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PHENOTYPIC EFFECTS OF LOADING ON RHOA EXPRESSION IN RAT SKELETAL MUSCLE

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RhoA exerts signaling involved in the formation of focal adhesion complexes and is a potentially important mediator of muscle gene regulation during overload induced growth. The purpose of this study was to determine rat soleus and plantaris muscle RhoA expression with unloading induced atrophy and functional overload. 7-week old male Sprague-Dawley rats (N=28) were randomly assigned to Cage Control (Cg/Con, n=9), 3 days of Hindlimb Suspension (HS, n=4), 3 days of Hindlimb Suspension with 1 day of re-loading (HS+1, n=5), and functional overload (ABL, n=5) or sham control (Con, n=5) groups. At the appropriate time animals were anesthetized and the plantaris (PLAN) and soleus (SOL) muscles were excised and frozen for analysis of RhoA expression. Expression of RhoA in the postural SOL muscle did not change from Cg/Con (1.00±.14) with HS (1.13±.15) or HS+1 (1.11±.14). RhoA expression tended to increase from Con (1.00±.30) after 3 days with ABL (3.74±1.1) in the SOL. Plantaris skeletal muscle RhoA expression significantly decreased (46%) from Cg/Con (1.00±.09) with HS (.54±.06), but significantly increased (150%) in the HS+1 (1.50±.04) group. PLAN RhoA expression also significantly increased at 3 days with ABL (2.15±.21) from Con (1.00±.13). Unaltered expression of RhoA in the SOL muscle with either unloading induced atrophy or the re-loading stimuli suggests a phenotypically distinct response of the postural slow twitch muscle.

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ACTIVATION OF NF-κB IN CARDIAC HYPERTROPHY INDUCED BY PRESSURE OVERLOAD IN RAT HEART.

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We have previously shown that pressure overload will induce myocardial NFκB activation. We postulated that NFκB activation might be involved in cardiac hypertrophy. To test our hypothesis, rat aortic banding was performed to induce cardiac hypertrophy. Control rats underwent a sham operation. Four weeks after aortic banding, the hearts were harvested and weighed. The nuclear and cytoplasmic proteins were isolated from the left ventricle. NFκB binding activity was determined by EMSA. The activities of IKKβ, NIK, and MEKK1 were determined by immunoprecipitation followed by kinase assay with appropriate substrates. Aortic banding significantly increased the ratio of heart/body weight (0.414 ± 0.06/100g body weight, n=8) compared to sham control (0.293 ± 0.057/100g body weight, n=6) and normal rat heart (0.304 ± 0.057/100g body weight, n=6). The levels of nuclear NFκB binding activity was very low in normal and sham controls but significantly increased in the aortic banding group (2.57 fold) compared to that of control. Aortic banding also increased the activities of IKKβ (2.01 fold), NIK (1.76 fold), and MEKK1 (1.69 fold) compared to that of control. The results suggest that activation of the NFκB pathway in pressure overload induced cardiac hypertrophy, may be a novel signaling pathway that may play an important role in the transition of cardiac hypertrophy to heart failure. This work is supported in part by AHA grant 005148B.

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Phosphoinositide 3-kinase activity leads to NF-κB activation through interacting with tyrosine-phosphorylated IκB-α and contributing to tyrosine phosphorylation of p65 NF-κB

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The role and mechanisms of PI3-kinase activity in NF-κB activation in silica-stimulated RAW 264.7 cells were investigated. Results indicate that the p85α subunit of PI3-kinase interacted with tyrosine-phosphorylated IκB-α in silica-stimulated cells. PI3-kinase specific inhibitors, such as wortmannin and LY294003, substantially blocked both silica-induced PI3-

kinase and NF-κB activation. The inhibition of NF-κB activation by PI3-kinase inhibitors was also observed in pervanadate-stimulated but not in LPS-stimulated cells. Furthermore, tyrosine phosphorylation of NF-κB p65 was enhanced in cells stimulated with silica, pervanadate, or LPS, and wortmannin substantially inhibited the phosphorylation event induced by these stimulants excluding LPS. Antioxidants, such as SOD, NAC and PDTC, blocked silica-induced PI3-kinase activation, suggesting that reactive oxygen species may be important regulatory molecules in NF-κB activation by mediating PI3-kinase activation. Our data suggest that p85 and p110 subunits of PI3-kinase play a role in NF-κB activation through interaction with IκB-α and contributing to tyrosine phosphorylation of p65 NF-κB.

