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LIPOPOLYSACCHARIDE (LPS)-INDUCED ALTERATIONS IN AIRWAY SMOOTH MUSCLE REACTIVITY TO EPITHELIUM-DERIVED RELAXING FACTOR (EpDRF) ARE OSMOLYTE-DEPENDENT. R.A. Johnston and J.S. Fedan. Dept. of Pharmacol. and Toxicol., West Virginia University, Morgantown, WV 26506 and PPRB, Health Effects Lab. Div., NIOSH, Morgantown, WV 26505.

Contractile and relaxant responses of guinea-pig tracheal smooth muscle can be measured *in vitro* using the isolated, perfused trachea (IPT) preparation, which allows agents to be added separately to the serosal (extraluminal; EL) or mucosal (intraluminal; IL) surfaces. In response to hyperosmolarity at the EL or IL surfaces, the epithelium releases EpDRF which relaxes the airway smooth muscle. Previously, we have shown in IPT precontracted with EL methacholine (3×10^{-7} M) that LPS (4 mg/kg, i.p.; 18 h) potentiates EpDRF-mediated smooth muscle relaxation in response to elevation of IL osmolarity with NaCl. The purpose of this study was to determine if potentiation of smooth muscle relaxation by EpDRF following LPS-treatment is dependent on the osmolyte used. When KCl and urea were used, there were no differences in maximum relaxation responses between control- and LPS-treated groups; however, the EC_{50} for urea-induced relaxation was increased after LPS-treatment. Relaxation responses to mannitol and NaCl were potentiated after LPS treatment, whereas the EC_{50}'s were not changed. These results suggest that the effect of LPS-treatment on the release and/or effects of EpDRF are dependent on the osmolyte used to elevate IL tonicity.

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Quantitation of Tracer Levels of Deuterium Enrichment in all Positions of Human Plasma Glucose by ^2H NMR

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When functioning hepatocytes are presented with ^2H_2O they generate glucose enriched with ^2H at multiple positions. The enrichment distribution is sensitive to the source of glucose (glycogen, glycerol or phosphoenolpyruvate). Hence its measurement provides a valuable insight into hepatic glucose metabolism. Since a tracer amount of ^2H_2O is safe, inexpensive and easily ingested, this measurement can be applied in humans provided that a reasonable amount of plasma glucose can be sampled (i.e. 10-20 ml of whole blood). Current GC-MS methods have the necessary sensitivity but do not provide a complete description of the glucose enrichment pattern. We therefore applied a new NMR method based on the monoacetone derivative of glucose (MAG). The ^2H NMR spectrum of MAG has fully resolved resonances allowing ^2H enrichment in all positions to be measured. Conversion of plasma glucose to MAG is simple and quantitative and the NMR analysis is sufficiently sensitive for quantitating enrichment levels of 0.1-0.5% from 5-10 mg of glucose (obtainable from 10-20 ml whole blood). We provided 5 overnight-fasted healthy subjects with tracer levels of ^2H_2O corresponding to 0.5% body water enrichment and 5-6 hours later withdrew 20 ml of blood for analysis. Enrichments relative to hydrogen 2 (H2) of glucose (set at 100%) and the standard errors were as follows: H1=48±5; H3=20±1; H4=42±6; H5=48±2; H6(R)=42±3 and H6(S)=42±3. The H5 and H6 relative enrichments are consistent with recent GC-MS measurements of ^2H enrichment in glucose from overnight-fasted individuals given ^2H_2O.

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NITRIC OXIDE RELEASE DURING HOECHST 33342-INDUCED APOPTOSIS. FL Kiechle, V Brovkovych, X Zhang and T Malinski. William Beaumont Hospital, Royal Oak, MI 48073 and Oakland University, Rochester, MI 48309

Hoechst 33342 (H342) induces apoptosis in BC3H-1 myocytes after a 3-hr latent period. To measure the release of nitric oxide (NO) from BC3H-1 myocytes in the presence of H342, the tip of a porphyrinic microsensor was placed on the cell surface. Maximal NO release occurred at 15-25 μg/mL H342 after 15 min with maximum NO concentration of 0.2 ± 0.01 μmol/L compared to 0.06 ± 0.03 μmol/L NO released in the presence of calcium ionophore A23187 [10 μmol/L, maximum constitutive nitric oxide synthase (cNOS) stimulating dose]. To determine if NO release was attributable to activation of both cNOS and inducible NOS (iNOS), the cells were pretreated with L-N^G-arginine methyl acid (L-NMA, 0.3 mmol/L, a cNOS inhibitor) or L-N-(1-iminoethyl)-lysine (Iso-lys, 50 μM, an iNOS inhibitor). Iso-lys significantly reduced H342-stimulated NO release by 90% while L-NMA had little inhibitory effect. However, pretreatment, of BC3H-1 myocytes with 50, 150 or 450 μM Iso-lys did not prevent H342-induced cell death by apoptosis. We conclude that H342-induced release of NO in BC3H-1 myocytes occurs mainly through activation of iNOS. However, the maximal release of NO is not required to initiate H342-induced apoptosis suggesting that a relatively small increase in NO is required to initiate the apoptotic pathway in BC3H-1 myocytes.

