979 RESTRAINT STRESS CAUSES PHOSPHORYLATION OF STAT3 IN LIVER OF C57BL/6J MICE THROUGH ACTIVATION OF ADRENERGIC RECEPTORS.

J. R. Turner, S. A. Benkovic, J. P. O'Callaghan and D. B. Miller. *TMBB, CDC-NIOSH, Morgantown, WV.*

Psychological stress elicits two classical responses: 1) it activates the hypothalamic-pituitary-adrenal axis, resulting in the release of glucocorticoids, and 2) it activates the sympathetic nervous system, which results in the release of norepinephrine and epinephrine. Although these two systems often work in tandem, our studies indicate that activation of the JAK-STAT signaling pathways in livers of mice subjected to restraint stress is solely controlled through the adrenergic stress response. Using a combination of focused-microwave irradiation to preserve protein phosphorylation state, and phospho-specific immunoblotting, we observed a 10-fold increase in PSTAT3tyr705 in the livers of mice restrained for two hours. This effect could be blocked by both alpha and beta adrenergic antagonists, but not by adrenalectomy. Additionally, alpha and beta adrenergic agonists (phenylephrine 10 mg/kg or isoproterenol 10 mg/kg s.c., respectively) mimic the stress response in vivo and trigger phosphorylation of liver STAT3. This activation appears to be controlled through the beta-adrenergic receptor, since blocking this receptor precludes PSTAT3 induction upon administration of phenylephrine, an alpha-adrenergic agonist. Histological analysis of livers from saline and phenylephrine treated mice showed a translocation of STAT3 from cytoplasm to the nucleus, respectively, and a blockade of translocation by propranolol pretreatment. Altogether, these data suggest that restraint stress triggers phosphorylation of liver STAT3 through a solely adrenergic pathway, which initially requires beta-adrenergic receptor activation. These findings support the idea the acute psychological stress can elicit STAT3 activation in the liver, and may therefore have ramifications on liver diseases or in modulating hepatotoxicity.
"The findings and conclusions in this report (abstract/presentation) have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy."

980 EFFECT OF *AHR* NULL ALLELES ON IRON-ENHANCED PORPHYRIA IN MICE.

A. G. Smith, B. Clothier, S. Robinson, R. E. Edwards, T. Chernova, P. Greaves and R. Davies. *MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom.*

A consequence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice is a hepatic dysfunction of heme synthesis (porphyria) similar to human porphyria cutanea tarda. Susceptibility to this type of porphyria in mice is associated with polymorphism of the *Ahr* gene, although not exclusively, and with a threshold for CYP1A2 expression but not always with the degree of induction. The porphyria is potentiated by iron and the heme precursor aminolevulinic acid (ALA) both of which may also cause porphyria. It is unknown whether *Ahr* expression is essential to the probable oxidative mechanisms of all hepatic porphyria developments. Male C57BL/6J *Ahr* +/+, +/- and -/- mice received TCDD (75 μg/kg orally) ± s.c. dosing of iron (800 mg/kg). After 5 weeks liver toxicity as shown by histology and plasma ALT and AST enzymes, occurred in +/+ mice and to a lesser extent in +/-. Hepatic toxicity in both strains was markedly enhanced by iron. Porphyria, as judged by uroporphyrin levels, occurred in +/+ and +/- mice especially with iron. Only mild toxic effects of TCDD were observed in -/- mice even with iron, and there was no influence whatsoever on uroporphyrin levels whatever the iron status. Even 5 weeks after TCDD CYP1A2 levels, as measured by MROD activity and by immunoblotting, were still massively induced in +/+ and to the same degree in +/- mice, but not in -/-. However, low basal expression of CYP1A2 (25-40% of wild type) was observed in *Ahr* -/- mice by RT-PCR and by immunoblotting. When iron-treated *Ahr* null mice were administered ALA in the drinking water (2 mg/ml) for 8 weeks without TCDD, uroporphyrin levels were markedly elevated although less than the wild type. In contrast, C57BL/6J *Cyp1a2* null mice are known to be totally resistant to the development of porphyria with this regimen. The results confirm that a minimum level of CYP1A2 may be required for porphyria development and demonstrate that although expression of *Ahr* appears essential for TCDD-induced porphyria it is not an absolute requirement for every porphyric response of the liver.

981 UROPORPHOMETHENE, THE INHIBITOR OF UROPORPHYRINOGEN DECARBOXYLASE CAUSING PORPHYRIA CUTANEA TARDA IDENTIFIED.

J. D. Phillips[2], J. P. Kushner[2], H. A. Bergonia[2], C. A. Reilly[1] and M. R. Franklin[1]. [1]*Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and* [2]*Medicine, University of Utah, Salt Lake City, UT.*

Porphyria cutanea tarda (PCT), the most common form of porphyria in humans, engaged the toxicology community when it was linked to human ingestion of hexachlorobenzene in Turkey in the 1950s. Porphyrin accumulation in the skin accounts for the photodermatitis. However, the disease is due to reduced activity of uroporphyrinogen decarboxylase (URO-D) in the liver, often in association with increased hepatic iron stores. Protein levels of URO-D do not change when catalytic activity is reduced, suggesting the possible presence of an inhibitor of URO-D. An inhibitor of URO-D has been identified in deproteinated liver cytosolic extracts from two murine models of PCT: wild type mice treated with polychlorinated biphenyls, together with iron and delta-aminolevulinic acid, and mice with one null allele of Uro-d and two null alleles of the hemochromatosis gene (Uro-d+/-, Hfe-/-) which develop PCT with no treatment. The inhibitor presence in the latter model indicates the inhibitor is chemically unrelated to PCBs. The inhibitor has now been characterized by solid phase extraction, chromatography, UV-vis spectroscopy and mass spectrometry and has proven to be uroporphomethene, a compound in which a single bridge carbon in the uroporphyrinogen macrocycle is oxidized, forcing the two adjacent pyrrole rings into a planar conformation. Synthetic uroporphomethenes were prepared by photo-oxidation of enzymatically-generated uroporphyrinogen I or III. Both uroporphomethenes inhibited recombinant human URO-D but the III isomer porphomethene was the more potent (2-3 fold) inhibitor. An inhibitor of rhURO-D has also been detected in cytosolic extracts of liver biopsy samples of patients with PCT. These studies support the hypothesis that the mechanism underlying clinical expression of the PCT phenotype appears to be the oxidation of uroporphyrinogen to uroporphomethene, which acts as a competitive inhibitor of urophorphyringen decarboxylation by URO-D.
Support:NIH DK20503

982 HEPATIC TRANSPORTER mRNA CHANGES ASSOCIATED WITH TREATMENT REGIMENS LEADING TO UROPROPHYRIA IN A MOUSE MODEL OF PORPHYRIA CUTANEA TARDA.

D. D. Arch[1], J. D. Phillips[2], J. P. Kushner[1] and M. R. Franklin[2]. [1]*Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and* [2]*Medicine, University of Utah, Salt Lake City, UT.*

Porphyria cutanea tarda (PCT) is characterized by increased body burdens of uroporphyrin, originating from hepatic accumulations as a result of severely reduced uroporphyrinogen decarboxylase (URO-D) activity. Active transport mechanisms have been implicated in the movement of porphyrins across cellular membranes and it was of interest to examine whether changes in the regulation of transporter transcription was occurring in the livers of animal models of the disease. We have investigated transporter mRNA changes following treatment of female mice possessing one null allele of Uro-d with a single dose of polychlorinated biphenyls (PCBs). The mice showed mean hepatic porphyrin levels of 6 nmol/g liver after 1 week and plateau levels of ~750 nmol/g at 2 and 3 weeks. When the mice also received delta-aminolevulinic acid (ALA) in their drinking water, the 1-week level was increased to 373 nmol/g and the 3-week level was increased to 1062 nmol/g if they also received an iron-dextran injection. Abcc3 mRNA levels correlated with porphyrin levels, with values of 134% (1 week) and 240% (2-3 weeks) of control for the PCB-alone treatment, and 191 and 276% respectively for the two further supplemented regimens. Slco1b2 showed an inverse correlation with porphyrin levels, reaching 67% depression with the treatment regimen that gave porphyrins of 1062 nmol/g. The only significant changes in Abcb1a, Abcb1b and Abcg2 mRNAs were the 301, 490 and 146% elevations, respectively, seen with the 3-week PCB/ALA/iron-dextran treatment regimen giving mean porphyrin levels of 1062 nmol/g. No significant changes were observed in Abcc2 from any treatment regimen. Abcb11 was significantly depressed with the 2-week PCB (37%) and 1-week PCB/ALA (60%) regimens, and therefore in a manner unrelated to porphyrin levels. Thus transcript levels of numerous transporters appear responsive not only to PCBs, but stimuli which in many cases arise as the disease-state develops.
Support:NIH DK20503

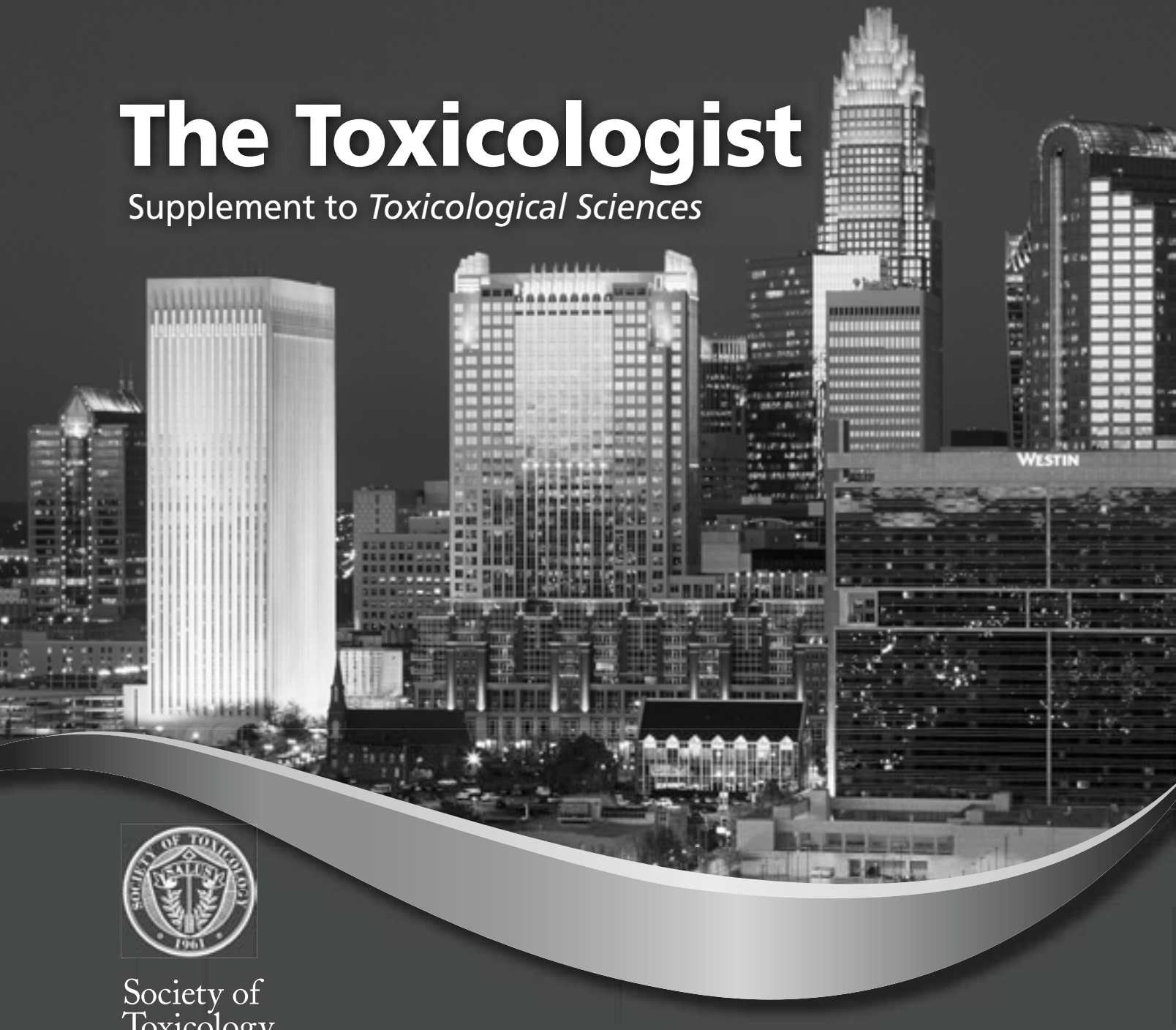
983 QUANTIFICATION OF PROLIFERATING LIVER SINUSOIDAL ENDOTHELIUM USING LASER-SCANNING CYTOMETRY.

R. Garrido, K. Toy, K. McClinchey, J. Wijsman, L. Obert and R. Dunstan. *Drug Safety R&D, Pfizer, Ann Arbor, MI.*

The liver has a major role in storage and metabolism of nutrients, and it is the main organ for drug biotransformation. Liver sinusoidal endothelial cells constitute the lining of the hepatic sinusoid and perform a wide range of important functions: exchange of blood components, endocytosis of xenobiotics, secretion of inflammatory mediators, and antigen presentation. Proliferation of sinusoidal endothelium in the liver has been related to drug toxicity, hemangiosarcoma formation and liver regeneration after acute liver injury.
Laser scanning-cytometry (LSC) analyzes fluorescently-labeled tissue sections in a fashion similar to flow cytometry. LSC collects tissue data by sequential scanning of microscopic fields, while preserving sample integrity and recording spatial localization of data within the tissue.

The Toxicologist

Supplement to *Toxicological Sciences*



Society of Toxicology

46th Annual Meeting and ToxExpo™

Charlotte, North Carolina

An Official Journal of the Society of Toxicology

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 96, Number 1, March 2007

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org