A Novel Role of GLP-1 Nanomedicine in Amelioration of Gut Inflammation

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Sorting Nexin 27 (SNX27) Is a Member of NHERF (Na+/H+ Exchanger Regulatory Factor) Family via PDZ Domain (PSD95, disc large, ZO1) Interaction. Sorting Nexin 27 (SNX27) is a protein specifically expressed in early endosomes, which is partly allievated in GLP-1 treated mice. Further, treatment with GLP-1 alleviated the increase in the expression of the pro-inflammatory cytokines in colonocytes of DSS mice e.g. IL-1β (Control: 20±5; DSS: 20±6; GLP-1: 2± GLP-1 (group 1, 5 μl); DSS: 20±6; SNX27: 20±3; GLP-1: 2±3). Western blot results showed that DSS decreased the expression of DRA as compared to control but GLP-1 abrogated this effect of DSS (Control: 1±9; DSS: 0±31; GLP-1: 1±20; GLP-1+DSS: 0±0±0.2). This data was further confirmed by FACS. Our data showed that GLP-1 nanomedicine is effective in reducing intestinal inflammation and the associated diarrhea. We speculate that GLP-1 nanomedicine could be used as a novel therapeutic approach to treat patients with Crohn’s disease and ulcerative colitis. (Supported by NIDDK and Dept. of Veteran Affairs)

3D Cell Culture of Caco2: A Better Model to Study the Functionality and Regulation of Intestinal Ion Transporters

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Traditional two-dimensional (2D) cell monolayers have been widely used as an in vitro model of intestinal epithelium. However, 2D system fail to represent the physiological complexity and pose limitations of this system by forcing cells to adopt a monolayer, flat and rigid surface. Thus, development of more advanced and reliable in vitro model systems is crucial as an alternative to conventional in vivo studies. In this regard, the three-dimensional (3D) culture system mimics physiological conditions that exist in vivo. Utilizing Caco-2 cells as a 3D model epithelium, recent studies have demonstrated the capability for testing drug permeability and epithelial morphogenesis. However, the suitability of 3D models for studies of intestinal epithelial ion transporters has not been addressed. Current studies were performed to validate 3D Caco2 model system for investigating expression of major intestinal ion transporters and their responsiveness to various inflammatory agents. To assess the responsiveness of ion transporters to various inflammatory agents, Caco-2 cells (10^4 cells/ml) were incubated with different concentrations of 3-phenylsulfonyl-1H-pyrido[3,4-b]indole (3-BSPI) and treated with 1 H-2-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 50 μM) for 24 hr to inhibit guanylyl cyclase, Rp-cGMP (50 μM) hr to inhibit PKG while 8-bromo cGMP (8-Brc) was used to activate PKG. In GLP-1+DSS treated mice, body weight loss was detected by anti-EGFP. In all interaction experiments, a mutant of DRA which lacks fluorescent labelled streptavidin. GST pull down assay. The fusion construct GST-PDZD2SNX7 was used to pull down EGFP-DRA from HEK/EGFP-DRA cells using magnetic glutathione beads. Precipitated EGFP-DRA was detected by anti-EGFP. ColP. The fusion construct mCherry-HA-SNX27 was transiently transfected into HEK/EGFP-DRA cells. SNX27 was immunoprecipitated using anti-HA-agarose. Co-immunoprecipitated EGFP-DRA was detected by anti-EGFP. In all interaction experiments, a mutant of DRA which lacks the C-terminal PDZ interaction motif (ETKFMNNS), served as a negative control. In the FLISA, the C-terminal of DRA bound to PDZD2SNX7 with a half-maximal binding of 50 nM. Thus, binding affinity of DRA to SNX27 was similar to NHERF and EKARR (n=3) In contrast, C-DRA-ETKFMNNS did not bind to PDZD2SNX7 (n=3). GST-PDZD2SNX7 pulled down EGFP-DRA but not EGFP-DRA-ETKFMNNS from cell lysates (n=6). EGFP-DRA but not EGFP-DRA-ETKFMNNS was co-immunoprecipitated with mCherry-HA-SNX27 (n=3). Summary and Conclusion: Three different assays (FLISA, GST pull down and CoIP) indicate that SNX27 binds to DRA via its PDZ domain. We have previously shown that DRA co-localizes with DRA in early endosomes, and that the PDZ interaction motif of DRA is required for this process. Because SNX27 is specifically located in early endosomes, it seems possible that it is involved in the recycling of DRA. Current investigations address the influence of SNX27 on the recycling of DRA and therefore the functional consequences of the interaction of the two proteins.

Protein Kinase G Mediated Regulation of Na-Glucose Co-Transport in NaH Exchange Silenced Intestinal Epithelial Cells

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Background: Previous studies have demonstrated that direct inhibition of intestinal epithelial cell brush border membrane (BBM) NaH exchange (NHE3) results in the stimulation of Na-glucose co-transport (SGLT1). Also previous in vitro and in vivo studies has demonstrated that constitutive nitric oxide (cNO) inhibits BBM NHE3 while stimulating SGLT1 in the BBM of rat intestinal epithelial cells (IEC-18) and rabbit villus cells. Also, NHE3 has been shown to regulate SGLT1 through cNOS in stable SRNA NHE3 transfected IEC-18 cells (NHE3KO). However, the molecular mechanism of regulation of SGLT1 by NHE3 by cNOS in human colonocytes is unknown. Hypothesis: Increased intracellular Ca++ likely mediates the stimulation of SGLT1 by cNO. Evidence: cNO inhibited with BAPTA in NHE3KO was restored by PKG activation with 8-Brc in these cells (control 1±0.9; NHE3KO 2297±180.2; NHE3KO+ BAPTA + 8-Brc: 2309±31.0 pmol/mg protein; n=3, p<0.05). Specific inhibition of cGMP formation with ODQ or cGMP inhibited with Rp-cGMP showed a reversal of stimulation of SGLT1 in NHE3KO cells (control 1875±15.4; NHE3KO 2395±55.7; NHE3KO+ ODQ 1842±47.9; NHE3KO+ Rp-cGMP 1810±30.0 pmol/mg protein; n=3, p<0.05). Western blot studies showed that PKG pathway inhibitors, Rp-cGMP or ODQ restored SGLT1 protein levels in NHE3KO cells. Conclusions: Increased intracellular Ca++ mediated stimulation of SGLT1 by cNO were up regulated by PKA. Unregulated cNO production activates protein kinase G mediated pathway, which in turn directly mediates the stimulation of SGLT1 in NHE3 silenced intestinal epithelial cells by increasing the number of co-transporters.

Effects of the Dipeptidyl Peptidase-4 Inhibitor, Sitagliptin, on Colorectal Carcinogenesis in a Model of Type 2 Diabetes

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Background: Patients with type 2 diabetes mellitus are known to have an increased risk of colorectal neoplasia compared with those without diabetes. Dipeptidyl peptidase-4 (DPP-4) inhibitors delay the degradation of active GLP-2 (DPP-4 inhibitor), which is involved in a number of physiological processes. DPP-4 inhibitors are therefore regarded as a new therapeutic agent for type 2 diabetes. Because the intristestinal hormone, GLP-2 is, is rapidly degraded and deactivated by DPP-4, DPP-4 inhibition may increase the risk of colorectal tumors by potentiating the effects of endogenous GLP-2. However.