

protein expression in response to an unanticipated postprandial carbohydrate load when glucose transport capacity is normally low. SGLT1 upregulation was due to a post-transcriptional increase in net protein synthesis. Moreover, rapid upregulation is not a general phenomenon because it was not observed for GLUT2.

Controlling mechanisms of cell migration in intestinal mucosa cells subjected to repetitive deformation

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INTRODUCTION: Mucosal barrier function and healing are impaired in patients with sepsis and ileus when fibronectin levels rise and peristalsis is diminished. Repetitive strain stimulates intestinal epithelial cell migration across fibronectin via intracellular ERK by an unknown mechanism that might be defective in such patients. We sought to identify the signal pathway mediating the motogenic effect of strain.

METHODS: We subjected human Caco-2 intestinal epithelial cells to 10 cycles/minute, 10% average deformation, resembling physiologic peristalsis or villous motility. We assessed migration via monolayer wound closure, and signaling by Western blot or immunoprecipitation.

RESULTS: Strain activated both PI3K and its downstream effector AKT. Blocking either kinase or reducing AKT by siRNA inhibited strain motogenicity. Strain also stimulated the known ERK substrate GSK, but inhibiting PI3K or AKT prevented this. Blocking GSK prevented strain motogenicity. Previous preliminary studies suggested that blocking the focal adhesion tyrosine kinases FAK and Src prevented the motogenic effect. Reducing FAK by siRNA also inhibited AKT phosphorylation. Src blockade or siRNA reduction did not. Strain phosphorylation of AKT was inhibited by point mutating FAK at the Tyr-397 autophosphorylation site but not at Src-dependent Tyr-577/578. Finally, ERK phosphorylation was prevented by inhibiting FAK and PI3K but not Src or AKT.

CONCLUSIONS: These results trace a complex pathway by which repetitive deformation stimulates intestinal epithelial motility across fibronectin. The pathway diverges but then converges upon GSK as a downstream mediator, so GSK may be a key pharmacological target. Manipulating this pathway may promote mucosal healing and maintain the mucosal barrier in critically ill patients.

Molecular hydrogen prevents intestinal I/R injury

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INTRODUCTION: Molecular hydrogen shows potential for reducing ischemia/reperfusion (IR) injury. We investigate the effect of hydrogen on intestinal IR injury.

METHODS: Rodents were subjected to SMA clamp IR (50min) and reperfusion with air or molecular hydrogen (2%) inhalation (30 pretreatment/50min ischemia/30min posttreatment). Histochemistry and IHC on jejunal muscularis whole-mounts quantified neutrophil and F4/80+ monocyte recruitment into the muscularis externa (N=4 each). Organ bath recordings measured jejunal circular muscle contractility to bethanechol (0.3-300 μ M, N=6 each). Orally fed FITC-fluorescent microspheres (0.4 μ M) within muscularis leukocytes assessed mucosal barrier function.

RESULTS: Few neutrophils were observed in air and hydrogen treated controls (Air=1.1 \pm 0.23 vs. Hydrogen=0.6 \pm 0.14). I/R resulted in a significant increase in neutrophils into the muscularis of air treated mice (46.2 \pm 8.19), which was significantly decreased by hydrogen inhalation (16.7 \pm 4.69). Monocytes were not observed within control tissues, but I/R air mice exhibited the appearance of dense monocytic plaques at 24 hours. Hydrogen treated animals did not exhibit the monocytic plaques. Bethanechol dose-response curves demonstrated no difference between air and hydrogen treated control mice or 3hrs after reperfusion. However, 24 hours after reperfusion hydrogen treatment resulted in markedly improved muscle contractility compared to air (Air-I50min/R24hrs=0.26 \pm 0.071 vs. Hydrogen-I50min/R24hrs=0.68 \pm 0.118 g/mm²/sec @ 100 μ M bethanechol). I/R resulted in a time dependent lumeno-lymphatic transference of microspheres in 30 minutes after reperfusion that was blocked by lymphatic ligation.

CONCLUSIONS: I/R injury causes a breakdown in mucosal barrier function, neutrophil and monocyte recruitment into the muscularis externa resulting in a suppression in muscle function, which is significantly prevented by molecular hydrogen.

De-differentiation of microsatellite unstable (MSI) colorectal cancer (CRC) is resistant to differentiation induction by the HDAC inhibitor butyrate as measured by intestinal alkaline phosphatase (ALPI) induction

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INTRODUCTION: MSI CRC is frequently characterized histologically as poorly differentiated. Histone Deacetylase inhibitors (HDACi) are cancer therapeutic agents that induce growth arrest, apoptosis and differentiation. Differentiation induction agents have been used effectively to treat hematopoietic malignancies. The purpose of our present study was to test the hypothesis that the HDACi butyrate induces differentiation of human colon cancer, as measured by the induction of the differentiation marker ALPI, independent of MSI status.

METHODS: An alkaline phosphatase assay, real-time PCR, and cDNA microarray of a panel of butyrate treated colon cancer cell lines were performed. Mithramycin, an inhibitor of Sp1/Sp3 binding, and a cyclohexamide assay tested ALPI induction inhibition.