart rate and blood pressure variability were evaluated in hypertensive patients before anti-hypertensive therapy and during enalapril or losartan treatment (cross-over study). Heart rate variability was assessed in a resting condition by power spectrum analysis to calculate the Low Frequency (LF) power, High Frequency (HF) power and LF/HF ratio in 19 hypertensive patients and 23 normotensive controls. Moreover, the coherence between the ta- chogram and the systickogram was evaluated, and the baroreflex gain (alpha-LF index) was obtained. Then a 24-h ambulatory blood pressure monitoring was performed. The 19 hypertensive patients were randomized to either enalapril or losartan treatment, and after 2 months were re-submitted to the RR variability and baroreflex study and to blood pressure monitoring. The subjects then crossed to the other anti-hyper- tension treatment and were re-evaluated after an additional two months. No significant difference was found either in LF power and HF power and LF/HF ratio between normotensive and hypertensive subjects whereas a slight though significant difference was observed in the alpha-LF index. In hypertensive patients, both the treatments with enalapril and losartan reduced blood pressure and had no effect on heart rate. No significant change was observed in autonomic balance or in baroreflex sensitivity during the two anti-hypertensive treatments.

Conclusions. In hypertensive patients, the angiotensin system or bradykinins do not seem to have any modulatory effect on the sympathetics/parasympathetic control of blood pressure in a resting condition. Moreover, since no change in heart rate was documented during the two anti-hypertensive treatments in presence of a reduction of blood pressure values, a resetting in baroreflex function was observed. However, baroreflex sensitivity was not influenced either by ACE-inhibition or AT1 receptor blockade. Key Words: Heart Rate Variability, Angiotensin II, Baroreflex sensitivity

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RAS POLYMORPHISMS AND ANGIOTENSIN PEPTIDES PLASMA LEVELS
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The aim of this study has been to analyse the influence of three polymorphisms of the Renin Angiotensin System (RAS), I/D from ACE, M235T from angiotensinogen and a1166c from AT1 receptor, on peptides plasma levels of Angiotensin I, II and 1-7 (AI, AII, A1-7). It was selected a homogeneous age population (m=20,67, SD=2,75) of 93 healthy subjects (43 men and 50 women). Peptides were separated by HPLC and quantified by RIA. Genotypes were determined by PCR and restriction enzyme analysis.

No differences on peptides plasma levels were found between the nine genotypes in the whole population. However when the subjects were distributed by sex it was observed a significant (p<0.01) higher level of A1-7 in men than women (37.76 vs 26.04 pg/mL) these differences were