(HPC) injury after exposure of rats to bacterial lipopolysaccharide and can magnify injury during chemical hepatotoxicity. *In vitro*, proteases, such as elastase and cathepsin G, released from activated PMNs damage HPCs. These proteases activate signal transduction pathways in other cell types. Therefore, the hypothesis was tested that killing of HPCs by PMNs requires activation of signal transduction pathways in HPCs. To this end, killing of primary rat HPCs by medium isolated from activated PMNs (PMN conditioned medium) was evaluated in the presence of inhibitors of various signaling pathways. The nonspecific serine/threonine kinase inhibitors, H7 and HA-1004, completely prevented killing of hepatocytes by PMN conditioned medium; whereas, selective inhibitors of protein kinase C, A, and G did not. In addition, genistein, a nonspecific tyrosine kinase inhibitor, prevented killing of hepatocytes by PMN conditioned medium. Finally, incubation of HPCs with PMN conditioned medium caused production of reactive oxygen species (ROS) in HPCs, and desferrioxamine, an iron chelator, completely prevented killing of HPCs by PMN conditioned medium. These results suggest that reactive oxygen species and activation of serine/threonine and tyrosine kinases in HPCs are important for their killing by activated PMNs. (Supported by NIH grants ES04139)

973 NITRIC OXIDE-MEDIATED SUPPRESSION OF FLAVIN-CONTAINING MONOOXYGENASE (FMO) ACTIVITIES IN CULTURED PRIMARY RAT HEPATOCYTES BY DESTABILIZING THE MRNA AND S-NITROSYLATION OF FMO1.

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Objectives: The present work is to investigate the effect of nitric oxide (NO)-donors treated exogenously on the metabolic activities of flavin-containing monooxygenase in cultured primary rat hepatocytes. Background: Overproduction of NO by inducible NO synthase (iNOS) is a common phenomenon in inflammation. At this time, the NO has been implicated as the mediator of decreased catalytic activity and expression of drug-metabolizing enzymes such as cytochrome P450s (CYPs) and flavin-containing monooxygenases (FMOs). Previous work in our laboratory suggests that FMO activities in rat liver treated with LPS are decreased by down-regulation of FMO1 mRNA expression *in vivo* (Park et al., 1999, Mol Pharmacol). Methods: Rat hepatocytes were isolated from rat livers perfused with collagenase. The cells were then treated for 4 h with NO-donors like SNAP or SIN-1. The FMO1 mRNA expression was compared after pretreatment of actinomycin-D to examine the effect of NO on the mRNA stability. The metabolic FMO activities were determined by ranitidine N-oxidation and thiobenzamide S-oxidation estimated with HPLC and spectrophotometer, respectively, in the absence or presence of DTT, a sulfhydryl-reducing agent. Results: FMO activities determined in hepatocytes treated with NO-donors were significantly suppressed to 20-40% of those in untreated control hepatocytes. However, the expression of FMO1 mRNA was not decreased or slightly decreased. Interestingly, stability of FMO1 mRNA after pretreated with actinomycin-D was decreased significantly by exposure to NO donors. Furthermore, the inhibition of *ex vivo* and *in vitro* FMO activities estimated with microsomes exposured to NO donors was reversed completely by addition of DTT. Conclusions: These results suggest that FMO activities in hepatocytes can be decreased both by enhanced unstability of FMO1 mRNA leading to decreased FMO1 expression and by reversible S-nitrosylation of the existing FMO1.

974 INVOLVEMENT OF PHOSPHATIDYLINOSITOL 3-KINASE IN HEPATIC STELLATE CELL ACTIVATION AND ANTIOXIDANT RESPONSE ELEMENT-REGULATED GENE INDUCTION.

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Liver fibrogenesis is dependant upon transdifferentiation of hepatic stellate cells (HSC) to a profibrogenic myofibroblastic phenotype. Electrophilic intermediates generated during prooxidant-induced liver injury, including 4-hydroxynonenal (4-HNE), purportedly modulate HSC gene expression and possibly HSC transdifferentiation. The extent to which 4HNE modulates HSC gene expression remains unclear. The objective of this study is to characterize the response of HSC to electrophiles, and the role of 4HNE in profibrogenic HSC activation. To this end, quiescent HSC were treated with 4HNE or tert-butylhydroquinone (tBHQ), both of which are known inducers of antioxidant response element (ARE)-regulated genes. Electrophilic agents elicit ARE-mediated gene induction through release of the cis-acting transcription factor Nrf2 from its cytoplasmic anchor protein (Keap1), allowing Nrf2 to translocate into the nucleus and participate in ARE-regulated gene induction. These studies demonstrate that both 4HNE and tBHQ induce nuclear translocation of Nrf2 and expression of GSTP1, a gene that is solely regulated by the ARE. This observation is further supported by Nrf2-mediated induction of luciferase in cultured HSC. In contrast to ARE induction, tBHQ but not 4HNE promotes myofibroblastic transdifferentiation of HSC. Pretreatment of cultured HSC with a phosphatidylinositol 3-kinase (PI3K) inhibitor blocked tBHQ-mediated ARE-dependant gene induction and HSC activation, but has no effect on HNE-mediated gene induction. These data implicate involvement of the mitogen-activated protein kinase (MAP kinase) pathway in tBHQ-mediated HSC activation and ARE gene regulation; while the trigger by which 4HNE mediates ARE-dependant gene induction is PI3K independent. We hypothesize 4HNE-mediated ARE activation may involve direct interactions between 4HNE with any of the 25 cysteine residues that compose Keap1. (This work is supported by NIH/AA05578-03 and NIH/AA09300.)

