Comparative Medicine

Vol 53, No 6
December 2003
Pages 616-621

Effect of Animal Bedding on Rat Liver Endosome Acidification

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Animal beddings, such as pine products, and environmental factors are known to induce liver drug-metabolizing cytochrome P450 enzymes. We observed that a change to pine-based rat bedding altered baseline and cAMP-stimulated rates of acidification in rat liver endosomes, apparently by decreasing ATP-dependent proton transport in the presence and absence of chloride. Although cAMP altered phosphorylation of protein kinase B and extracellular signal-regulated kinases 1 and 2 (ERK 1,2) and p38 mitogen-activated protein kinases, changes in housing conditions did not affect baseline or cAMP-stimulated values of these or other selected signaling molecules. We conclude that compounds in rat bedding may alter not only drug metabolism, but also aspects of endocytosis.

Previous studies have documented effects of environmental changes, especially animal bedding, on rat liver drug metabolism (22, 25). The best characterized effects appear to be induction of cytochrome P450 (CYP) enzymes by exposure to volatile organic compounds in pine- or cedar-based bedding (22, 25), that are potentially mediated by nuclear receptors (9). In our studies of endocytosis, we have documented that the rates of ATP-dependent proton transport (acidification) in rat liver endosomes are increased while endosome maturation and trafficking are altered by cAMP and agents that affect heterotrimeric G-protein signaling, including cholera (CTX) and pertussis toxins (16, 17). Subsequent to institutional changes in animal husbandry practices, we observed that rat liver endocytosis appeared to be altered. We undertook the study reported here to identify and characterize environmental effects on liver endosomes and their ion transport properties. Previous studies by ourselves and others have identified cAMP-dependent changes in various signal transduction pathways in rat liver (19, 20, 23, 24) and have suggested that cAMP, phosphoinositide 3 kinase (PI-3 kinase), protein kinase B (PKB), glycogen synthase kinase-3α, β (GSK-3α, β), and the mitogen-activated protein kinase (MAPK) p38 may regulate endosome trafficking and function in liver and other cell types (1-5, 8, 10, 19, 20, 23, 24). Further, estrogen and aromatic hydrocarbons, both of which activate nuclear receptors, have been reported to alter MAPK signaling (13). Therefore, we also explored whether changes in endosome acidification due to housing conditions were associated with changes in representative members of several major signal transduction pathways.

Materials and Methods

Materials. A 70,000-Da fluorescein isothiocyanate (FITC)-dextran conjugate and other chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.), fluorescein antibody was purchased from Molecular Probes, Inc. (Eugene, Oreg.), CTX was obtained from List Biological Laboratories, Inc. (Campbell, Calif.), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE) and immunoblot analysis (western blot) supplies were purchased from Bio-Rad Laboratories (Hercules, Calif.), Amersham Life Science (Little Chalfont, England), and Pierce Chemicals (Rockford, Ill.). Polyclonal antibodies to total and phosphorylated PKB, GSK-3β (total) or GSK-3α, β (phospho), p38 MAPK, and c-Jun (including antibodies that detect phosphorylation at either Ser63 or Ser73), polyclonal antibodies to total extracellular signal-regulated kinases 1 and 2 (ERK1,2) MAPK, to phosphorylated Raf-1 (at Ser259) and monoclonal antibodies to phosphorylated ERK1,2 MAPK and to phosphorylated p70 S6 kinase/p85 S6 kinase were obtained from Cell Signaling Technology (Beverly, Mass.). Secondary anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase (HRP) were obtained from Jackson Immunoresearch Laboratories, Inc. (West Grove, Pa.). Primary (1°) antibodies were used at a dilution of 1:1,000, and secondary (2°) antibodies were used at a dilution of 1:1,250, except for antibodies to c-Jun (1°, 1:100; 2°, 1:500), phospho p70 S6 kinase/p85 S6 kinase (1°, 1:800; 2°, 1:1,000), phospho ERK1,2 (1°, 1:1,250) and phospho RAF-1(Ser259) and total GSK (1°, 1:2,000).

Animals. Male Wistar-Furth rats (200 to 250 g) were obtained from a single breeding colony from Harlan, Inc. (Indianapolis, Ind.) and were designated specific pathogen-free by the supplier on the basis of results of serologic studies for 14 virus and bacteria species, polymerase chain reaction (PCR)-based tests for four virus and bacterial species, culturing for respiratory and enteric bacteria, and microscopic examination for endo- and ectoparasites, as detailed at http://www.harlan.com/us/index.htm.

Animals received humane care according to the *Guide for the Care and use of Laboratory Animals* (National Academy of Sciences, 1996). All work was approved by the IACUCs at the University of Michigan and the Ann Arbor VA Hospital.

Animals were housed in one of two animal care facilities (University of Michigan [UM] and Veterans Administration [VA]) for at least five days before the study. Both facilities housed animals in plastic cages with contact bedding and supplied with filtered air and city water; however, the standard

Received: 5/5/03. Revision requested: 6/04/03. Accepted: 7/07/03.
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