repair. TGF-β1 may also play a key role in vascular remodeling process and myocardial hypertrophy through hypertension. Data exist that TGF-β1 and angiotensin regulate the expression of each other and TGF-β1 increases renin release from juxtaglomerular cells in the kidney. It has recently been shown that peripheral blood mononuclear cells from patients with essential hypertension produce more TGF-β1 than normotensive controls and a correlation of blood pressure and circulating levels of TGF-β1 was found in patients with hypertension due to end stage renal disease. We therefore evaluated if TGF-β1 levels are also elevated in patients with essential hypertension.

We studied 80 patients with essential hypertension, 30 normotensive patients without cardiovascular disease served as controls. A solid phase TGF-β1 sandwich ELISA (Quantikine human TGF-β1 ELISA, R&D- Systems) was used to determine TGF-β1 protein levels.

In patients with essential hypertension, levels of active TGF-β1 (58; range: 10-388 pg/mL vs. 21; 9-35 pg/mL, p<0.05) and total TGF-β1 (7211; 731-33012 pg/mL vs. 948; 72-2762 pg/mL, p<0.01) are significantly higher than in normotensive controls. Neither active TGF-β1 nor total TGF-β1 correlated with systolic or diastolic blood pressure levels (r< 0.14 for all parameters). Furthermore we could not show a correlation between TGF-β1 levels and indices of target organ damage, i.e. microalbuminuria (MAU), left ventricular hypertrophy (LVH), in essential hypertension (p = 0.372 for LVH and p = 0.765 for MAU).

In sum, our data show that levels of TGF-β1 are increased in essential hypertension. The mechanisms and consequences of elevated TGF-β1 deserve further investigations.

Key Words: Elevation, Transforming Growth Factor Beta, Essential Hypertension

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EFFECTS OF ANGIOTENSIN II AND INSULIN ON ERK 1/2 IN HUMAN SKIN FIBROBLASTS
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Several studies indicate that insulin-resistance frequently correlates with hypertension. In order to identify a common pathophysiological pathway between the metabolic disorders and the vascular dysfunction, we investigated the interactions between Ang II and insulin on extracellular signal-regulated kinases (ERK1/2) activation in skin fibroblasts from normotensive subjects (NT), from insulin-sensitive (IS) and insulin-resistant (IR) patients with essential hypertension (HT).

Skin fibroblasts from NT (n = 5), IS-HT (n = 4) and IR-HT (n = 3) were cultured and used after four passages. ERK 1/2 expression and phosphorylation were measured by Western Blot using specific antibodies, respectively anti-ERK 1/2 and anti-pERK 1/2.

ERK 1/2 were similarly expressed in skin fibroblasts in all the groups. Ang II (250 nM) induced a maximal increase in ERKs phosphorylation within 2 minutes (39% ± 6% for ERK1, 57% ± 10% for ERK2; p<0.01). The maximal effect on ERK 1/2 phosphorylation was obtained at 1μM Ang II, Erk1 being approximately 100% of the effect of Ang II on ERK1/2 phosphorylation was completely abolished by Losartan (1μM), an Ang II-type 1 receptor inhibitor, but not by PD123395 (1μM), an Ang II-type 2 receptor inhibitor. PD98059 (1μM), a MEK-inhibitor, reduced the Ang II-induced ERK 1/2 phosphorylation by approximately 50%. ERK 1/2 phosphorylation evoked by Ang II (250nM, 2 min) was significantly higher in fibroblasts from HT, than in fibroblasts from NT (ERK1: 41% ± 8% vs 33% ± 11%; ERK2: 64% ± 13% vs 34% ± 10%, p<0.05).

There was no difference on Ang II-induced ERK 1/2 activation in fibroblasts from IS-HT and IR-HT. Insulin concentration-dependently increased ERK1/2 phosphorylation. The effect of insulin (500pM, 10min) on ERK1/2 phosphorylation was not significantly different in NT and HT. Ang II (250nM, 2min) and Insulin (500pM,10min) had an additive effect on ERK 1/2 phosphorylation only in NT, which was absent in IS-HT and IR-HT. Our results suggest that ERK 1/2 hyperresponsiveness in fibroblasts from HT, elicitable by Ang II, may have a role in the pathogenesis of essential hypertension. The similar behaviour of ERK 1/2 phosphorylation after Ang II and insulin in IS-HT and IR-HT proves that insulin-resistance associated to hypertension does not affect the ERK1/2 cascade.

Key Words: ERK 1/2, Angiotensin II, Hypertension

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MULTIPLE ROLES FOR RHO KINASE IN CULTURED HUMAN VASCULAR SMOOTH MUSCLE CELLS
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Rho kinase (ROCK) is a direct downstream effector of the monomeric GTPase rho and has recently been shown to play an important role in neointimal formation in response to vessel injury, adding to the role in vessel contractility established earlier. This study, using human vascular smooth muscle cells (SMCs), examined the functional involvement of ROCK in the mitogenic and chemotactica processes which underlie wound healing and remodelling.

Cultured saphenous vein SMCs were derived from patients undergoing bypass surgery using an explant method. Mitogenesis, chemotaxis, cell morphology and the distribution pattern of F-actin were determined using labelled thymidine incorporation, blind-well migration assays, phase contrast microscopy and fluorescence microscopy of cells stained with FITC-labelled phallolidin respectively.

Treatment of cells with the specific ROCK inhibitor Y27632 (1μM-30μM) dose-dependently inhibited DNA synthesis in response to platelet-derived growth factor-BB (PDGF-BB, 10 ng/ml) and foetal calf serum (FCS, 15% vol/vol). DNA synthesis in response to PDGF-BB and FCS displayed a Y27632 insensitive component of 31±7% and 42±5% (mean±standard error of the mean, n=4 cell strains) of the untreated control values respectively. In contrast, peak stimulated chemotaxis in response to PDGF-BB (2 ng/ml) was completely abolished (n=4) at a concentration of 10μM Y27632. When cells were plated sparsely (1000/cm²) to allow full spreading, subsequent addition of Y27632 (10μM and 30μM) resulted in time-dependent alterations in cell morphology. The most prominent feature was the development of neuron-like protrusions, appearing over a timescale of 1-4 hours after addition of ROCK inhibitor. These protrusions were found to contain elongated actin filaments, indicating that ROCK inhibition could initiate actin polymerisation.

It is concluded that ROCK has multiple functional roles in human SMCs. DNA synthesis proceeds via ROCK dependent and independent pathways whereas chemotaxis is critically dependent on ROCK activity. Active ROCK plays an important role in the maintenance of normal cell morphology, at least in part by regulation of actin polymerisation and organisation. Inhibition of ROCK activity, leading to altered regulation of the actin cytoskeleton and loss of control of morphology could explain the Y27632 induced abolition of chemotaxis, which requires a co-ordinated series of morphological rearrangements to effect motility. Highly localised endogenous regulation of ROCK activity most likely plays an important part in the regulation of SMC chemotaxis.

Key Words: chemotaxis, mitogenesis, cell morphology

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EFFECT OF PERMISSIVE AND NON-PERMISSIVE TEMPERATURES ON RESPONSES OF TEMPERATURE SENSITIVE (TS) SV40 TRANSFORMED HUMAN VASCULAR SMOOTH MUSCLE CELLS
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In view of the limited availability of human vascular smooth muscle cells, we recently developed an immortalized cell line (SM1) by infection...