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TNF-α-Induced IL-6 Release by Human Urothelial Cells is Mediated by NF-κB and MAP Kinase Signaling

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We examined whether TNF-α stimulated the release of IL-6 by urothelial cells and whether MAP kinase and NF-κB signaling pathways were involved. Human urothelial cells immortalized by human papillomavirus 16 E6 (E6 HUC) were seeded in 12 well culture plate and grown to 60-70 % confluence. IL-6 in media was measured by ELISA. Activation of MAP kinase and NF-κB were evaluated by immunoblotting (IB). Results: At 6 h, unstimulated release of IL-6 was 39.8 ± 5.8 pg/ml. The release was 61.9 ± 5.6, 96.8 ± 4.3 and 130.4 ± 3.9 pg/ml, in the presence of 1, 3 and 10 ng/ml TNF-α, respectively (n = 6). IB analysis revealed increased phosphorylation of ERK and degradation of IκB-α, an inhibitory subunit of NFκB, in response to TNF-α. PD 98059, a specific blocker of the ERK pathway, inhibited TNF-α-induced increased IL-6 release. An agonistic antibody against TNF-α receptor I (TNFRI) also increased release of IL-6. TNF-α treatment had no effect on cell proliferation. In conclusion, TNF-α increases release of IL-6 in a concentration-dependent manner, by activating TNFRI and both MAP kinase (ERK) and NFκB signaling pathways are involved (supported by NIH RO1 DK 49471).

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UVA Radiation Modulates Nuclear Factor kappa B (NFκB) Activation in Human Lens Epithelial Cells

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Repeated exposure to ultraviolet radiation contributes to cataractogenesis via mechanisms that include free radical production and aberrant gene expression within lens cells of the eye. NFκB is a transcription factor known to be regulated by oxidative stress, but its role in lens cell biology has not been thoroughly explored. We investigated the effects of UV radiation on NFκB activity in a human lens epithelial cell line, designated HLE SRA 01/04. HLE cells were exposed to UVA (900 J/m²), UVB (400 J/m²), UVC (150 J/m²) radiation or control for 30 min. Gel shift analysis of HLE nuclear proteins indicate that UVA activated NFκB to a much greater extent than UVB or UVC. Exposure of HLE cells to UVA radiation (0-900 J/m²) followed by a 30 min incubation results in a dose-dependent activation of NFκB. However, UVA irradiation above 1200 J/m² results in NFκB inhibition. UVA-induced NFκB activation in HLE cells was evident 10 min post-irradiation, and maximal at 60 min. Western blot analysis of IκB phosphorylation revealed that UVA activates NFκB via a mechanism involving the phosphorylation of IκB-α. Our results indicate that UVA activates NFκB in a time and dose-dependent manner via signaling through IκB-α.

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NF-κB Signals is Downregulated in Ground-based Microgravity Cultured Lymphocytes (Jurkat)

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Human lymphocytes flown on the Space Shuttle respond poorly to mitogen stimulation and populations of the lymphoblastoid T cell line, Jurkat, manifest growth arrest, increase in apoptosis and time- and microgravity-dependent increases in the soluble form of the cell death factor, Fas/APO-1 (sFas). The underlying mechanism has not been investigated yet. Previous evidence indicates that activation of the transcription factor NF-κB is a

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ABSTRACTS
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