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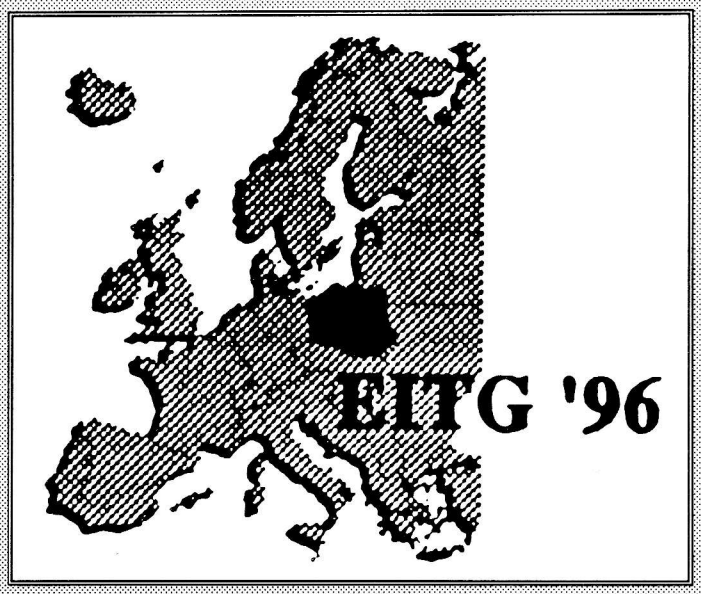

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CHARACTERIZATION AND LOCALIZATION OF A PROBENECID SENSITIVE ORGANIC ANION TRANSPORTER IN HT29 CLONE 19A CELLS.

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Recently, we have identified an organic anion transport mechanism in the human colon carcinoma cell line HT29 cl.19A (1). This transport system mediates the extrusion of Fluo-3 and Fura-Red from the cytosol, and therefore decreases dye loading of the cells with these fluorescent indicators. Intracellular accumulation of Fluo-3 and Fura-Red is enhanced by the addition of probenecid, but is not affected by multidrug reversing agents (1). This transport system could resemble the probenecid sensitive anion exchanger that has been characterised in the basolateral membrane of small intestinal epithelial cells and that is involved in bile acid transport (2). Therefore, we determined the localisation of the organic anion transport system in HT29 cl.19A cells and studied the effects of low concentrations of bile acids on transporter activity by measuring Fluo-3 efflux.

In the first set of experiments, we used Fluo-3 loaded cells that had been cultured on permeable supports. Fluo-3 efflux was determined by measuring time dependent and probenecid sensitive Fluo-3 release to the apical and the basolateral compartment, respectively. In the second set of experiments, we used Fluo-3 loaded cells that had been grown on cover slips for 2 days. Fluo-3 efflux was determined by measuring intracellular Fluo-3 fluorescence, using a quantitative fluorescence microscopy system that contained a photomultiplier tube connected to an inverse microscope.

Fluo-3 release to both the apical and basolateral compartment saturated after 20 min. At this point 2.4 ± 0.7 and 1.5 ± 0.4 nmol Fluo-3 (means ± SE, n=5) had accumulated in the basolateral and apical compartment, respectively. 2 mM probenecid at the apical side significantly inhibited Fluo-3 extrusion into this compartment by 33 ± 11 % (mean ± SE, n=3) after 20 min incubation. Efflux of Fluo-3 to the basolateral compartment remained unchanged under these conditions. Addition of 2 mM probenecid to the basolateral compartment did not alter Fluo-3 efflux to both sides of the cell layer. The decrease in intracellular fluorescence intensity was linear during the first 5 min. Incubation with medium containing 0.2 % (v/v) dimethyl sulfoxide (control) resulted in a decrease in intracellular Fluo-3 intensity of 16.4 ± 3.2 % (mean ± SE, n=8) during this time interval. 10 µM chenodeoxycholic acid, and dehydrocholic acid did not significantly alter Fluo-3 efflux. However, Fluo-3 efflux was significantly reduced in the presence of 10 µM deoxycholic acid and lithocholic acid (6.4 ± 3.3 % and 8.0 ± 1.8 %, respectively, n=5, $p<0.05$).

These results indicate that, although some bile acids influence Fluo-3 efflux, the transport system involved in this process is not related to the probenecid sensitive bile acid transporter in the basolateral membrane of small intestinal epithelium cells, as the probenecid sensitive organic anion transporter in HT29 cl.19A cells is located in the apical membrane.

This work was supported by the Institut Danone für Ernährung, München, Germany.

(1) Abrahamse and Rechkemmer Pflügers Arch. 429: R56, 1995
(2) Weinberg et al. J. Clin. Invest. 78:44-50, 1986

THE ABILITY OF SHEEP RUMEN EPITHELIUM TO TRANSPORT AND METABOLISE HISTAMINE.

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Certain roughage may contain large amounts of histamine (1). Secondly, bacteria are able to produce histamine in the rumen during ruminal acidosis and increased blood histamine levels have been described in this disease (2). Therefore, this Ussing chamber study aimed at the evaluation of histamine transport across rumen epithelium and the influence of ruminal pH. Since histaminase is known to be present in rumen epithelium (3), special interest was put to the efficacy of intraepithelial histamine metabolism.

Methods: *(I)* Stripped ruminal epithelia of sheep were incubated (short-circuited) with buffered bathing solutions containing 0.08 mM histamine. Flux rates were determined using ^{3}H-histamine. To simulate ruminal acidosis, the mucosal buffer solution was acidified from pH 7.4 to pH 5.1. *(II)* Efficacy of intraepithelial histamine metabolism was determined at mucosal pH 7.4 and 5.1 under open circuit conditions. Having a buffer solution with 0.08 mM histamine on one side of the epithelium and a solution containing no histamine on the other, fluxes of histamine-associated radioactivity were compared with histamine fluxes measured by HPLC (Nucleosil® 7C_6H_5, gradient: 25 mM NaH_2PO_4 with increasing [5 % to 50 % v/v] acetonitrile; fluorescence of OPA derivative: exc. 350 nm, em. 450 nm).

Results: *(I)* Taken from radioactivity, histamine was net secreted ($P < 0.01$) at pH 7.4 ($J^{Hist}_{ms} = 0.49 \pm 0.03$ nmol·cm^{-2}·h^{-1}; $J^{Hist}_{sm} = 0.71 \pm 0.07$ nmol·cm^{-2}·h^{-1}; n = 24). Acidification to pH 5.1 lead to increased ($P < 0.05$) unidirectional and net fluxes of histamine ($J^{Hist}_{ms} = 1.01 \pm 0.16$ nmol·cm^{-2}·h^{-1}, $J^{Hist}_{sm} = 1.60 \pm 0.12$ nmol·cm^{-2}·h^{-1}, n = 6). *(II)* Measured by HPLC, much smaller ($P < 0.01$) histamine fluxes were calculated at pH 7.4 ($J^{Hist}_{ms} = 0.006 \pm 0.003$ nmol·cm^{-2}·h^{-1} [0.4 % of radioactivity; n = 4]; $J^{Hist}_{sm} = 0.038 \pm 0.005$ nmol·cm^{-2}·h^{-1} [7.1 % of radioactivity; n = 4]; n = 6). However, at pH 5.1, histamine metabolization dropped markedly and a highly increased ($P < 0.01$) part of histamine was transported unmetabolized ($J^{Hist}_{ms} = 0.24 \pm 0.06$ nmol·cm^{-2}·h^{-1} [28 % of radioactivity; n = 4], $J^{Hist}_{sm} = 0.52 \pm 0.03$ nmol·cm^{-2}·h^{-1} [46 % of radioactivity; n = 4]; n = 6). At both mucosal pH, the ms histamine transport was relatively smaller ($P < 0.01$) than the sm transport and metabolism was more effective ($P < 0.05$) for the ms transport.

Conclusions: Rumen epithelium seems to be able to secrete histamine. However, a far more important protective mechanism against histamine absorption is the very effective intraepithelial histamine metabolization which is even higher for the ms transport. Secretion at mucosal pH 5.1 points to pH-dependent histamine dication entrapment on the mucosal side. Following mucosal acidification, histamine transport in either direction is increased and intraepithelial histamine metabolism is markedly reduced. This leads to significant transport of unmetabolized histamine under acidotic conditions.

(1) M. Okamoto et al., J Dairy Sci **47**: 1231-1236, 1964
(2) F.A. Ahrens, Am J Vet Res **28**: 1335-1342, 1967
(3) J.O. Dickinson and W.G. Huber, Am J Vet Res **33**: 1789-1795, 1972

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FUNCTIONAL CHARACTERISTICS OF TRANSPORT OF DIFFERENTLY CHARGED DIPEPTIDES BY THE CLONED INTESTINAL PEPTIDE TRANSPORTER PEPT1.

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PepT1, isolated from a rabbit intestinal cDNA library is the first cloned mammalian peptide transporter (1, 2). Its substrates are di- and tripeptides as well as a number of peptide derived drugs with di- or tripeptide backbone including ß-lactam antibiotics or ACE-inhibitors. Among the substrates of PepT1 there´s a great heterogeneity with regard to the net charges of the compounds being transported. We have shown recently (3) that in the case of differently charged ß-lactams only their neutral species seems to be transported. In this study we investigated whether this holds true also for anionic (Gly-Asp) zwitterionic (Gly-Gln) and cationic (Gly-Lys) dipeptides. **METHODS:** *Xenopus laevis* oocytes were injected with 5 ng of PepT1 cRNA or water (controls) and transport of ^{3}H-Gly-Gln in the presence of Gly-Asp, Gly-Gln or Gly-Lys was measured after 3 days. In addition the two-electrode voltage clamp technique was used to determine substrate mediated inward currents and intracellular pH measurements were performed with dextran coupled SNARF-1 as a fluorescent probe to assess substrate linked proton flow. **RESULTS:** Kinetic analysis of ^{3}H-Gly-Gln uptake into oocytes expressing PepT1 at its transport optimum at pH 6.5 revealed an apparent K_m of 142 ± 18 µM and a V_{max} of 203.1 ± 8.2 pmol · oocyte^{-1}· 10 min^{-1}. At pH 6.5 Gly-Gln and Gly-Asp inhibited uptake of 25 µM ^{3}H-Gly-Gln with EC_{50}-values of 112 ± 22 µM and 156 ± 20 µM respectively, whereas Gly-Lys did not inhibit Gly-Gln uptake. In two-electrode voltage clamp experiments K_m values of 133 ± 12 µM for Gly-Gln, 270 ± 23 µM for Gly-Asp and 1.52 ± 0.21 mM for Gly-Lys were determined at pH 6.5. All three dipeptides displayed very similar V_{max} values with respect to the maximal current flow and similar intracellular acidification rates. These data suggested that the peptides are transported by PepT1 only in their zwitterionic forms. In countertransport experiments with oocytes preloaded with the three dipeptides at pH 6.5 and assayed for influx of ^{3}H-Gly-Gln at pH 7.4 only Gly-Gln and Gly-Lys stimulated uptake to more than 40% whereas Gly-Asp was not able to stimulate uptake. Using the same experimental approach of preloading in voltage-clamp experiments it was shown that Gly-Asp only yielded minimal outward currents when oocytes were superfused with substrate free buffer pH 7.4, whereas Gly-Gln and Gly-Lys generated outward currents of 50 ± 6 nA · oocyte^{-1} and 42 ± 8 nA ·oocyte^{-1} respectively. **CONCLUSION:** Our data suggest that only the neutral species of charged dipeptides are transported by PepT1. The pH dependent shift of the ionic species inside the oocyte towards its negatively charged form precludes Gly-Asp from countertransport, whereas Gly-Gln remains in neutral form and Gly-Lys shifts towards the neutral form which in both cases enabels countertransport.

1. Nature 386, 553-556, 1994
2. Pflüger´s Arch - Eur J Physiol 429, 146-149, 1994
3. J Pharmacol Exp Ther, in press, 1996

RELATIONSHIP BETWEEN TEER AND PARACELLULAR MARKER PERMEABILITY ACROSS MONOLAYERS OF TWO DIFFERENT CACO-2 CELL SUBPOPULATIONS.

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Differentiated monolayers of the Caco-2 TC 7 clone - isolated from late passage numbers of the parental Caco-2 clone (P 198) - and the Heidelberg heterogeneous Caco-2 cells (Caco-2 HB) of P# 50-60 were compared concerning permeability characteristics of different model compounds and transepithelial electrical resistance (TEER). TEER was measured using 'chopstick' electrodes (Millicell® ERS, Millipore, Germany). TEER was considerably higher in Caco-2 HB compared to TC7 monolayers (912.4±32.6 Ωcm^2 vs. 149.2±2.6 Ωcm^2). Surprisingly, higher TEER-values in the Caco-2 HB cells were not related to lower paracellular marker permeability; apparent permeability was even slightly higher in Caco-2 HB compared to TC7 monolayers (4.97±0.028 10^{-7}cm/s vs. 3.8±0.035 10^{-7}cm/s and 1.16±0.21 10^{7}cm/s vs. 0.1±0.014 10^{-7}cm/s for ^{14}C-mannitol and lucifer yellow, respectively). There was no difference in ^{14}C-testosterone apparent permeabilities (transcellular transport) between Caco-2 HB and TC7 monolayers. Light microscopic examinations revealed that intracellular lumina were much more prevalent in the Caco-2 HB but not in TC7 cells which might cause differences in TEER. However, there was a negative correlation between TEER and paracellular marker permeability in Caco-2 HB monolayers. Damaged filters could be easily detected by TEER values below 700 Ωcm^2. Incubation with Verapamil (0.5 mM), Propranolol (1 mM) or Quinidine (0.5 mM) for 1h induced a significant increase of TEER by 50-100% without considerable change in paracellular marker permeabilities. In spite of large differences in absolute TEER values relative changes were similar in TC7 and Caco-2 HB monolayers. It is concluded that TEER is a suitable indicator for integrity of untreated monolayers within a *single* subpopulation but absolute TEER values do not appear to be a good tool to compare paracellular permeability properties of *different* Caco-2 cell subpopulations.

DIFFERENCES IN THE ACTIVE SECRETORY TRANSPORT OF TALINOLOL AND PROPRANOLOL ACROSS CACO-2 MONOLAYERS.

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The ß-blockers talinolol [1], celiprolol and propranolol [2] are known for active exsorption in vivo. In vitro results suggested active secretory transport of talinolol [3] and celiprolol [4] by P-glycoprotein. The less pronounced in vivo secretory transport of propranolol in comparison to celiprolol was assumed to be due to higher transcellular transport because of its higher lipophilicity [3]. In the present study we investigated the transport of the lipophilic ß-blockers talinolol (determined by HPLC) and [^{3}H]-propranolol across Caco-2 monolayers in absorptive (a→b) as well as secretory (b→a) direction alone and in presence of an excess of 100 µM verapamil, 1 mM propranolol (unlabelled), 1 mM metoprolol, 1mM quinidine* and 100µM nicardipine* (*: only propranolol). Results were compared to those obtained from celiprolol [4]. Additionally, n-octanol/water partition coefficients were determined for propranolol, talinolol and celiprolol at pH 7.0. Transport rates were significantly higher in the secretory compared to the absorptive direction for both talinolol and propranolol. Apparent permeability (a→b vs. b→a) was 1.4±0.2 10^{-7} vs. 8.0±0.7 10^{-6} cm/s and 1.4±0.1 10^{-5} vs. 2.7±0.1 10^{-5} cm/s for talinolol and propranolol, respectively. Talinolol b→a transport was significantly inhibited and a→b transport was significantly stimulated by verapamil. Propranolol and metoprolol induced less pronounced changes in transport rates. Propranolol b→a transport, however, was neither inhibited by P-glycoprotein modifiers (verapamil, quinidine, nicardipine) nor by ß-blockers. Interestingly, compared to controls propranolol transport rates were significantly higher in both directions in presence of all substances given in excess. In connection with the results obtained for celiprolol [4] it might be concluded that talinolol and celiprolol but not propranolol b→a fluxes are inhibitable by P-glycoprotein modifiers. Moreover, a→b flux was much higher for propranolol compared to talinolol, celiprolol a→b flux was slightly higher compared to talinolol. N-octanol/water partition coefficients at pH 7.0 (log D: -0.52, 0,7 and 1,17 for celiprolol, talinolol and propranolol, respectively) indicate that both talinolol and propranolol are lipophilic ß-blockers whereas celiprolol is of lower lipophilicity. Therefore, lipophilicity may not explain the observed differences. Talinolol and celiprolol, however, are closely related concerning their chemical structure containing an ureido-phenyl group instead of the naphtyl group of propranolol. Considering the structural requirements for P-glycoprotein substrats, it might be possible that talinolol and celiprolol have an exceptional position among the group of ß-blockers. It is concluded that propranolol active secretory transport is mediated by a transport system other than P-glycoprotein which might additionally be involved in the secretory transport of talinolol and celiprolol.

1. Gramatté Naunyn Schmiedeberg's Arch. Pharmacol. 351: (Suppl.) R 1 (1995)
2. Kuo et al. Pharm. Res. 11: 648-653 (1994)
3. Wetterich et. al. Naunyn Schmiedeberg's Arch. Pharmacol. 351: (Suppl.) R 1 (1995)
4. Karlsson et. al. Br. J. Pharmacol. 110: 1009-1016 (1993)

THE PROABSORPTIVE EFFECT OF MORPHINE IS MEDIATED *VIA* NITRIC OXIDE.

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Nitric oxide (NO) has been shown previously to mediate a proabsorptive tone in the intestine even under various secretory conditions. Opiates, on the other side, are the most useful antidiarrhoeal agents, revealing antisecretory and proabsorptive effects.

The present experiments were performed (1) to investigate whether morphine uses NO formation in the rat jejunum in vivo to exert its proabsorptive effect and (2) whether morphine stimulates NO production in isolated epithelial cells of the rat jejunum.

Infusion of L-NAME (25 mg/kg, i.v.) or PGE_2 (12 µg/kg, close i.a.) both produced a comparable secretory response in ligated jejunal loops in anaesthetized rats in vivo. Close i.a.-infusion of morphine dose-dependently (24 - 760 µg/kg) reversed the secretory effect of PGE_2, showing total inhibition of secretion at the dose of 240 µg/kg, but lacking any effect at the dose of 24 µg/kg. L-NAME-induced secretion, however, was already totally blocked by a concentration of 24 µg/kg morphine. On the other hand, the antisecretory effect of morphine (240 µg/kg) on PGE_2 -induced secretion was blocked in the presence of L-NAME.

In further experiments, NO formation was measured in freshly prepared jejunal epithelial cells by measuring nitrite after reduction of nitrate, using Cayman's Nitrate/Nitrite Assay Kit. In this preparation, the calcium ionophore A23187 induced a three fold increase in NO formation, suggesting the presence of constitutive NO synthase. L-arginine (10 mM) induced a two-fold increase in nitrite concentration and enhanced the effect of A23187. Morphine dose-dependently (10 -100 µM) increased NO formation, and in the presence of L-arginine the effect of morphine was enhanced.

These results indicate that morphine counteracts the inhibitory effect of L-NAME on NO formation, induces NO formation by itself and exerts ist proabsorptive effect via this mechanism.

EVALUATION OF LOCAL TRANS- AND PARACELLULAR EPITHELIAL CONDUCTIVITIES WITH VOLTAGE-SCANNING TECHNIQUES.

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Differentiation of trans- and paracellular conductivities has proven difficult. We present a method for quantification of trans- and paracellular conductivities. This method has two advantages: First, it does not depend on complex electrical circuit models of the epithelium, and second it allows localized assessment of individual cells and junctional sites.

A low-resistance subtype of Madin-Darby Canine Kidney cells (C-11 strain), named MDCK–2 cells, resembling the intercalated cells of renal collecting ducts (Kersting et al., Cell Physiol. Biochem. *3*, 42, 1993), were plated on *Cellagen CD 24* culture plate inserts. After 3 – 4 days of cultivation, confluent monolayers were investigated on a microscope stage in a 4-electrode Ussing-type chamber continuously perfused with oxygenated Ringer's solution (37 °C). The cells' mean diameter was 14 µm and their borders were clearly visible indicating dilated intercellular spaces. With minor modifications, voltage-scanning techniques were used as described by Köckerling et al. (Am. J. Physiol. *264*, C1285, 1993). In brief, an AC bridge system with synchronous demodulation was used to measure above the epithelium the potential gradient generated by sinusoidal current that was passed through a pair of silver wire electrodes. The voltage drop over a distance of 10 µm perpendicular to the epithelial plane was measured with glass microelectrodes that were controlled by a micromanipulator.

Potential gradients were recorded in the centers of 3 adjacent cells and at 3 tight junctions of these cells (n=8), and transformed into apparent trans- and paracellular conductivities, G^c_A and G^p_A. The true trans- and paracellular conductivities, G^c and G^p, contributed as $G^p_A = G^c + k_1 \cdot G^p$ and $G^p_A = G^c + k_2 \cdot G^p$, with k_1 and k_2 describing the contributions of G^p at the spots recorded from. Local transepithelial conductivity was determined with the scanning electrode 30 µm above the epithelium and artifacts due to incomplete confluence or edge damage were subtracted. Intercellular space width was reduced by mucosal addition of 40 mM sucrose. This provided a second set of data with decreased G^p but unchanged G^c, allowing the computation of true conductivities. The mathematical model was validated by the small variation of the parameters, $k_1 = 0.514 \pm 0.067$ and $k_2 = 1.61 \pm 0.20$.

Addition of sucrose reduced the transepithelial conductivity from 17.1 ± 2.0 mS/cm^2 to 9.4 ± 1.5 mS/cm^2. With symmetrical Ringer's, trans- and paracellular conductivities were determined as $G^c = 3.6 \pm 0.7$ mS/cm^2 and $G^p = 13.5 \pm 1.6$ mS/cm^2, respectively. After mucosal addition of sucrose, G^p decreased to 5.7 ± 1.0 mS/cm^2. The results show that, with symmetrical Ringer's, the paracellular pathway is about 4 times more permeable than the transcellular pathway. Thus, MDCK-cells (C-11 strain) form monolayers that can be classified as true leaky epithelia, as defined by a paracellular conductivity being higher than that of the transcellular pathway.

INTERLEUKIN-1β STIMULATES SHORT CIRCUIT CURRENT IN HUMAN DISTAL COLON.

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Cytokines have been implicated in the pathogenesis of various intestinal diseases e.g. inflammatory bowel disease and HIV-enteropathy. For this reason the influence of interleukin-1β (IL-1) on intestinal ion transport and barrier function was investigated.

In Ussing experiments the effect of IL-1 on short circuit current (I_{SC}) and transepithelial resistance (R^t) was determined. Measurements were performed on partially stripped tissue preparations (muscularis propria removed).

Serosal addition of IL-1 dose-dependently increased I_{SC} with a minimum effective concentration of 0.1 ng/ml ($p<0.05$; n=9). Maximum I_{SC} increase of 2.1±0.3 µmol·h^{-1}·cm^{-2} was obtained after 43±4 min at 20 ng/ml ($p<0.001$; n=10). R^t was 96±4 Ω·cm^2 and did not change within this time. Involvement of the enteric nervous system (ENS) in IL-1 action was investigated with the ENS blocker tetrodotoxin (TTX, 10^{-6} M). Pre-incubation (15 min) of the tissues with TTX had no inhibitory effect (ΔI_{SC}=1.8±0.3 µmol·h^{-1}·cm^{-2}; n.s.) indicating that IL-1 action is not mediated by the ENS. The role of arachidonic acid products in IL-1 action was tested by pre-incubation experiments with the cyclooxygenase blocker indomethacin (10^{-5} M) and the 5-lipoxygenase inhibitor ICI 230487 ($5 \cdot 10^{-8}$ M). Indomethacin completely blocked IL-1 action, while 5-lipoxygenase inhibition failed to prevent the IL-1 effect (ΔI_{SC}=2.1±0.5 µmol·h^{-1}·cm^{-2}; n.s.). The following experiments served to determine, whether IL-1 exhibits its effect directly at the colonocytes or by liberation of mediators from the subepithelium. First, IL-1 had no effect on the highly differentiated intestinal epithelial cell line HT-29/B6 suggesting that IL-1 did not act directly at the epithelial cells. Second, IL-1 elicited the same I_{SC} increase (ΔI_{SC}=2.4±0.7 µmol·h^{-1}·cm^{-2}; n.s.) on totally stripped tissues (submucosa additionally removed). From these results we assume that IL-1 stimulates the liberation of secretory mediators from lamina propria cells localized close to the colonocytes.

Conclusion: IL-1 dose-dependently increased I_{SC} in partially stripped human distal colon. Indomethacin blocked the IL-1-mediated secretory response suggesting prostaglandins to be mediators of IL-1 action. Since IL-1 had no effect on HT-29/B6 intestinal epithelial cell line and total stripping also had no effect on IL-1 action, we conclude that IL-1 stimulates prostaglandin liberation (most likely PGE_2) in lamina propria subepithelial cells. In contrast, lipoxygenase products and the enteric nervous system are not involved in IL-1 action.

By its secretory effect IL-1 could contribute to diarrhea occurring e.g. in inflammatory bowel disease and HIV-infection.

LIPID ABSORPTION IN THE SMALL INTESTINAL BRUSH BORDER MEMBRANE.

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Dietary lipids with a critical micellar concentration smaller than 10^{-7} to 10^{-8} are absorbed at the level of the small intestinal brush border membrane in a protein-mediated way. This kind of lipid are transported along the small intestine as lipid dispersions. The dispersed system prevailing at the site of lipid absorption, i.e., at the brush border membrane is the mixed bile salt micelle containing in addition to bile salts the major dietary lipids and the products of fat hydrolysis such as 2-monoacylglycerols, fatty acids, cholesterol and lysophospholids. Unambiguous evidence was presented that absorption of dietary cholesterol in the upper small intestine is a protein-mediated process. More recently our laboratory showed that long-chain cholesteryl esters and triacylglycerols are also incorporated in the brush border membrane in a protein-mediated manner, and that these compounds, contrary to the current textbook view, need not necessarily be hydrolysed prior to their absorption. We have isolated, purified and partially characterized some proteins responsible for the lipid uptake activities of the brush border membrane. The solubilization of brush border membrane vesicles with detergents and the reconstitution of these activities to well-defined proteoliposomes were carried out successfully. The reconstitution combined with immunostaining techniques allow us to assign these activities to integral membrane proteins the active centres of which are exposed on the outer or luminal surface of the brush border membrane.

EFFECTS OF THE K/H-ATPASE ON THE SURFACE pH OF COLONOCYTES OF THE DISTAL COLON OF GUINEA PIGS.

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The lipid solubility of weak electrolytes like short-chain fatty acids (SCFA) depends on their degree of dissociation. Thus, the proton activity at the luminal surface should be a determining factor for the absorption of these compounds by passive diffusion across the hindgut epithelium. The importance of proton secretion by an apically located, ouabain-sensitve K/H ATPase for the absorption of SCFA had been shown by flux measurements (Engelhardt et al., J. Physiol. (London) 460 (1993) 455-466).

We developed a method to continuously measure the proton activity at the luminal surface of the gastrointestinal mucosa. We used the pH-sensitive fluorescent dye 5-hexadecanoyl-amino fluorescein (HAF) to monitor the surface pH. This dye consists of a C16-fatty acid moiety linked to fluorescein and could be integrated in the apical membrane of the colonic cells by adding the dye to the perfusion solution. The fluorescence intensity of the labelled epithelium fixed in a microperfusion chamber was measured using an inverted fluorescence microscope. By excitation at two different wavelengths and calculation of the ratio of the two fluorescence intensities we obtained a concentration independent measure of the surface pH.

The influence of an apically located, ouabain-sensitive K/H ATPase on the surface pH could be shown in bicarbonate-free solutions. Reduction of the buffer concentration in bicarbonate-free medium from 21 mM to 5 mM gave a decrease for pH_s of 0.14 ±0,03 (n=3). This decrease could be abolished by the addition of 0.1 mM ouabain. In solutions containing 21 mM bicarbonate or 21 mM HEPES as buffer ouabain had no effects on the surface pH. Thus the K/H ATPase in the apical membrane of the coloncytes is active under resting conditions although its action can be masks by solutions with high buffer capacity.

GASTRIC MONOSACCHARIDE ABSORPTION: REGULATION BY pH.

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Elder and Lomas (1) showed that the absorption of glucose from the antral pouch of a surgically modified but unanaesthetised dog could be completely inhibited by raising the surface pH of an autotransplanted fundic pouch. As the fundic pouch was denervated its signal to the antral pouch had to be hormonal. The stomach is not regarded as a glucose absorbing organ though alkalinising its mucosal surface reduces intestinal glucose transport (see (2)). The aims of the current study were to see (a) if gastric glucose absorption occurred in herbivores and omnivores and if it was inhibited by raising the luminal pH and (b) if the mechanisms in the stomach could clarify the inhibitory process that occurs in the small intestine.

Measurements were made on anaesthetized animals in vivo by perfusing the stomach in situ with a single pass procedure. Buffered glucose was perfused for 1 hour at pH 3.3 (control) followed by 1 hour at pH 7.3, at 0.2 ml/minute. 10 minute samples were analysed to calculate the luminal loss using each animal as its own control. Sheets of gastric mucosal tissue obtained by procedures analogous to those described in (2) were used to estimate luminal loss and serosal gain of sugars during 1 hour incubation at 37°C.

Glucose perfused through guinea pig stomach in vivo at 2.5 and 7.5mM showed an increased luminal loss when the pH was raised to 7.3 (56 ± 5%(n = 5) and 53 ± 8% (n = 5) respectively), the opposite effect to that obtained by Elder and Lomas (1). Perfusion at 20 and 50mM glucose did however show decreased luminal loss (26 ± 5% (n = 6) and 26 ± 8 (n = 6) respectively) when the luminal pH was raised. Stimulation of luminal loss at 2.5 and 7.5 mM glucose and inhibition with 50 mM glucose were also observed with the rat in vivo.

Maintaining the luminal pH at 3.3 but infusing CCK-8 Sulphate intravenously during a second hour of perfusion inhibited luminal loss by 34 ± 11% (n = 6) in the guinea pig, a result that is consistent with the idea that a hormone released by alkalinization could affect transport in the stomach as it does in intestine (3).

^{14}C - sucrose added as a marker of paracellular transport showed increasing luminal loss in vivo as the luminal glucose concentration was increased; an effect that was also produced in vitro with guinea pig mucosal sheets. A relatively small percentage of the in vitro luminal loss of glucose appeared on the serosal side (20 - 45%) and probably did so by paracellular transport. The gastric mucosae appear to be more leaky than their intestinal counterparts and were more leaky at higher glucose concentrations. The results suggest that significant monosaccharide absorption could occur via the stomach, possibly by a paracellular route that is subject to regulation.

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DIFFERENT EFFECTS OF HISTAMINE AND CARBACHOL IN THE ISOLATED GASTRIC MUCOSA OF EEL, *Anguilla anguilla.*

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In our previous studies it was shown that the isolated gastric mucosa of eel, Anguilla anguilla, develops a mucosa negative short circuit current (I_{sc}),due to a Cl^- secretion in excess to H^+ secretion (non acidic Cl^- secretion), and secretes small but finite amounts of HCl. Both I_{sc} and the acid secretion rate (J_H) are independently stimulated by histamine and carbachol. In addition it was suggested that the two secretagogues modulate different transport mechanisms operating on the serosal membrane.

In this paper we try to identify the mechanisms modulated by the two secretagogues by employing the short circuit current and the pH stat methods.

Ion substitution experiments clearly showed that the J_H in both carbachol and histamine stimulated tissues as well as in control tissues (spontaneously secreting) is strictly dependent on the presence of serosal Cl^-, indicating that Cl^- accompanies H^+ secretion to mantain electroneutrality.

The H^+-linked Cl^- secretion might be mediated by a serosal Cl/HCO_3 exchange system, as serosal DIDS ($5{\cdot}10^{-4}$M) completely blocked the spontaneous as well as the secretagogues stimulated acid secretion.

On the contrary the stimulation of I_{sc} produced by histamine (10^{-4}M) or by carbachol (10^{-4}M) seems due to different mechanisms. In fact the carbachol stimulated I_{sc} was completely nullified by the serosal addition of DIDS suggesting that Cl/HCO_3 exchange mediates also the non acid Cl^- secretion. On the contrary both the control and the histamine stimulated I_{sc} were unaffected or increased by the serosal addition of DIDS whereas were reduced by 60% by serosal bumetanide (10^{-5}M), known inhibitor of the Na-K-2Cl triporter. The I_{sc} that remained after the maximal inhibition of bumetanide was nullified by the addition of DIDS in the serosal bath.The best way to explaine these results is that two different mechanisms responsible for non acidid Cl^- transport operate on the serosal membrane in the control tissues: the bumetanide sensitive Na-K-2Cl and the DIDS sensitive Cl/HCO_3.

Histamine would stimulated both mechanisms, while carbachol would act only on the Cl/HCO_3 exchange. The increase of I_{sc} sometimes observed in control or histamine stimulated tissues following DIDS treatment as well as the recovery of I_{sc} in the presence of the stilbenic inhibitor would suggest that the inhibition of a Cl/HCO_3 induces a stimulation of the Na-K-2Cl as a homeostatic reaction.This hypothesis is supported by the observation that the stimulation of I_{sc} produced by serosal DIDS was nullified by the addition of bumetanide.

The bumetanide sensitive transport mechanism is not likely to be responsible of the Cl^- transport associated to H^+ secretion as the loop diuretic did not modify J_H.

Differences in the action mechanism of histamine and carbachol are also suggested by the observation that while J_H stimulated by histamine was not affected by serosal Na^+ substitution, the J_H stimulated by carbachol was strictly dependent on the presence of Na^+ in the serosal solution.

Several hypothesis were evaluated to explain the Na^+ dependence of the J_H stimulated by carbachol. The most likely seems the presence of a base loader mechanism (a Na/H antiport or a $NaHCO_3$ cotransport) that increases the pH_i and hence stimulates the activity of the Cl/HCO_3 exchange to extrude HCO_3 for mantaining pH_i and to supply Cl^- for secreting HCl during carbachol treatment.

EFFECT OF GENISTEIN, A PROTEIN TYROSINE KINASE INHIBITOR, ON CHLORIDE SECRETION IN RAT DISTAL COLON.

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The protein tyrosine kinase inhibitor, genistein, caused an increase of short-circuit current (Isc) across the rat distal colon in forskolin-pretreated tissues, suggesting a synergistic interaction of the drug with cAMP-dependent secretion. In the absence of forskolin, genistein had a dual effect on Isc, it increased Isc in tissues with a low baseline, but decreased Isc in tissues with a high baseline Isc. The secretory effect of genistein was dependent on the presence of Cl^- and was blocked by inhibitors of Cl^- secretion like bumetanide, an inhibitor of the Na^+-K^+-Cl^--cotransporter, or 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), a Cl^- channel blocker. Unidirectional flux measurements revealed that genistein inhibited Na^+ and Cl^- absorption and induced net Cl^- secretion. The protein tyrosine phosphatase inhibitor vanadate suppressed the secretory effect of genistein. In contrast, genistein caused an inhibition of carbachol-induced, i.e. Ca^{2+}-mediated secretion.

Whole-cell patch-clamp experiments confirmed the synergistic effect of genistein on cAMP-induced Cl^- currents. In the presence of forskolin, genistein caused a depolarization concomitant with an increase in membrane inward current. In addition, genistein caused an inhibition of a basal K^+ conductance and inhibited the Ca^{2+}-dependent K^+ conductance stimulated by carbachol. These results suggest a complex role of the protein tyrosine kinase pathway in the control of colonic Cl^- secretion, an antagonistic action on the cAMP- and a synergistic action on the Ca^{2+}-pathway as revealed by the opposing effects of genistein. The physiological importance of this regulation remains to be clarified.

INFLUENCE OF PHOSPHORYLATION-ACTIVE COMPOUNDS ON PARACELLULAR TRANSPORT IN CACO-2 AND IEC-18.

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The primary physiological role of the intestinal epithelium is to separate the mucosal (external) and serosal (internal) compartments. Passive transport of hydrophilic compounds over the intestinal epithelium takes place by the paracellular pathway and is mainly restricted by the tight junctional complex. Only recently is has been recognized that several physical and chemical agents can alter the permeability of the paracellular route. One of the postulated mechanisms to regulate tight junctional permeability is tyrosin-phosphorylation of junctional proteins. In this study we investigated the phosphorylative status of junctional proteins and the effect on paracellular permeability. The test compounds were the PKC activator TPA, the protein tyrosine phosphatase inhibitor phenylarsine-oxide (PhAO) and the protein tyrosine kinase (PTK) inhibitors Erbstatin Analogue and H7.

The intestinal epithelial cell lines Caco-2 and IEC-18 were cultured for 19-23 days on Transwell polycarbonate filters (0.4 μm pore size). Both cell lines formed a confluent monolayer as confirmed by electrical resistance measurements ($TER_{Caco\text{-}2}$ = $\pm$600 $\Omega.cm^2$, $TER_{IEC\text{-}18}$ = $\pm$50 $\Omega.cm^2$) and the presence of ZO-1 and periferal F-actin. Transport studies were performed in apical to basolateral direction with the radioactively labelled tracers ^{3}H-Mannitol and ^{14}C-PEG-4000. The test compounds were incubated before or during the transport studies. Cytotoxicity was tested by LDH leakage and neutral red absorption. Lokalization and morphology of ZO-1 protein, F-actin and tyrosin-phosphorylated proteins was determined using immunocytochemistry and CSLM.

Incubation of IEC-18 with TPA resulted in a clear increase of transport of both tracers after 2 hours of exposure, without concomitant cytotoxicity. TPA did not result in a disturbance of the ZO-1 morphology, however, expression of phosphotyrosine seemed to relocate towards the cell periphery, indicating an increased phosphorylation of junctional proteins. Increased permeability was also found with PhAO, with the effect was being more pronounced in IEC-18. However, all effective concentrations showed cytotoxicity after prolonged exposure (4 hr). Erbstatin and H7, were not found effective in decreasing TER or increasing paracellular transport allone. We will test these compounds, and PhAO, in co-incubations with TPA and will present the results in the near future.

CYTOCHALASIN E INHIBITS ACTIVE TRANSPORT OF SUGARS IN RAT INTESTINE.

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Cytochalasins are fungal compounds with well-established effects as cytoskeleton inhibitors by their actions on actin filaments. Colchicine also modifies cytoskeleton activity by inducing microtubule depolymerization. Cytochalasin B has specifically been shown to bind to the equilibrative sugar transporter GLUT 2 inhibiting sugar transport across basolateral membrane of the enterocytes. In previous studies, an enhancing effect of cytochalasin E or colchicine on mannitol paracellular permeability in rat intestine *in vitro* has been reported, the effect being attributed to cytoskeleton alteration and tight jnction opening. To further investigate, the effects of those cytoskeletal inhibitors on galactose transport in rat intestine are presently studied *in vitro*.

In experiments with Ussing-type chambers, the presence of 0.5 mmol/L colchicine both in mucosal (M) and serosal (S) mediums does not modify mucosal to serosal galactose flux (M-S) although it significantly enhances the opposite S-M one. When 20 µmol/L cytochalasin E is added to both M and S sides an about 50 % inhibition of galactose M-S flux is observed without significant changes in S-M flux. The inhibitory effect requires the presence of cytochalasin E in the mucosal medium since it does not appear when cytochalasin E is only present in the serosal side. The uptake of 2 mmol/L galactose by everted intestinal rings after a 30 minute incubation is clearly diminished by 20 µmol/L cytochalasin E although some sugar accumulation in the total tissue water still remains. In Na^+-free mediums, no effect of cytochalasin E is observed, which suggests that the inhibition is exerted on the Na^+-dependent transport. By using everted intestinal sacs, the uptake of galactose by the luminal surface of the tissue and the sugar transference to the serosal medium can be measured since galactose and cytochalasin E are only present in the incubation (M) medium. The accumulated sugar into the tissue and the transferred galactose to serosal are both clearly inhibited by mucosal cytochalasin E after 30 min incubation. In shorter experiments (5 min) only the inhibition of sugar penetration in the tissue is significantly seen.

The results seem to indicate that cytochalasin E inhibits Na^+-sugar cotransporter activity, which could be modulated by the cytoskeleton.

LONGITUDINAL PATTERN OF DIGESTIVE AND ABSORPTIVE FUNCTIONS IN THE SMALL INTESTINE OF RATS AFTER DIETARY EXPOSURE TO CADMIUM CHLORIDE.

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Experimental findings in vitro and in vivo suggest at least an impairment of digestive and absorptive functions in the small intestine by luminally administered Cd in the micro to millimolar range. In the rat, diets with up to 1 µmol Cd/g are well tolerated, however. Therefore it can be assumed that the impairment, particularly for macro nutrients might not be this drastic or might be compensated by adaptive changes. To elucidate whether small intestinal functions are altered, we studied the effect of dietary Cd on the longitudinal pattern of mucosal enzymes and the in-vitro uptake of α-methyl glucoside in the small intestine of female Sprague-Dawley rats. Three groups of rats (8 animals each) were employed, a control group and two groups receiving dietary $CdCl_2$ either at 0.3 µmol or 1 µmol Cd/g of diet. After two weeks of feeding these diets, rats were killed; the entire small intestine was removed, rinsed with ice-cold saline and divided into 12 segments of equal length. Mucosal scrapings from each segment were used to measure mucosal Cd levels and enzyme activities (sucrase, lactase, alkaline phosphatase and glycylleucine hydrolase). Sugar uptake was determined in vitro in all segments by the tissue accumulation method. Although Cd levels in the mucosa were high (>100 µg Cd/g wet wt. at 1 µmol Cd/g of diet), most enzyme activities were only slightly changed. With no changes in their longitudinal distribution pattern, significant decreases in activity were particularly observed in the very proximal segments, only lactase activity was decreased by about 20% on average over the entire length of the small intestine. Sugar uptake was impaired only in the proximal segments. These findings suggest that cadmium even at dietary levels of 1 µmol/g, which are on average 1000-fold higher than the maximal permissible Cd concentration in human diets or animal feed, do not lead to a drastic impairment of digestive and absorptive functions in the small intestine and that in the rat the presently observed, mostly proximal impairments are easily compensated by unaltered distal functions. Certainly, absorption of micronutrients, for which an impaired proximal function cannot be compensated, e.g. iron, might be critical in this respect.

EFFECT OF NOCODAZOLE ON THE INTESTINAL SECRETION OF EXTRACELLULAR XENOBIOTICS AND INTRACELLULAR METABOLITES.

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In the isolated mucosa of guinea pig small intestine mounted in a flux chamber[1] 1-naphthyl sulphate (1-NS) is secreted from the blood side into the luminal solution. 1-NS which was formed intracellularly from 1-naphthol (1-N) added to the incubation solutions is excreted across the brush border as well as the luminal membranes of the enterocytes. The distribution to the luminal and blood side solution depends on the side of 1-naphthol administration in a characteristic pattern suggesting compartmentation of intracellular 1-naphthol metabolism[2].

In the teleost renal proximal tubule the secretion of organic anions is inhibited by the microtubule disrupting agent nocodazole [NOC][3]. To get further insight into the secretory routes of extra- and intracellular xenobiotics the influence of NOC [0.1 to 20 µM] on the intestinal secretion of 0.1 mM ^{35}S-labeled 1-NS added initially to the blood side solution has been studied in guinea pig isolated mucosa. 1-NS-secretion was diminished in a concentration dependent manner. Maximal inhibition of jejunal secretion to 10 % of control values was achieved using 10 µM NOC during a 30 min preincubation and 15 min incubation period. The colon was less sensitive to NOC, and revealed an inhibition to 20-25 % of control values after a 60 min preincubation.

After luminal incubation for 15 minutes with 1-N [130 µM] and sulphate [1 mM] 75 % of intracellularly formed 1-NS could be detected in the luminal solution, 23 % in the mucosa and 2 % in the blood side solution.With blood side administration of 1-N, about 50 % of 1-NS was found in the mucosa, the other half was equally distributed between the blood and the luminal solution. The main effect of NOC (10 µM) was an inhibition of the release of 1-NS to the luminal side to one half or one tenth of control values after 1-N administration to the luminal and the blood side, respectively. The release to the blood side and especially the tissue content were increased in a compensatory manner. 1-naphthyl glucuronide which is formed in addition to 1-NS behaved in a comparable manner. The effect of NOC was independent on the side of its administration to the mucosa.

In similar experiments no influence of NOC on the secretion of the cardiac glycoside digoxin and the absorption of sulphate and glucose could be detected under the above conditions.

The results suggest that the functional integrity of microtubules is crucial for the intestinal secretion of organic anions like sulphoconjugates and glucuronides, whereas other compounds like digoxin use NOC insensitive pathways.

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EFFECT OF NITRIC OXIDE SYNTHASE BLOCKADE ON NET FLUID TRANSPORT AND MUCOSAL PERMEABILITY IN THE JEJUNUM OF THE RAT *IN VIVO*.

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Almost all secretory states in the small intestine in vivo are at least partly mediated via the enteric nervous system (ENS). In the rat small intestine inhibition of NO synthase has been reported to induce a transient net fluid secretion and/or an increase of epithelial permeability (Nylander et al, Am J Physiol 2228:G1029-1038, 1993, Kubes Am J Physiol 262: G1138-1142, 1992). The aim of the present study was to investigate if ENS was involved in any of these effects.

^{14}C-mannitol and ^{51}Cr-EDTA were administered intravenously to anaesthetized rats after extirpation of the kidneys. A jejunal segment, 10-15 cm, was perfused with a modified Kreb´s solution containing either 30 mM mannitol or glucose. Net fluid transport (NFT) was reflected as changes in the continuously recorded perfusate volume. The passive transport of ^{14}C-mannitol and ^{51}Cr-EDTA from plasma to the lumen was calculated from the concentrations of the two probes in plasma and luminal perfusate and expressed as clearance values (µl(minxg)$^{-1}$). Inhibition of nitric oxide synthase was achieved by a bolus i.v. injection of L-NNA (N-ω-nitro-L-arginine), 10 mg/kg followed by a slow infusion at a rate of 3 mg(kg x h)$^{-1}$.

Administration of L-NNA elicited a transient net fluid secretion and a 2-3 fold increased clearance of the two probes with both perfusates. These effects were accompanied by an increased clearance ratio from 0.65-0.70 to 0.80-0.85 for the two perfusates. Prior infusion of L-arginine markedly reduced the L-NNA effect on NFT. Blockade of the nervous reflexes within ENS by hexamethonium (10 mg/kg), a nicotinic receptor antagonist, had no effect per se on clearance or clearance ratio of the two probes but enhanced net fluid absorption. L-NNA administration in this situation only induced a small secretory response. Also the leakage of the probes to the lumen was significantly reduced with the Kreb´s mannitol solution. However, the increase in permeability during the Kreb´s glucose solution was unaffected and the clearance ratio was further enhanced.

In conclusion, inhibition of endogenous release of NO induced a short lasting net fluid secretion and an increased epithelial permeability. The secretory response and the increase in permeability in the absence of glucose was to a major part mediated via the ENS. However, the permeability increase seen with the glucose containing solution was not dependent of ENS. The passive transport to the lumen seems at least partly to takes place via solvent drag. However, it is not known if the fluid secretion and the permeability changes are located to the same or different parts of the intestinal epithelium.

LACK OF BUTYRATE IS ASSOCIATED WITH INDUCTION OF BAX AND SUBSEQUENT APOPTOSIS IN THE PROXIMAL COLON OF GUINEA PIG.

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Butyrate stimulates proliferation and suppresses differentiation in normal colonic epithelial cells. While the underlying mechanism is unclear, we investigated certain molecular effects of butyrate on colonocytes *in vitro*.

Tissue sheets from guinea-pig proximal colon were mounted in Ussing chambers and incubated in the presence and absence of butyrate. Colonic tissues were analyzed by scanning electron microscopy, DNA analysis and Western blots.

Following incubation for 150 min without butyrate, the morphology of the colonic mucosa demonstrated degeneration of colonocytes and massive apoptoses. Simultaneously, these colonocytes exhibited a significant oligonucleosomal DNA fragmentation. In contrast, addition of physiological concentrations of butyrate (10 mM) to colon sheets revealed no detectabel DNA fragmentation within 150 min. While induction of apoptosis in the butyrate-deprived coloncytes appears primarily in the G_0/G_1 phase of the cell cycle, the intracellular signaling targest remain unclear. Western blot analysis revealed little if any difference in the level of Bcl-2 expression in colonocytes incubated with or without butyrate for up to 15 min. In contrast, expression of Bax proteins continuously increased after 60 min without butyrate and reached a 5-fold induction after 150 min when compared to cells incubated in the presence of butyrate.

In conclusion, the removal of butyrate from colonocytes *in vitro* induces increased expression of Bax proteins paralleled by rapid apoptosis.

DUODENAL MUCOSAL BICARBONATE SECRETION AND PERMEABILITY.

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The gastroduodenal mucosa is covered by a continuous layer of visco-elastic mucus gel which provides a physical barrier between the apical cell surfaces and the lumen (1). Prevention of the migration of large molecular weight bacterial toxins would make their release at the mucus-cell interface a prerequisite for effects on the mucosa. We therefore studied the migration of choleratoxin (subunit A, M_w 27 kD + five subunits B, M_w 11.7 kD = 85 kD) and toxins from Helicobacter pylori (M_w VacA 94 kD and M_w CagA 120 kD) across the surface mucus gel. Effects of glucagon (M_w 3.5 kD), a known stimulant of duodenal mucosal bicarbonate secretion, were tested for comparison. The bicarbonate secretion increases the pH at the duodenal cell-mucus interface and protects against luminal acid (2).

Assuming that mucosal bicarbonate secretion and/or mucosal permeability (^{51}Cr-EDTA clearance) could be affected by the toxins, these parameters were used as indices of the appearance of the toxins at the cell surface. Blood-to-lumen ^{51}Cr-clearance is, furthermore, a good estimate of mucosal integrity (3). Toxins or glucagon were applied luminally and experiments performed in intact rat duodenum and after removal of the surface mucus gel by perfusion with papain-containing solution (5-10 U/ml) for 30 min. Water extracts from either of two Helicobacter pylori cytotoxin-producing strains (88-23 and 17874) were then perfused for 120 min or purified cholera toxin (1-8 µg/ml) for 30 min. Glucagon (0.5 or 50 µg/ml) was perfused for 60 min. The absence of a continuous mucus gel was confirmed at the end of each experiment by examination of unfixed sections by light microscopy.

Luminal glucagon (50 µg/ml) stimulated the bicarbonate secretion, indicating some migration of this peptide across the intact mucus gel. Removal of the mucus gel was, however, required for a response to the lower (0.5 µg/ml) concentration of the glucagon. Cholera toxin caused a dose-dependent (up to four-fold) increase whereas Helicobacter pylori toxins only weakly stimulated duodenal mucosal bicarbonate secretion in papain-treated mucosae. Neither these toxins nor the treatment with papain alone affected mucosal permeability. The effect of cholera toxin was significantly smaller, and Helicobacter pylori toxin did not affect duodenum with an intact mucus gel. The presence of an intact mucus gel thus decreases migration of cholera toxin to the epithelial surface and release of Helicobacter pylori toxin at the at cell-mucus interface seems a prerequisite for effects.

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INTESTINAL ABSORPTION OF CYCLOSPORIN A AND ITS MODULATION BY P-GLYCO-PROTEIN IN MAN. G. Fricker, J. Drewe*, Ch. Beglinger*, *Institute of Pharmaceutics and Biopharmacy, Heidelberg, Germany and *Medical Outpatient Clinic and Division of Gastroenterology, Kantonsspital, Basel, Switzerland.*

Cyclosporin A (CSA) interacts with p-glycoprotein mediated transport in the liver and in kidney proximal tubules (1,2). Therefore, we investigated in *in vitro* studies and in an intubation study with healthy volunteers, whether CSA also interacts with p-glycoprotein during its intestinal uptake after oral administration. Intestinal interaction of CSA might be a reason for the rather low and highly variable absorption of CSA in animal and man. Human Caco-2 cell monolayers served as *in vitro* model throughout our studies.

CSA permeation through the cell monolayers from the apical to the basolateral side was apparently saturable up to a concentration of 0.5 μM. At higher concentrations an overproportional increase of permeation was seen. The permeation was significantly higher, when CSA was given from the basolateral side of the cell system, as compared to permeation after administration to the apical side. Permeation of CSA from the apical to the basolateral side was increased in the presence of the p-glycoprotein inhibitors PSC-833, vinblastine and daunomycin. Basolateral to apical permeation of CSA exhibited a dose dependent decrease in the presence of vinblastine. When the transport of daunomycin across the Caco-2 cell monolayers was determined, the permeation from the basolateral side to the apical side of the monolayer was decreased in the presence of PSC-833 and CSA.

When the enteral absorption of CSA in the GI-tract was determined in healthy volunteers, a decrease of the blood AUC of CSA could be observed dependent on the location of absorption in the rank order stomach > jejunum/ ileum > colon. The decrease of the fraction absorbed exhibited a marked correlation (r=0.994) to the extent of expression of mRNA for p-glycoprotein in the various locations of the GI-tract (stomach < jejunum < colon).

In summary, the data give evidence that CSA is a substrate for p-glycoprotein in the GI-tract, which might explain the local differences as well as the high variability in CSA absorption.

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KINETICS OF D-GLUCOSE AND D-FRUCTOSE TRANSPORT ACROSS THE BASOLATERAL MEMBRANE OF THE CHICKEN JEJUNUM. C. Garriga, M. Moretó, J.M. Planas, *Unitat de Fisiologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain.*

We have studied the transport kinetics of D-glucose and D-fructose by the basolateral membrane of the jejunum of the chicken. White Leghorn chickens, 5-6 weeks old were used. Membrane vesicles were isolated from the basolateral domains of the jejunum using a $MgCl_2$-based method(1). The enrichment in ouabain-sensitive K^+-activated phosphatase activity was 7.5-fold while the activity of sucrase (a brush border marker enzyme) was unchanged, indicating that our vesicle preparation was mainly composed of BLMV. The time-course of D-glucose and D-fructose uptake was linear for the first 10 s incubation and reached equilibrium at min 30. The intravesicular space was 2.3 μL/mg protein. The nonlinear regression analysis of D-glucose and D-fructose influx indicates that both sugars are taken up by a single, saturable transport system. The table shows the kinetic data (mean ± standard error) of 4 separate experiments:

Kinetic constant	D-GLUCOSE	D-FRUCTOSE
Vmax (pmol/mg.s)	2368 ± 425	3790 ± 319
Km (mmol/L)	17.3 ± 3.3	40.4 ± 3.3
$Ki_{D\text{-}glu}$(mmol/L)	17.4 ± 1.6	18.7 ± 1.8
$Ki_{D\text{-}fru}$(mmol/L)	40.3 ± 4.4	38.1 ± 0.1

The transport of both hexoses was independent of external Na^+ and sensitive to theophylline and cytochalasine B. Neither L-glucose nor α-methyl-D-glucose affected the initial influx of D-glucose or D-fructose. However, both 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose significantly reduced the transport of D-glucose and D-fructose. Trans-stimulation of D-fructose uptake (more than 30%) was observed in vesicles pre-loaded with D-glucose and 2-deoxy-D-glucose but not in vesicles preloaded with L-glucose. The results indicate that the basolateral membrane of chicken jejunum has a transport system with characteristics of a GLUT2 isoform, which is responsible for the movement of both D-glucose and D-fructose out of the enterocyte across the basolateral membrane.

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ENTERAL ABSORPTION OF SOMATOSTATIN ANALOGS: STRUCTURE-ABSORPTION RELATIONSHIPS. G. Fricker, E. Küsters[1] J. Drewe[2], *University of Heidelberg, Heidelberg, Germany, [1]Sandoz Pharma AG, Basel and [2]Kantonsspital, Basel, Switzerland.*

The intestinal absorption of a series of glycosylated somatostatin analog peptides was compared using rat enterocyte brush border membranes as an *in vitro* test system and rats as an *in situ* absorption model. Derivatives of the cyclic octapeptide octreotide with mono- di- and trisaccharide residues were used througout the studies. The nonglycosylated peptide was taken up into the membrane vesicles by transport mechanisms comprising both saturable uptake and passive diffusion. The uptake of octreotide was inhibited by the glycosylated analogs, but not by the bicyclic octapeptide α-amanitin, which exhibits some structural similarity, but is not absorbed in rats. The inhibition of octreotide permeation into the vesicles decreased in the presence of derivatives with an increasing length of the carbohydrate residues. The lipophilicity of the peptides was determined by measurement of $P_{octanol/buffer}$. Comparison of the extent of inhibition of octreotide transport with the lipophilicity of the glycosylated analogs suggested, that the capability to form hydrogen bonds is rather the limiting factor for absorption than just the lipophilicity of the peptides. To evaluate, whether the vesicle system is predictable for the *in vivo* situation, the extent of absorption of the peptides was determined after *in situ* administration of the peptides into the jejunum of rats and into a mesenteric vein, subsequent determination of plasma concentrations and calculation of the absorption efficiency. A linear relation between inhibitory capacity in the vesicle system and *in situ* absorption efficiency was found, suggesting that the membrane vesicles comprise a useful tool to predict the bioavailability of somatostatin analog drugs *in vivo*.

ATYPICAL SEROTONERGIC CONTROL OF ION TRANSPORT IN THE RAT DISTAL COLON UNDER CONTROL CONDITIONS AND IN A MODEL FOR IBS. J. Goldhill, P. Pichat, I. Angel, *Synthelabo Recherche (L.E.R.S), Rueil Malmaison, France.*

5HT receptor antagonists are effective in the treatment of irritable bowel syndrome (IBS), an illness that is characterized by abdominal pain, diarrhoea and/or constipation. The efficacy of this class of compounds may result from antinociceptive or antispasmodic effects. In addition diarrhoea and/or constipation may result from altered epithelial transport, and as 5HT has previously been shown to evoke intestinal secretion, 5HT receptor antagonists may also modify ion transport during IBS. To develop 5HT antagonists as antisecretory agents for use in IBS it is first neccessary to elucidate 5HT control of secretion under healthy conditions and secondly to determine changes during IBS. To address these points, short-circuit current (SCC) generated across muscle stripped rat colonic epithelium was continuously monitored using the classic Ussing chamber technique. After a 30min incubation period in the presence or absence of specific antagonists, cumulative concentration response curves to 5HT were assessed. Groups of control or stressed rats were used in these experiments, the latter having previously been used as a model for IBS. Animals were stressed on 3 consecutive days for 2 hours through mild restraint of their forelimbs. Under control conditions 5HT evoked a reproducible increase in SCC (E_{max} 56±4μAmps/cm^2, pD_2 4.8). This response was unaffected by the mixed $5HT_1/5HT_2$ receptor antagonist, methysergide (10μM), the $5HT_3$ antagonist, ondansetron (1μM), the $5HT_4$ receptor antagonist, GR113808 (10nM) or by cisapride (3μM). Methiothepin (0.1μM) reduced the maximal response to 5HT (67±9 vs 32±8 μAmps/cm^2; $p<0.05$) without affecting the pD_2. Chronic stress also reduced the maximal response to 5HT (41±9 vs 20±2 μAmps/cm^2; $p<0.05$) without affecting the pD_2. We conclude that under the present conditions, 5HT stimulates secretion in the rat colon through an atypical methiothepin-sensitive receptor. This response is attenuated in an animal model for IBS, and may result in reduced fluid output and consequent constipation.

OVERLAPPING OF SPONTANEOUS, STEROID-, AND SECRETAGOGUE-INDUCED K^+ (Rb^+) SECRETION IN SURFACE AND CRYPTS OF RAT DISTAL COLON.

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Second to Cl^- secretion, K^+ secretion is one of the main driving forces for secretory diarrhea. In voltage scanning experiments we have localized Cl^- secretion both in colonic crypts and surface epithelia (Köckerling et al. 1993, AJP 264: C1294). Aim of this study was the differentiation of spontaneous, cAMP, and aldosterone induced electrogenic K^+ secretion and its localization. Voltage scanning and Ussing chamber flux experiments were performed in rat late distal colon in the presence of mucosal amiloride (100 µM). Bidirectional $^{86}Rb^+$ fluxes were measured to corroborate that changes in short circuit current (ΔI_{SC}) indeed represent K^+ secretion.

Electrogenic K^+ secretion was determined by mucosal addition of the K^+ channel blocker TEA (20 mM). Under unstimulated control conditions, this resulted in ΔI_{SC}= 0.8±0.1 µmol·h^{-1}·cm^{-2} and ΔJ^{Rb}_{net}= 0.6±0.1 µmol·h^{-1}·cm^{-2} (n=11). Voltage scanning (n=10) showed, that there is a small spontaneous K^+ secretion which is significant in surface epithelium (ΔG^S= 0.3±0.1 mS/cm^2) but not in crypts (ΔG^C= -0.1±0.1 mS/cm^2). After in vitro preincubation with aldosterone (3 nM) for 6 h, TEA unmasked K^+ secretion in surface epithelium only (ΔG^S= 1.1±0.3 mS/cm^2) but not in crypts (ΔG^C= 0.1±0.3 mS/cm^2). Spontaneous K^+ secretion may represent intrinsic corticosteroid action.

Epinephrine (5 µM, both sides), which stimulates K^+ secretion via cAMP without accompanying Cl^- secretion, resulted in ΔI_{SC}= -0.6±0.1 µmol·h^{-1}·cm^{-2} and ΔJ^{Rb}_{net}= -0.5±0.1 µmol·h^{-1}·cm^{-2} (n=9). TEA block yielded ΔI_{SC}= 1.6±0.1 µmol·h^{-1}·cm^{-2} and ΔJ^{Rb}_{net}= 1.2±0.1 µmol·h^{-1}·cm^{-2}. About half of this K^+ secretion has to be attributed to spontaneous secretion, therefore. After correction for spontaneous K^+ secretion, voltage scanning yielded epinephrine-induced conductivity changes of ΔG^S= 0.4±0.1 mS/cm^2 and ΔG^C= 0.7±0.2 mS/cm^2, indicating that cAMP-mediated K^+ secretion may originate from both structures.

Addition of epinephrine (5 µM) to tissue preincubated with aldosterone (3 nM, 6 h) resulted in ΔI_{SC}= -0.6±0.1 µmol·h^{-1}·cm^{-2} and ΔJ^{Rb}_{net}= -0.4±0.1 µmol·h^{-1}·cm^{-2} (n=9). TEA block then yielded ΔI_{SC}= 2.0±0.2 µmol·h^{-1}·cm^{-2} and ΔJ^{Rb}_{net}= 1.6±0.1 µmol·h^{-1}·cm^{-2}. After subtraction of spontaneous and cAMP-induced K^+ secretion, aldosterone-induced K^+ secretion was 0.4 µmol·h^{-1}·cm^{-2} ($p<0.01$). This indicates that aldosterone-induced K^+ secretion is in part additional to cAMP-induced K^+ secretion. Rb^+ net fluxes consistently measure about 80% of ΔI_{SC}-derived K^+ secretion.

RAT ILEAL RESPONSE TO 5-HYDROXYTRYPTAMINE (5-HT) *IN VITRO* INVOLVES MORE THAN ONE 5-HT RECEPTOR SUBTYPE.

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5-HT induces secretion in the small intestine, but the receptors responsible are not yet fully identified. Recent *in vivo* studies suggest that more than one 5-HT receptor subtype contributes to the response (1) and this investigation aimed to determine whether this also occurred *in vitro*.

Sheets of distal ileum were mounted in Ussing chambers and their electrical activity monitored. Serosal 5-HT (10^{-4}M) caused similar rises in short-circuit current (SCC) in intact and stripped (outer muscle layers and myenteric plexus removed) sheets (52±4(48) and 58±2(52) µA/cm^2, $P>0.05$). These effects were concentration-dependent with maximum SCC changes of 51±9(8) µA/cm^2 in intact sheets and 67±8(8) µA/cm^2 in stripped sheets, with corresponding EC50 values of 20.0 (15.3-26.2) and 2.5(1.9-3.2) µM (geometric mean (95% confidence interval), $P<0.001$). 10^{-4}M 1-phenylbiguanide (PBG), a selective 5-HT$_3$ agonist, increased the SCC by 21±3(29) µA/cm^2 in intact sheets, with a significantly smaller change in stripped sheets (11±2(24) µA/cm^2, $P<0.05$). Responses to PBG were less than those obtained with equimolar 5-HT ($P<0.001$ in both cases). 5-methoxytryptamine (5-MT, 10^{-4} M), an agonist at all receptors except 5-HT$_3$, was more effective in stripped sheets (stripped: 48±2(24) µA/cm^2; intact: 14±2(28) µA/cm^2, $P<0.001$). As both PBG and 5-MT induce a response, more than one 5-HT receptor subtype must contribute to 5-HT-induced secretion. The component mediated by 5-HT$_3$ receptors is totally neurogenic as tetrodotoxin (TTX, 10^{-5}M) abolished the response to 10^{-4}M PBG in intact sheets, whilst the response to 10^{-4}M 5-HT was inhibited by only 57±3(4)% ($P<0.001$). Some of the neural elements involved in the 5-HT response are in the myenteric plexus as the TTX-induced inhibition was reduced to 25±8(8)% in stripped sheets ($P<0.05$).

These secretory responses are subject to desensitisation *in vitro* and a second application of agonist fails to increase the SCC. Prior desensitisation to PBG (10^{-4}M) reduced the response to 10^{-4}M 5-HT by 32±11(8)% in intact sheets ($P<0.05$), but had no effect in stripped sheets ($P>0.05$, n=8). Desensitisation to 5-MT (10^{-4}M) abolished the 5-HT response in stripped sheets ($P<0.001$, n=8), but caused only a 42±12(8)% inhibition in intact sheets ($P<0.01$).

5-HT-induced ileal secretion involves the stimulation of more than one 5-HT receptor subtype. 5-HT$_3$ action is entirely neurally-mediated, primarily via the myenteric plexus, whilst other receptor subtypes activate both neural and non-neural pathways.

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COMPARISON OF THE SECRETORY RESPONSE TO 5-HYDROXYTRYPTAMINE (5-HT) IN THE JEJUNUM AND ILEUM OF THE RAT *IN VITRO*.

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Although the ability of 5-HT to induce intestinal secretion is well-established, the nature of the response is still far from clear and it is becoming apparent that considerable regional variations exist. The present study compares the secretory effects of 5-HT in the jejunum and ileum of the rat.

Intact sheets of proximal jejunum and distal ileum (excluding the last 5cm) were mounted in Ussing chambers and their electrical activity monitored. Serosal application of 5-HT (10^{-4}M) caused a maximum response, increasing the short-circuit current (SCC) by 79±4(53) µA/cm^2 in the jejunum and 52±4(48) µA/cm^2 in the ileum ($P<0.01$). Cumulative concentration response curves yielded EC50 values of 18.7 (10.3-34.1, n=5) and 20.0 (15.3-26.2, n=8) µM respectively (geometric mean (95% confidence interval), $P>0.05$).

The ionic basis of the increased SCC differed in the two regions. Cl-free (gluconate) conditions or serosal furosemide (10^{-3}M) had no effect in the jejunum ($P>0.05$ in both cases), but reduced the ileal response to 5-HT (10^{-4}M) by 48±6(8)% ($P<0.001$) and 48±8(6)% ($P<0.01$) respectively. Removal of HCO_3 (phosphate) reduced the 5-HT (10^{-4}M)-induced rise in SCC in the jejunum by 83±5(8)% ($P<0.001$), but was without effect in the ileum ($P>0.05$).

Neural involvement in the response to 5-HT (10^{-4}M) was greater in the jejunum, where tetrodotoxin (TTX, 10^{-5}M) caused an 86±2(4)% inhibition compared with a decrease of 57±3(4)% in the ileum ($P<0.001$). In the jejunum the neural elements involved were located in the myenteric plexus as TTX failed to inhibit the 5-HT response in stripped sheets where this plexus has been removed ($P>0.05$). In the ileum however, a significant neural component remained in stripped sheets as TTX caused a 25±8(8)% inhibition of the response to 5-HT (10^{-4}M), although this was significantly smaller than that observed in intact sheets ($P<0.05$).

These studies indicate that while both jejunum and ileum respond to 5-HT, there are important differences in the nature of the response. In the jejunum the rise in SCC is predominantly due to a stimulation of HCO_3 secretion, while in the ileum Cl secretion is responsible. Neural involvement is greater in the jejunum where the myenteric plexus plays a major role. In the ileum however, both the myenteric plexus and other intramural neural elements contribute to the response.

WHAT ARE THE DIFFERENCES IN FUNCTION OF THE INTESTINAL PEPTIDE TRANSPORTER PepT1 AND ITS RENAL COUNTERPART rPepT2?

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PepT1 and rPepT2 were isolated from rabbit intestinal and kidney cortex cDNA libraries by expression cloning in *Xenopus laevis* oocytes (1-3). Both cRNA´s when expressed in oocytes induce a transport activity that is characterized by electrogenic peptide/H^+ symport. Sequencing of the open reading frames of the cDNA´s of PepT1 and rPepT2 identified gene products of 707 (PepT1) and 729 (rPepT2) amino acids. Hydrophathy analysis predicted both gene products to have 12 membrane spanning domains and a large extracellular loop between membrane domains 9 and 10. In vitro translation of the cRNA´s in the presence of microsomes (for core glycosylation) revealed proteins with apparent molecular weights of 78 kDa (PepT1) and 107 kDa (rPepT2). Although both transporters have been shown to transport dipeptides, any differences in transport function have not been investigated systematically. We therefore employed flux studies with radiolabeled substrates and the two electrode voltage clamp to determine similarities and differences in the transport modes of PepT1 and rPepT2. **METHODS**: Carrier function was assessed in *Xenopus* oocytes expressing the proteins after injection of 5 ng of the transporters cRNA and in HeLa cells transfected with the cDNA of PepT1 and rPepT2 in a eukaryotic expression vector under control of a cytomegalovirus promotor. Electtrophysiological studies where carried out in oocytes clamped to a membrane potential of -60mV and current voltage relationships were obtained as described previously (3). **RESULTS:** Studies with the dipeptides ^{3}H-glycine-(L)-glutamine and ^{3}H-(D)-phenylalanine-L-alanine and the ß-lactam antibiotic ^{3}H-cefadroxil showed that PepT1 has a pH optimum for uptake at 6.5 whereas rPepT2 function is maximal at pH ≤ 5.5. Kinetic analysis of transport as a function of substrate concentration revealed that rPepT2 has a at least 15 times higher affinity for cefadroxil than PepT1 whereas for both dipeptides the affinity of rPepT2 is only fivefold higher. With respect to substrate recognition and stereospecificity both transporters discriminate between peptides containing L- or D-amino acids but rPepT2 appears not to transport peptid mimetics like enalapril and captopril. In contrast, PepT1 has a much lower affinity for dibasic dipeptides like glycyl-lysine and glycyl-arginine at all pH vaues. Two electrode voltage clamp experiments established that both transporters operate electrogenic with all substrates but rPepT2 generally generates lower maximal currents under V_{max} conditions.

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THE LIPID CONTENT OF THE RAT MOTHER'S DIET ALTERS NUTRIENT ABSORPTION IN THE SUCKLING OFFSPRINGS. E. Jarocka-Cyrta, M. Keelan, N. Perin, M.T. Clandinin, A.B.R. Thomson, *Divison of Gastroenterology and Nutrition and Metabolism Group, University of Alberta, Canada.*

The nutrient coposition of the diet is important in modulating the form and function of the intestine. The adaptability of the intestine may be altered by dietary lipids. Changes in dietary lipids consumed by the nursing mother result in variations in the lipid content of her milk. Accordingly, the *in vitro* uptake of varying concentrations of D-glucose and D-fructose (4-64 mM) and single concentrations of medium or long chain fatty acids and cholesterol were assessed in 18-21 day old suckling rats. Pregnant rats were fed one of five diets during the period of lactation: a diet with a n6/n3 ratio of 7.3:1, a diet with a n6/n3 ratio of 4:1, a diet with 1.23% arachidonic acid (AA), a diet with 1.17% AA and 0.70% docosahexanoic acid (DHA), and a diet with 0.77% as DHA. The individual lipid constituents of the diet influenced the uptake of sugars and lipids. Fructose uptake in suckling rats was greater when the nursing mothers were fed the low as compared with the high n6/n3 diet. The jejunal uptake of glucose was lower when the mothers were fed a diet containing 0.7% DHA, but when 1% AA was added to this DHA diet the reduced glucose uptake was restored to normal. Adding 1% AA to the mother's diet reduced the jejunal and ileal uptake of stearic acid (1*:0), and jejunal uptake of linoleic acid (18:2). The inhibitory effect of AA on the uptake of 18:0 and 18:2 was prevented by adding 0.7% DHA to the AA diet. The uptake of oleic acid (18:1) was lower in the jejunum of the sucklings when the nursing mothers were fed a diet containing 0.7% DHA. These differences in uptake were not explained by variations in body weight or in the intestinal morphology. Thus, changes in the lipids in the diet of the lactating mother rat alter the normal development of nutrient transport in their nursing offspring.

DIABETES AND THE REGULATION OF INTESTINAL GLUT5 AND GLUT2. G.L. Kellett, C.P. Corpe, M. Basaleh, J. Affleck, *Department of Biology, University of York, York, U.K.*

Fructose is sweeter, more soluble and less glucogenic than either glucose or sucrose and has accordingly been recommended as a substitute for them in the diets of diabetic and obese people. We have therefore studied the adaptation of intestinal fructose transport to diabetes.

Highly purified brush-border (BB) and basolateral (BL) membrane vesicles (MV) were prepared from mucosal scrapings collected from the jejunum of an anaesthetised rat. The isoforms of the facilitative glucose transporter family present were then identified by 10% SDS-PAGE and Western blotting, using rabbit polyclonal antibodies specific for the C-termini of the rat isoforms GLUT 1 to 5 provided by Dr GW Gould (Glasgow), detection was by ^{125}I-goat anti-rabbit IgG and autoradiography. The major fructose transporters identified in normal rats were GLUT5 in BBMV and GLUT2 in BLMV; low levels of GLUT2 were detected in BBMV. After 10 days of diabetes (65 mg kg^{-1} streptozotocin I.V.), GLUT5 levels in BBMV were enhanced 3-fold, while GLUT2 levels were enhanced 7-fold in BBMV and 2-fold in BLMV.

Transport of 1 mM-fructose by GLUT5 in BBMV was not inhibited by 100 mM-D-glucose or 0.1 mM-phloretin. In contrast, transport of fructose by GLUT2 in BLMV was strongly inhibited by both, so that residual transport was not significantly different from the diffusive component measured with 1 mM-L-glucose. Overall fructose transport in BBMV was enhanced 57 % ($P < 0.05$) after 10-days diabetes. The differences in stereospecificity between GLUT5 and GLUT2 were used to detect the presence of GLUT2 in BBMV from 10-day diabetic rats as a D-glucose-inhibitable component that was not observed in BBMV from normal rats. The findings with vesicles were confirmed in perfusion studies of whole intestine. Luminal perfusion of jejunal loops *in vivo* with 5 mM-fructose revealed that fructose transport was inhibited in diabetic compared with normal rats, probably because the mucosal concentration of glucose, which inhibits fructose metabolism, is much higher in diabetic rats. Nevertheless, the presence of GLUT2 in the brush-border membrane of diabetic rats could be detected as a phloretin-inhibitable component of fructose transport not detectable in normal rats.

NUTRIENT TRANSPORT IN WEANLING RATS ADAPTS TO CHANGES IN DIETARY LIPIDS, E. Jarocka-Cyrta, M. Keelan, N. Perin, M.T. Clandinin, A.B.R. Thomson, *Divison of Gastroenterology and Nutrition and Metabolism Group, University of Alberta, Canada.*

The intestine of adult rats adapts in response to isocaloric changes in the lipid composition of the diet. Varying the lipid content of the diet of nursing dams results in alterations in sugar and lipid uptake into the intestine of their suckling offspring. In this study we wish to determine whether the same alterations in dietary lipids resulted in adaptation of the intestinal transport in weanling rats. The *in vitro* uptake of varying concentrations of D-glucose and D-fructose (4-64 mM) and single concentrations of medium or long chain fatty acids and cholesterol were assessed in six week old weanling rats. Their nursing mothers had been fed one of the same five diets onto which the offspring had been weaned at three weeks of age, and the same diets were continued for three weeks. These diets contain 15-84 % of total fatty acids (w/w) as 18:2n-6 and a n6/n3 ratio of 7.3:1, a diet with 17.61 % of total fatty acids as 18:2n-6 and a n6/n3 ratio of 4:1, a diet with 16.24 % of total fatty acids as 18:2n-6 and 1.23 % arachidonic acid (AA), a diet with 16.79 % of total fatty acids at 18:2n-6, 1.17 % AA and 0.70 % docosahexanoic acid (DHA), and a diet with 16.04 % of total fatty acids at 18:2n-6 and 0.77 % as DHA. The individual constituents of the diet influenced the uptake of sugars and lipids, as compared with animals fed high n6/n3. The jejunal and ileal rates of uptake of higher concentrations of D-glucose were greater in weanling rats fed 1 % AA, and lower fed in those fed 1 % AA plus 0.7 % DHA, or low n6/n3. These changes were due in part to alterations in the passive permeability of the intestine to glucose (as measured with L-glucose), and also were in part due to variations in the values of the maximal transport rate (Vmax). Fructose uptake was higher in 1 % AA and in 0.7 % DHA, but was lower in 1 % AA plus 0.7 % DHA, and was lower in low as compared with high n6/n3. The incremental change in free energy associated with the uptake of fatty acids was lower in the jejunum in 1 % AA plus 0.7 % DHA than in the other diet groups, but was higher int he ileum. The jejunal uptake of 18:0 was lower in 0.7 % DHA and in 1 % AA plus 0.7 % DHA, as compared with AA alone, whereas the ileal rates of uptake of long chain fatty acids was unaffected by diet. Animals fed 1 % AA plus 0.7 % DHA gained more weight, in part due to a greater ratio of weight gain per food intake; and yet the reduced nutrient uptake in 0.7 % DHA and low n6/n3 did not result in changes in weight gain since, the reduced food intake was compensated for by an increased ratio of weight gain for food intake. The differences in nutrient uptake in the various dietary lipid groups were not explained by changes in the villus surface area. Thus, the intestine of weanling rats adapts its nutrient transport in response to variations in dietary lipids, and the lipid composition of the diet of weanling animals may have important nutritional consequences.

ENTEROCYTE DIFFERENTIATION AND GLYCOSYLATION OF MEMBRANE GLYCOPROTEINS DURING ONTOGENY. J. Kolínská, J. Kraml*, M. Zákostelecká, L. Kadlecová*, D. Hiršová*, *Institute of Physiology ASCR, *the 1th Medical Faculty of Charles University , Prague, Czech Republic.*

Small intestine represents a unique system for studying the mechanism of development at various stages of differentiation. During ontogeny the small intestine undergoes profound physiological and morphological changes in the period between birth and weaning which proceeds in rat during the second and third week of life. The activities of enzymic glycoproteins located in the intestinal brush-border membrane dramatically change and the glycoforms switch from predominantly sialylated glycoconjugates to those expressing predominanty fucose. The principal contributor to developmental decline of bound sialic acid expression is the α2,6-sialyltransferase (α2,6-ST). The important marker of enterocytes is the enzyme dipeptidyl peptidase IV (DPP IV). In enterocytes it is associated predominantly with the brush-border membrane where it plays its role in terminal digestion of proteins. It is a good marker of developmental decline in sialylation, being less sialylated in the postweaning period.

Glucocorticoid hormones play an important role during the fetal period in the process of organ differentiation and maturation. In rats serum levels of these hormones increase near the weaning period and their administration induces striking changes in morphology and function of intestinal epithelium; among them the most important is the precocious appearance of sucrase-isomaltase, a brush-border marker of the adult mucosa. Concerning glycoconjugates glucocorticoids play a key role in inducing the precocious appearance of fucosyltransferase and in accelerating the originally continuous decrease in sialylation and sialyltransferase activity, documented recently at the molecular level by a significant decrease in the α2,6-ST mRNA level after hydrocortisone administration to suckling rats. We used antiglucocorticoids which fully antagonize the effect of glucocorticoids through competitive binding to the glucocorticoid receptor. Thus, we showed that early postnatal development of enzyme glycoproteins, viz. glucoamylase, lactase and DPP IV of the brush-border membrane, is under the control of endogenous glucocorticoids. We described an inhibition of hydrocortisone-induced precocious appearance of sucrase-isomaltase and a loss of sialic acid bound to DPP IV by an antiglucocorticoid and transcriptional inhibition of α2,6-ST via the glucocorticoid receptor pathway. The use of antiglucocorticoids eliminated the difficulty in controlling simultaneously both endo- and exogenous sources of glucocorticoids in suckling rats, and also many of the complications resulting from the interaction of glucocorticoids with other endocrine systems.

Transcriptional regulation of α2,6-ST is seen also under the effect of inflammatory cytokines IL-1 and IL-6, indicating the possibility that enterocytes respond to injury and inflammation, as α2,6-ST belongs to acute phase proteins.

GLUCOSE-SENSITIVE SHORT-CIRCUIT CURRENT IN SHEEP RUMEN EPITHELIUM.

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Glucose transport in the rumen epithelium has been considered to be of minor importance, because ingested carbohydrates are fermented to short chain fatty acids inside the forestomach. However, previous studies pointed to the fact, that there is a significant amiloride-insensitive but sodium-dependent Isc (1). Although this might be due to an electrogenic Na^+ channel (1), existence of an electrogenic Na^+/glucose co-transport might be another explanation for this observation. Therefore we tested, if part of Isc across the rumen epithelium is glucose-sensitive.

Methods: Stripped ruminal epithelium of sheep was preincubated for 6h in a buffer solution containing 10 mM glucose and 10 mM n-butyrate and was then transferred to a glucose-free bathing solution in Ussing chambers. The electrophysiological parameters Isc and tissue conductance (Gt) were measured under short-circuit conditions. After 30 min of equilibration 10 mM D-glucose or 3-O-methyl-α-D-glucose (3-OMG) were added either to the mucosal or the serosal bathing solution and phlorizin, a selective competitive inhibitor of the Na^+/glucose co-transporter, was added at a concentration of 0.1 mM.

Results: Addition of D-glucose to the mucosal bathing solution caused an immediate and significant ($p<0.01$) increase in Isc that reached 220 ± 24 % (means ± S.E.M) of basal Isc (0.25 ± 0.04 $\mu Eq^* cm^{-2}*h^{-1}$) after 15 min. Following addition of phlorizin to the mucosal bathing solution, Isc decreased within 5 min to 37 ± 5 % of basal value before addition of phlorizin. The Gt of the glucose treated group showed no significant change in comparison with the control group. Similar effects were seen after mucosal addition of 3-OMG, a glucose derivative that cannot be metabolized in the cell. Isc increased by 183 ± 24 % and decreased again after mucosal addition of phlorizin. Addition of D-glucose to the serosal bathing solution resulted in a significant ($p<0.01$) increase in Isc of 445 ± 86 % of basal Isc (0.24 ± 0.04 $\mu Eq*cm^{-2}*h^{-1}$). The addition of 3-OMG and phlorizin showed no effect on Isc at the serosal side of the epithelium.

Conclusions: D-glucose has a significant ($p<0.01$) influence on Isc. To explain the observed effects of glucose on Isc, two different mechanisms can be taken into account: firstly, an electrogenic glucose dependent transporter and secondly, a metabolic effect of glucose. The stimulating effect of 3-OMG on Isc and the inhibitory influence of phlorizin after mucosal addition of D-glucose or 3-OMG favours the model of an electrogenic glucose-sensitive transporter at the mucosal side of the rumen epithelium. Further studies will show, whether this is a Na^+/glucose co-transporter. The marked increase in Isc after serosal addition of D-glucose and the missing effect of 3-OMG at the serosal side indicate an additional metabolic influence of D-glucose on ion transport after entering the cell. The physiological relevance of a glucose transporter in rumen epithelium should be further investigated.

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MUTATION OF GLUTAMINE 457 TO ARGININE IN THE Na^+/GLUCOSE COTRANSPORTER CAUSES GLUCOSE-GALACTOSE MALABSORPTION IN A SWEDISH FAMILY.

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The Gln457Arg mutation in the Na^+/glucose cotransporter gene (SGLT1) is one of the 23 missenses mutations identified so far that cause glucose-galactose malabsorption. Gln457Arg is a homozygote mutation that has been found in 3 members of a Swedish pedigree. The Gln457 residue is conserved in all but one of the members of the SGLT1 family, and is located in transmembrane domain 11 of the transporter. We have expressed the mutant protein in *Xenopus* oocytes and used a combination of techniques to determine the cause of the defect in SGLT1 function. No α-methyl-D-glucose (αMDG) transport was found using tracer uptake or electrophysiological measurements, but Western blot analysis revealed that Gln457Arg SGLT1 was present in amounts comparable to the wild-type transporter. Although sugar was not transported, electrophysiological methods showed presteady-state currents and these results, together with the freeze-fracture studies, indicated the presence of the mutant protein in the plasma membrane. Neither the apparent valence or the $V_{0.5}$ (membrane potential for 50% maximal charge) were changed, but the relaxation time constant (τ) was decreased to a third of the wild-type value (6 vs. 18 ms). αMDG and Phlorizin reduced the Na^+-leak current through the mutant protein with an apparent affinities of 52 mM and 0.22 μM respectively, i.e. about two orders of magnitude lower than that for the wild-type transporter.

We conclude that the mutation of Gln to Arg at position 457 eliminate Na^+/glucose cotransport by reducing the affinity for sugar and preventing the conformational changes of the Na^+-sugar-loaded protein from the outside to the inside of the membrane.

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EFFECTS OF $CdCl_2$ ON TRANSEPITHELIAL ELECTRICAL PARAMETERS IN THE INTESTINE OF THE TELEOST FISH, *Anguilla anguilla*.

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Cadmium has been shown to produce ion disturbances and osmoregulatory impairment in fish. Since intestine is one of the tissues involved in the ion and osmotic regulation of marine teleosts, in the present study the action of $CdCl_2$ on the absorptive function of the eel, *Anguilla anguilla*, intestine was evaluated by estimating the rate of net Cl^- absorption expressed as short circuit current (Isc, $\mu A \cdot cm^2$). In tissues mounted in Ussing style chamber Isc and transepithelial potential difference (Vt, mV) responded in a concentration dependent manner to the addition of $CdCl_2$ to the serosal or mucosal bathing solutions. The dose response curve indicated that the maximal inhibition of Isc and Vt was obtained at a concentration of 10^{-4} M. The effect was more pronounced from the serosal side (90% inhibition) with respect to the luminal one (60% inhibition). Higher concentration of heavy metal could not be tested because of its precipitation in Ringer solution. To understand the nature of Isc inhibition induced by $CdCl_2$, the response of electrical parameters to $CdCl_2$ exposure was examined in tissues reduced in non-trasporting conditions by different experimental manouvres known to block Cl^- transport at different cellular levels. Hence, bumetanide which is known to inhibit Cl^- transport by blocking the luminal Na-K-2Cl cotransporter and Cl^--conductive channels at the basolateral membrane of the enterocyte, added to the luminal (10^{-5} M) or the serosal solution (10^{-4} M), abolished the response of Isc and Vt to $CdCl_2$ treatment. Similar results were obtained when tissues were preatreated with ouabain (serosal, 10^{-4} M) or when Na^+ or Cl^- were omitted from the bathing media.

Furthermore, $CdCl_2$ alters the permselectivity of the tight junctions since the magnitude of the diffusion potentials evoked by an imposed serosa-mucosa NaCl gradient (2:1) was strongly reduced by addition of $CdCl_2$ to either serosal or mucosal solution.

The results suggest that $CdCl_2$ acts on the transport process responsible for Cl^- absorption in eel intestine. Therefore, one of the factors contributing to the toxic effect of heavy metal on fish could be related to the ion-balance disturbances of body fluids subsequent to the altered osmoregulatory function of the intestine in marine teleosts.

DIPEPTIDE TRANSPORT BY EEL (*Anguilla anguilla*) INTESTINAL BRUSH BORDER MEMBRANE VESICLES.

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Using brush border membrane vesicles (BBMV) isolated from eel intestine, we have previously demonstrated that the dipeptide glycyl-glycine was translocated into the vesicular space by a H^+/dipeptide cotransport mechanism (1) with characteristics similar to those found in intestinal and renal epithelium of higher vertebrates (2).

The purpose of the present study was to further elucidate fish intestinal absorption mechanism(s) of dipeptides, by testing other molecules of the same group such as D-phenilalanine-L-alanine (D-Phe-L-Ala) and glycyl-L-proline (Gly-L-Pro).

Dipeptide transport in isolated eel (*Anguilla anguilla*) intestinal BBMV, was analised both by measuring the uptake of tritiated D-phenilalanine-L-alanine and glycyl-L-proline and by monitoring the dipeptide-dependent H^+ accumulation with the pH-sensitive fluorescent dye, acridine orange.

It was found that in the absence of a transmembrane electrical potential ($K^+_{in} = K^+_{out}$ + valinomycin), the uptake of both radiolabelled dipeptides [^{3}H]-Gly-L-Pro and [^{3}H]-D-Phe-L-Ala , was not significantly different either in the presence or in the absence of an inwardly directed transmembrane proton gradient ($pH_{out} = 5.4$; $pH_{in} = 7.4$). In addition no dipeptide dependent proton accumulation could be observed by monitoring the acridine orange fluorescence quenching. On the other hand when an inside negative electrical membrane potential was imposed (K^+_{in} = 100 mM; K^+_{out} = 1 mM + valinomycin), the uptake of the two radiolabelled peptides was strongly increased and further stimulated by the additional presence of a transmembrane proton gradient. It was also found that in the presence of an electrical membrane potential, there was a H^+ flux into the vesicular space specifically associated with the presence of both Gly-L-Pro and D-Phe-L-Ala in the extravesicular medium.

The kinetics parameters (J_{max}; Km) of the H^+/dipeptides cotransporter were measured by both radioactive and fluorescent tracers: Km_{app} was found to be around 1-2 mM for both dipeptides. Different dipeptides strongly inhibited [^{3}H]-Gly-L-Pro uptake, as well as the cephalosporin antibiotic cephalexin, suggesting that, in the eel intestine, dipeptide molecules and cephalosporin antibiotics may share a common transport system.

These results further strengthen that the electrical membrane potential difference plays a basic role for the absorption of the dipeptides through the luminal membrane of the eel intestinal absorbing cells.

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MOTILIN RECEPTORS IN ENTEROCYTES FROM RABBIT JEJUNUM.

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Motilin is a 22 aminoacid gastrointestinal peptide which has been shown to have a physiological role in the regulation of the fasting motility pattern. Motilin has also shown to inhibit the intestinal absorption of amino acids either "in vivo" or "in vitro". Recent results carried out in rabbit jejunum have shown that motilin inhibits the brush border Na^+-dependent intestinal transport of L-leucine by acting indirectly from the basolateral membrane. This inhibition seems to involve fast intracellular processes mediated by protein kinase C. These findings suggest that the motilin effect in the intestinal epithelium might be initiated by the binding of the hormone to specific receptors located in the basolateral membrane of the enterocyte. Since the existence of motilin receptors in the intestinal epithelium remains unknown, and the complex motilin-receptor might be involved in the regulation of the intestinal transport of nutrients in the rabbit, the aim of the present work has been to study whether motilin receptors are present in both brush border (BB) and basolateral (BL) membranes of the enterocyte in rabbit jejunum. BB and BL membranes were prepared simultaneously from the same sample of frozen rabbit jejunum by the procedure of Touzany et al. (1994) with slight modifications. Iodination of motilin was performed usingthe Hunter and Greenwood method (1962) with slight modifications. Binding of ^{125}I-motilin to enterocyte membranes was determined by modifying the procedures previously described by Bormans et al (1986) and Dharmsathaphorn et al (1983). The results obtained in the present work show that ^{125}I-motilin binded specifically to basolateral (BL) membrane but it did not bind to the brush border (BB) of the rabbit jejunum enterocyte. The ^{125}I-motilin dissociation constant (Kd) was 95.58±15.0 pM and the receptor density (Bmax) was 2.54±0.40 fmol/mg protein. The binding of ^{125}I-motilin to BL membrane was competitively inhibited by both unlabelled motilin and erythromycin. The IC_{50} were (2.1±0.4) 10^{-8} M and (1.3±0.1) 10^{-6} M for motilin and erythromycin respectively, and the Ki were (6.83±1.3) 10^{-9} M for motilin and (4.32±0.33) 10^{-7} M for erythromycin. Saturation and competition binding studies showed interaction at only one class of binding sites in BL membrane.

REGULATION OF 11β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN RAT SMALL AND LARGE INTESTINE.

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11ß-hydroxysteroid dehydrogenase (11ß-OHSD) is considered as a key enzyme for the mineralocorticoid selectivity of the tissue. In the rat intestine, 11ß-OHSD activity is already high on the second postnatal day and does not alter until adulthood (1). In contrast, the activity in the ileum is low during the first two weeks of life and than increases (1). The developmental profile of ileal 11ß-OHSD reflects the developmental profiles of plasma levels of corticosterone, aldosterone and insulin. To examine whether the development of 11ß-OHSD activity might be regulated by these hormones we have performed *in vivo* and *in vitro* experiments and characterized 11ß-OHSD activity in ileum and distal colon of rat. *In vitro study:* The intestinal segments of 7-day-old rats were aseptically removed and split longitudinally into two halves. One half served as a control, the other half was incubated in the presence of dexamethasone (5.10^{-7} M), aldosterone (5.10^{-7} M), or insulin (10^{-6} M). The explants were incubated according to Kolínská et al. (2) and 11ß-OHSD activity was measured as a percentage of conversion of [^{3}H]corticosterone to 11-dehydrocorticosterone using high performance liquid chromatography (3). *In vivo study:* To test the sensitivity of 11ß-OHSD to corticosteroids, 18-day-old adrenalectomized rats were treated with dexamethasone or deoxycorticosterone acetate, DOCA (600 or 500 μg/100 g BW/day) for 7 days; controls received vehicle alone. Rats were sacrificed, distal colon and ileum removed and 11ß-OHSD activity measured as previously described (3).

In vitro study. Corticosteroids and insulin stimulated 11ß-OHSD in both large (distal colon) and small intestine (ileum). Data are presented in a Table (% of conversion of corticosterone to 11-dehydrocorticosterone, means ± SEM). Mineralocorticoid receptor antagonist spironolactone (5.10^{-5} M) did not inhibit the effect of aldosterone in distal colon and had a weak effect in ileum. *In vivo study.* Dexamethasone administration increased the activity of 11ß-OHSD by 259 % in ileum and by 138 % in distal colon. DOCA stimulated the enzyme activity by 387 % in ileum and by 202 % in distal colon. We conclude that 11ß-OHSD activity in immature rats is under multifactorial regulation of corticosteroids and insulin.

	Ileum	Distal colon
Controls	1.20 ± 0.4	13.4 ± 2.8
Aldosterone	13.0 ± 3.1	22.4 ± 2.5
Dexamethasone	12.6 ± 2.4	15.5 ± 1.8
Insulin	5.40 ± 0.8	13.8 ± 1.9

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EXPRESSION OF Cl/HCO_3 ANTIPORT FROM RAT JEJUNUM IN XENOPUS LAEVIS OOCYTES.

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A Cl/HCO_3 exchange, recently evidenced in basolateral membranes from rat jejunum, could represent the mechanism by which HCO_3 exits the cell during its absorption (1). This Cl/HCO_3 antiporter is positively affected by the presence of sodium, but sodium is not transported (2). In view of this feature it could represent a different isoform expression of the AE gene family; we have attempted to express the carrier in *Xenopus laevis* oocytes and to identify the size of the mRNA encoding the protein. RNA was obtained from the jejunal mucosa using a chloroform/phenol method (3) and poly $(A)^+$ RNA was exctracted by affinity chromatography on an oligo(dT)-cellulose column. Poly $(A)^+$ RNA was size fractionated using a continuous sucrose gradient. The mean size of the fractionated RNA was determined by electrophoresis on a denaturing 1% agarose gel. Poly $(A)^+$ RNA and fractionated RNA were injected in healthy *Xenopus laevis* oocytes of stage V-VI; water injected oocytes served as controls. Both not injected and water-injected oocytes show a DIDS-insensitive capacity for Cl transport, suggesting an endogenous mechanism that does not involve Cl/HCO_3 exchange. In the poly $(A)^+$ RNA-injected oocytes, the rate of Cl uptake was increased when compared with the control and the increment was abolished by DIDS. After fractionation, the maximal stimulation of Cl uptake was found with a fraction containing mRNA of 2-4 Kb in length. To exclude the possibility that Cl moves through a DIDS-sensitive conductive pathway, Cl gradient was inverted and the effect of anthracene-9-carboxylix acid was tested. