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EXPRESSION OF KCl COTRANSPORTER (KCC3) IN THE CENTRAL NERVOUS SYSTEM: CONCURRENCE WITH MYELINATION

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Northern blot analysis of multiple mouse tissues revealed high expression of KCC3 in the brain. We investigated the cellular localization of KCC3 in the central nervous system using a purified polyclonal antibody directed against a 20 residue N-terminal peptide. The antibody was first tested by Western blot analysis using 60 μg crude membrane protein isolated from total brain. The antibody recognized a protein doublet of 150-155 kDa, consistent with KCC3 being a glycoprotein. Western blot analysis of dissected regions of the brain revealed abundant expression in structures containing white matter tracts, such as corona radiata and spinal cord, but extremely low in regions devoid of white matter, i.e superficial cortex, hippocampus. A similar pattern was observed when probing the membrane with a monoclonal antibody to CNPase (oligodendrocyte marker). We then examined KCC3 expression during postnatal development and observed low expression at birth and increasing levels to adulthood. These results are consistent with KCC3 being expressed in maturing oligodendrocytes. Indirect immunofluorescence with anti-KCC3 rabbit polyclonal antibody and a panel of mouse monoclonal antibodies revealed expression of the cotransporter in both cell bodies and processes of oligodendrocytes, as demonstrated by co-staining with anti-CNPase and anti-MBP. No KCC3 signal was detected in GFAP positive cells (astrocytes) nor MAP2 positive cells (neurons) with the exception of Purkinje cells in the cerebellum. The cotransporter also labeled the basolateral membrane of choroid plexus epithelium. Supported by NIH NS36758.

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THE NEUROENDOCRINE EFFECT OF DEHYDROEPIANDROSTERONE (DHEA) ON ZUCKER RATS SELECTED FOR FAT FOOD PREFERENCE

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When allowed a choice, Zucker rats consume a higher proportion of fat than is usually provided in their normal diet of rat chow. Over the short term, using a single meal paradigm, the percentage of fat calories ranges from 20 to 80% of caloric intake. When either lean or obese male Zucker rats are treated with intraperitoneal injection of DHEA, they consume fewer calories. However, the magnitude of this effect depends upon their initial preference for fat; the higher their fat preference, the greater the decrease of caloric intake and the more pronounced the effect is on fat consumption in particular. This effect is demonstrable with doses as low as 25-50 mg DHEA/Kg of body weight. Administration of DHEA 2 hours before decapitation leads to changes in the content of monoamine neurotransmitters in select regions of the hypothalamus known to control food intake; norepinephrine, epinephrine and dopamine are lowered in DHEA-treated animals. It is hypothesized that exogenous DHEA causes the acute release of norepinephrine, epinephrine and dopamine in select regions of the hypothalamus and this release causes a decrease in food intake, particulary fat macronutrient intake. Supported by the American Hea

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LUNG SURFACTANT DECREASES IL-1β AND TNF-α PRODUCTION AT THE POST-TRANSCRIPTIONAL LEVEL IN LPS-STIMULATED ALVEOLAR MACROPHAGES . K.M.K. Rao, T. Meighan and L. Bowman. PPRB/HELD/NIOSH, Morgantown, WV 26505.

We have shown previously that lung surfactant inhibits nitric oxide production at the post-transcriptional level in lipopolysaccharide(LPS)-stimulated rat alveolar macrophages (Miles et al., Am.J.Physiol. 276:L186, 1999). In this study, we examined the effect of lung surfactant (200 μg phospholipid/ml), isolated from rat lung lavage, on the production of two pro-inflammatory cytokines, IL-1β and TNF-α, by LPS-stimulated rat alveolar macrophages. After 22 h incubation with LPS plus lung surfactant, IL-1β and TNF-α production was reduced by 77% and 95%, respectively. mRNA levels were measured by Northern blot analysis, performed 4 hours after LPS-stimulation using digoxigenin-labeled probes. There was no difference in IL-1β or TNF-α mRNA levels between cells stimulated with LPS with or without lung surfactant. Nitric oxide, IL-1β and TNF-α are known to be regulated at both the transcriptional and post-transcriptional levels. It is interesting that lung surfactant inhibits the production of all three pro-inflammatory molecules at the post-transcriptional level. This suggests that the post-transcriptional regulation of these three molecules may share a similar mechanism.

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SIGNAL RECOGNITION PARTICLE (SRP) ANTIBODY (AB) AND HEPATITIS C VIRUS (HCV) INFECTION IN POLYMYOSITIS (PM). K. Ravakhah. Huron Hospital, Cleveland, Ohio 44112

Introduction: SRP antibody, an autoantibody to 54-K Da protein of the SRP is present in up to 4% of PM patients. HCV infection is associated with different rheumatic/immunologic disorders including PM. A case of PM with anti-SRP and HCV infection is reported. Case Report: A 50 y/o AA woman with a history of iatrogenic hypothyroidism developed Raynaud's phenomenon. It was rapidly followed by generalized weakness and weight loss. She had tachycardia and muscle wasting. Lab Results: AST 320/u/l, ALT 416 u/l, ESR 31 mm/hr, Hbs Ag (-), Hbs Ab (-), HA Ab (-), HC Ab (+), HC PCR (+), HLA DRW6/DRW52. Creatinine Kinase and aldolase were both elevated. A deltoid biopsy showed widespread muscle fiber degeneration and regeneration with foci of endomysial inflammation. Electromyogram - Nerve conduction velocity were consistent with necrotizing PM. Anti SRP (+), other PM specific and associated Ab (-). She was treated with prednisone and methotrexate. After a prolonged and complicated hospitalization she was discharged home wheelchair bound. Discussion: HCV infection has been associated with autoantibody production. Several autoimmune/rheumatic diseases have been reported in HCV infected patients. The association of anti-SRP PM and HCV infection in certain patient populations (HLA, race) is suggested.

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BLOOD PRESSURE RESPONSES TO NITRIC OXIDE INHIBITION IN SODIUM REPLETE AND DEPLETE RATS. G.P. Reams, O. Villarreal, R.H. Freeman. Univ. of Missouri and HS Truman VAH, Columbia, MO 65212

Acute inhibition of Nitric Oxide (NO) induces a marked increase in mean arterial pressure (MAP) of normotensive rats. However, the effects of NO inhibition on MAP in normotensive compared to hypertensive animals on a low sodium diet with an activated renin-angiotensin system are unclear. Normotensive Sprague-Dawley (SD) rats and Spontaneously Hypertensive (SHR) rats (n=9 for each group) were assigned to either a normal sodium (0.28%) or a sodium deficient (0.02-0.03%) diet for two weeks. For the acute experiment, rats were anesthetized, fitted with catheters, and L-NAME (185μmol/kg I.V. Bolus) was administered. MAP (mmHg) at baseline and peak response post L-NAME are shown:

	SD		SHR	
	Baseline	L-NAME	Baseline	L-NAME
Low Na^+	144±3	190±2*	165± 6	220±6*
Normal Na^+	148±2	173±2*	190± 4	223±3*

*p<0.05 vs. baseline; +p<0.05 Low Na^+ vs. Normal, Na^+

NO inhibition increased MAP in all groups. However, the absolute increase in MAP was two-fold greater (p<0.05) in the sodium deplete SD compared to the sodium replete SD. This differential response was not observed in the SHR. These data suggest an important interaction of NO-synthesis and dietary sodium for blood pressure regulation in the normotensive, but not the spontaneously hypertensive rat.

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KININ B_1 RECEPTOR ACTIVATION STIMULATES TYPE I COLLAGEN SYNTHESIS *VIA* CTGF mRNA STABILIZATION. DA Ricupero, JR Romero*, DC Rishikof and RH Goldstein (SPON: ME FABRY). Boston University School of Medicine and *Harvard Medical School, Boston, MA 02118.

The kinin B_1 receptor is up-regulated in tissue injury. However, its role in postinflammatory fibrosis is unknown. We have shown that IMR-90 fibroblasts express kinin B_1 and B_2 receptors. We now report that B_1 receptor stimulation by des-arg^{10}-kallidin produced a rise in cytosolic Ca^{2+} that was dose dependent (EC_{50}=1.9nM) and blocked by the antagonist, des-arg^{10}-leu^9-kallidin. This antagonist did not interfere with a B_2 receptor-mediated rise in cytosolic Ca^{2+}. In addition, a B_2 receptor antagonist, HOE-140, did not alter the Ca^{2+} transient induced by des-arg^{10}-kallidin. Our data also show that activation of the B_1 receptor by des-arg^{10}-kallidin caused stabilization of connective tissue growth factor (CTGF) mRNA. In addition, a rise of α1(I) collagen mRNA and an increase in type I collagen synthesis were demonstrated. These events were not observed in B_2 receptor-activated cells. The B_1-mediated increase in α1(I) collagen mRNA was time- and dose-dependent with a peak response observed at 20 hours with 100nM des-arg^{10}-kallidin. The increase of CTGF mRNA was also time- and dose-dependent with a peak response observed at 6 hours with 100nM des-arg^{10}-kallidin. The increased CTGF mRNA was blocked by incubation with a B_1 receptor antagonist. The transcriptional inhibitor, actinomycin D, did not block the des-arg^{10}-kallidin-induced increase in CTGF mRNA indicating that des-arg^{10}-kallidin induces stabilization of CTGF mRNA. These results suggest that engagement of the B_1 receptor during inflammatory events increases CTGF expression and contributes to fibrogenesis.