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A soluble PDGF receptor inhibits fibroblast proliferation

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Platelet-derived growth factor exerts powerful effects upon mesenchymal cells, causing synthesis, directed migration and proliferation. Excessive mesenchymal cell acccumulation within organs can lead to fibroproliferative disorders. Among these is obliterative bronchiolitis (OB), a fibroproliferative disorder occurring after lung transplantation, characterized by accumulation of mesenchymal cells within small airways. In this study, we created a mutant soluble form of the PDGF b receptor and tested its effects on fibroblast viability and proliferation. Methods: A mutant receptor consisting only of the extracellular binding domain was cloned from the full length cDNA by site directed mutagenesis. After stable transfection into CHO cells, conditioned medium (CM) was harvested and purified by wheat germ agglutination and immunoaffinity chromatography. The purified CM was tested for its effects on NIH3T3 cells and fibroblasts isolated from an animal model of OB. Results: Addition of the soluble PDGF receptor diminished NIH3T3 cell replication in full serum by 40%. For OB airway fibroblasts, the PDGF soluble receptor diminished cell growth by 18% in full serum and 58% in 1% serum. Conclusions: A soluble PDGF receptor can abrogate fibroblast proliferation. Mutant receptors may lead to novel therapies for fibroproliferative disorders.

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GLUCOCORTICOIDS ALTER UT-A UREA TRANSPORTER PROTEIN ABUNDANCES IN THE KIDNEY. J.D. Klein, P. Rouillard, J.L. Bailey and J.M. Sands. Renal Division, Emory University, Atlanta, GA 30322.

Glucocorticoids (GC) play a key role in protein degradation *in vivo*. To study the role of GC on UT-A proteins, we adrenalectomized (ADX) rats and gave them 0.9% saline to drink for 10 days; some rats received a physiologic replacement dose (5ng/100g bw, SC, bid) of dexamethasone (DEX) for the last 7 days. The kidneys were divided into inner medullary (IM) tip, IM base, outer medulla (OM), and cortex. Tissue lysates were probed for UT-A1, UT-A2, and UT-A4 proteins by western blot. ADX increased the 97 and 117 kDa UT-A1 proteins in IM tip by 25% and 36%, respectively. These increases were abolished by DEX replacement. In contrast, DEX-replaced ADX rats had a 167% increase in a 56 kDa UT-A protein, most likely UT-A4, in the IM tip. DEX replacement was also increased this 56 kDa protein in IM base and OM by 77-187%. In cortex, DEX increased a 51 kDa UT-A protein by 58%. Rats fed a low protein diet (LPD) for 14 days had a 4.4 fold increase in GC levels compared to rats fed a normal protein diet, and a comparable increase in UT-A1 protein abundance in the IM tip. There was a small, but not statistically significant, increase in UT-A4 protein in the IM tip of LPD rats, and no change in IM base or OM. In cortex, the 51 kDa UT-A protein was increased 170% in LPD. We conclude that GC regulate UT-A abundance in an isoform specific manner and contribute, in part, to the mechanism(s) by which a low-protein diet alters UT-A protein abundance. (Supported by NIIDDK)

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DIFFERENTIAL RESPONSE OF UT-A GLYCOPROTEINS TO ACIDOSIS. J.D. Klein, P. Rouillard, R. Duchesne, and J.M. Sands. Renal Division, Emory University, Atlanta, GA 30322.

We showed that uremia up-regulates UT-A protein abundance in liver (JASN '99). Since uremic rats are also acidotic, we tested the effect of acidosis on UT-A1 proteins in kidney. Rats were made acidotic by adding HCl to their food for 7-days; control (Ctrl) rats had NaCl added. HCl feeding reduced blood pH from 7.29 ± 0.01(Ctrl) to 7.16 ± 0.02 (HCl); n=8, p<0.01. Acidosis increased the abundance of the 117 and 97 kDa UT-A1 proteins by 169 and 68%, respectively, in the inner medullary (IM) tip. In the IM base, the 97 kDa isoform was increased 75%. The acidotic animals also exhibited a 144% increase in urine volume and a decrease in urine osmolarity. Therefore, we limited the acidotic rats' water intake by pairing them to Ctrl rats, with HCl-fed rats determining food intake and Ctrl rats determining H_2O intake. HCl rats with restricted H_2O intake showed a urine volume and osmolarity comparable to Ctrl rats. Both the 97 and 117 UT-A1 proteins were decreased relative to the acidotic rats receiving H_2O *ad libitum*. However, while the 97 UT-A1 isoform was reduced to near Ctrl levels, the 117 kDa glycoprotein remained 114% elevated relative to Ctrl rats (n=4, p<0.05). We conclude that acidosis up-regulates the abundance of the 117 kDa UT-A1 protein. The different response to acidosis of the two UT-A1 glycoproteins suggests that they may be independently regulated or have differing functions. (Support: NIDDK).