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Control of claudin intercellular binding compatibility by extracellular loop domains

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Tight junction permeability is regulated by proteins in the claudin family. In order to form a tight junction, claudins on one cell bind to claudins on an adjacent cell through head-to-head interactions. Previous work suggests that claudins fall into compatibility groups, however, the rules which dictate claudin head-to-head binding specificity have not been defined. Characterizing claudin compatibility is complicated by the fact that most epithelial cells express four or more claudin isoforms. We have found that HeLa cells endogenously express many tight junction components (e.g. occludin, ZO-1, ZO-2, ZO-3, and JAM) however, they are deficient in claudin expression. Thus, we used HeLa cells as a null background to examine claudin head-to-head compatibility. Cells were stably transfected with different human claudins and the ability of different claudins to interact was examined in a co-culture system. Claudin interactions were detected by immunofluorescence co-localization and confirmed by co-immunoprecipitation. Using this approach, we found that claudin-3 has the capacity to bind to itself and claudin-1, but not claudin-4. To further define claudin compatibility, we produced a series of claudin chimeras, based on a claudin-4 backbone, where either one or both of the extracellular loop (EL) domains were replaced with EL domains from claudin-3. A chimera containing both EL domains (claudin-4/3e1+e2) had the capacity to bind wild type claudin-1 or claudin-3. Also, we found that replacing either the first EL domain (claudin-4/3e1) or second EL domain (claudin-4/3e2) was sufficient to enable binding to wild type claudin-1 or claudin-3. This suggests that claudin head-to-head compatibility can be controlled by a single EL domain. Also, the two EL domains may act as independent motifs to regulate intercellular binding between claudins in tight junctions.

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Pharmacological Manipulation of Tight Junctions in Rat Colonic Mucosa in Vitro

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A comparison was made between two tight junction openers, sodium caprate (C10) and cytochalasin D (CD) regarding effects on transepithelial electrical resistance (TEER) and paracellular flux of mannitol across rat colonic mucosa *in vitro*. In addition, mucosae were pre-treated with a myosin light chain kinase inhibitor (ML-9) in order to determine if it has a moderating influence on the actions of both agents. Muscle-stripped rat colonic sheets were mounted in Ussing chambers bathed in oxygenated Krebs-Henseleit solution at 37°C. TEER and the apparent permeability coefficient (Papp) of [^{14}C]-mannitol were recorded for 120 minutes. ML-9 (50μM) was added 20 minutes before the addition of C10 (10mM) or CD (4μg/ml). TEER was reduced in both C10- and CD-treated mucosae compared to controls (91.5 and 83% respectively, $P<0.0001$). TEER was reduced to a lesser extent in ML-9 pre-treated C10-exposed mucosae (82%, $P<0.05$) compared with C10 controls, and also in ML-9 pre-treated CD (69%, $P<0.01$) compared to CD controls. The Papp of mannitol in the presence of C10 (1.61×10^{-5} cm/sec, $P<0.001$) and CD (9.23×10^{-6} cm/sec, $P<0.05$) treated tissue was significantly increased compared to untreated controls (1.69×10^{-6} cm/sec). In the presence of ML-9 however, the Papp of mannitol was increased to a lesser extent in C10 (7.11×10^{-6} cm/sec, $P<0.01$) and CD (5.70×10^{-6}, $P<0.05$) treated colons compared to C10 and CD-treated tissues in the absence of ML-9. ML-9 had no effect on basal TEER values. Inward short-circuit current responses to the secretagogue, carbachol (10μM) were retained in each mucosae following exposure to all three agents. C10 and CD therefore both decrease TEER and increase Papp in rat colonic mucosa *in vitro* while ML-9 can offset these effects. Myosin light chain kinase inhibition may be a potential mechanism to increase barrier function in leaky epithelia.

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Demonstration of Compromised Ileal Structure and Function Following Severe Acute Trauma to the Left Forebrain in the Isoflourane Anaesthetized Rat

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The aim of this study was to examine the hypothesis that severe acute trauma to the forebrain can impact intestinal physiological function. Rats with and without a severe neurotrauma (NT) were studied electrophysiologically for effects on intestinal function *in vitro*. Immunohistochemistry was also carried out to determine morphological changes in the intestine as a result of NT compared to controls. Following a craniotomy in isoflourane anaesthetised rats (n=5), NT (1.3m/s velocity) was produced directly on exposed medial prefrontal cortex *via* a controlled cortical impact using a pneumatically driven impactor, resulting in a local 2.62 mm deformation. Control rats (n=5) were subjected to a craniotomy but did not receive a NT. Anaesthesia was maintained for a further 6 hours before euthanasia by isoflourane overdose. Muscle-stripped rat ileal and colonic segments were dissected and mounted in Ussing Chambers and bathed in oxygenated Krebs-Henseleit solution at 37°C. Transepithelial electrical resistance (TEER) and the apparent permeability coefficient (Papp) of [^{14}C]-mannitol were recorded for 120 minutes. TEER of Ileal and colonic mucosae showed no differences between NT and control rats, nor did the Papp of colonic tissue from either group. In contrast, following severe NT, Papp in the ileum was statistically increased from $4.7\pm0.4\times10^{-6}$cm/sec in controls to $7.02\pm1.7\times10^{-6}$cm/sec in NT rats ($P<0.05$). Furthermore, independent immunohistochemical analysis showed damage to ileal but not colonic sections from NT rats. This study showed that NT results in damage to the small intestinal mucosa along with reduced barrier function, while colonic parameters were unchanged. This may have implications for the oral absorption of nutrients by patients with acute head injury, while allowing the passage of endotoxins across the gut that might normally be excluded by intact barrier function.

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Cell-cell junction dynamics at the blood-testis barrier (BTB) are regulated by α_2-macroglobulin (α_2-MG) via the c-Jun N-terminal kinase (JNK) signaling pathway

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Proteolysis is crucial to anchoring junction restructuring events but it is limited to the cell-matrix interface in virtually all epithelia examined to date. However, a surge in α_2-MG, a protease inhibitor, was detected at the Sertoli-Sertoli and Sertoli-germ cell interface in the seminiferous epithelium during cadmium chloride ($CdCl_2$)-induced BTB disruption in adult rats. Furthermore, this $CdCl_2$-induced primary damage to Sertoli cell tight junctions (TJ) led to a secondary disruption of Sertoli-germ cell adherens junctions (AJ), causing germ cell depletion from the epithelium. It was thus suggested that α_2-MG was a critical player in BTB dynamics. To delineate the role of α_2-MG in the BTB, changes in several signal transducers that were known to affect junction dynamics in the testis were examined. Both phospho-p38 MAP kinase and phospho-JNK were induced during the $CdCl_2$-mediated BTB disruption. However, a blockade of p38 MAP kinase by SB202190 failed to affect changes in α_2-MG level during BTB restructuring, ruling out the involvement of p38 in this event. Yet, when rats were pretreated with dimethylaminopurine (DMAP), a JNK inhibitor, at 8 μmol/testis prior to $CdCl_2$ treatment (3 mg/kg b.w., i.p.), the surge in α_2-MG was significantly reduced, while damages in TJs and AJs at the epithelium were worsened when examined histologically and by fluorescent microscopy. These analyses were consistent with immunoblotting results in which a significant loss of occludin, ZO-1, N-cadherin and β-catenin from the BTB was detected. In summary, BTB dynamics are regulated by at least two signaling pathways: the p38 MAPK pathway determines the levels of TJ- and AJ-integral membrane proteins whereas the JNK pathway regulates the homeostasis of proteases and protease inhibitors at the cell-cell interface of the BTB.

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Cystic fibrosis deficient cell complementation

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Cystic Fibrosis is a lethal disorder caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. The trafficking of the ΔF508-CFTR protein from the endoplasmic reticulum is impaired compared to the wild-type (WT) CFTR. This membrane protein is involved with cAMP-dependent Cl- ion transport and appeared to be involved with the regulation of other channels and cell signaling pathways. To better understand the role that CFTR plays in CF pathology, CF cell lines have been complemented with a WT- and DF508-CFTR cDNA using an episomal expression system pCEP4 (InVitrogen), that contains the Epstein-Barr virus (EBV) origin of replication, oriP, and nuclear association antigen, EBNA-1. The vector used is maintained as an episome when transfected cells are maintained under selective pressure. WT-CFTR cDNA containing either the open reading frame (ORF) or the entire CFTR cDNA (4.7 or 6.2 Kb, respectively) were introduced in the pCEP4 vector into CF cell lines carrying mutations in the CFTR gene with genotypes of ΔF508/ΔF508 or ΔF508/Q2X. Two cell lines were transfected: the CFSMEo- DF508.Q2X) derived from airway submucosal gland tissue isolates and the CFBE41o-(αF508/ΔF508) derived from bronchial tissue isolates. Cells were transfected by electroporation (AMAXA) with the normal and mutated plasmid and transferred to medium containing hygromycin B (Hyg B). The transfection with the DF508-CFTR cDNA will control for vector regulated CFTR expression. Hyg B resistant clones appeared within 10 days, and were isolated and expanded and then characterized for the expression of vector-specific CFTR mRNA by RT-PCR. Electrophysiological analysis of the complemented cell lines by patch clamp is underway. These cell lines represent a significant tool for investigating the role of CFTR in multiple cell functions in different tissue and genetic venues. This work is supported by grants from the Cystic Fibrosis Foundation, Pennsylvania Cystic Fibrosis, Inc.