975 THE ROLE OF THE ALPHA$_1$ ADRENERGIC RECEPTOR IN THE RESTRAINT-INDUCED PHOSPHORYLATION OF STAT3.

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Stress causes physiological changes in most organ systems in intact organisms. The Janus kinases-signal transducers and activators of transcription (JAK-STAT) signaling pathways are believed to be crucial in these changes but have received limited investigation utilizing *in vivo* stress models. We previously showed that the restraint of mice results in a substantial activation of STAT-3 in liver but the mechanism responsible for this activation is unknown. One critical component of the response to stress involves activation of the sympathetic nervous system and release of the catecholamines epinephrine and norepinephrine. In this work we used agonists and antagonists of the alpha adrenergic receptor to examine the role of the catecholamines in the activation of STAT-3 in liver. C57Bl6J mice were restrained in centrifuge tubes (2.5 cm inner diameter) for 2 or 4 hours and killed immediately afterward by focused microwave irradiation. The phosphorylation state of STAT3 was determined by western blotting and chemiluminescence detection using phospho state-specific antibodies. In confirmation of our previous work restraint produced large increases in p-STAT3. Phentolamine, an alpha-adrenegic antagonist given (10 mg/kg s.c.) 30 minutes before the start of restraint completely blocked the phosphorylation of STAT3. Further, the alpha1-adrenergic agonist phenylephrine when given (10 mg/kg s.c.) to unrestrained mice stimulates phosphorylation of STAT3 by more than 10 fold within 2 hours of the injection. Our data suggest that catecholamines acting through alpha adrenergic receptors play a crucial role in the activation of STAT3 induced by restraint.

976 15-DEOXY-PROSTAGLANDIN J2 ENHANCES ALLYL ALCOHOL-INDUCED TOXICITY IN RAT HEPATOCYTES.

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Allyl alcohol (AA) induces hepatotoxic responses that are potentiated by small doses of bacterial lipopolysaccharide (LPS). Previous research from this laboratory has shown that cyclooxygenase-2 is involved in the augmentation of AA liver damage by LPS *in vivo*. Studies *in vitro* showed a significant enhancement of hepatocyte (HC) killing by prostaglandin D2 (PGD2), but no effect by PGE2. In studies presented here, 15-deoxy-PGJ2 (15d-PGJ2), a non-enzymatic product of PGD2, enhanced AA cytotoxicity *in vitro* to a much greater extent than PGD2. HCs were freshly isolated from rat livers, adhered to tissue culture wells and exposed to PGs for 2 h. HCs were then exposed to various concentrations of AA for up to 90 min. HC killing was assessed from the release of alanine aminotransferase (ALT). The concentration required for half-maximal cytotoxicity by AA (EC50) was reduced by half with 15 μM 15d-PGJ2 (from 50 μM to 25 μM AA). Though 15d-PGJ2 has been identified as a peroxisome proliferator-activated receptor γ (PPARγ) agonist, it did not appear to act through PPARγ to elicit this activity because a synthetic PPARγ agonist neither augmented AA cell killing by itself nor affected the activity of 15d-PGJ2. 15d-PGJ2 has been suggested to act *via* cyclic AMP (cAMP) in macrophages, but in HCs augmentation of cAMP activity with IBMX, or inhibition of protein kinase A with HA-1004, had no effect on enhanced cell killing by 15d-PGJ2. Protein synthesis was markedly decreased by 15d-PGJ2 in HCs, but inhibition of protein synthesis did not appear to cause cell death, as shown by a lack of ALT release with treatment of cells with cyclohexamide alone. 15d-PGJ2 inhibited translocation of the p65 fragment of nuclear factor kappa B (NF kappa B) from the cytosol to the nucleus in a manner additive with AA. In addition, an inhibitor of NF kappa B enhanced AA-induced HC death. Together, these results indicate that 15d-PGJ2 augments HC killing by AA, and the mechanism may be related to the inhibition of NF kappa B activation. (Supported by NIEHS 08789)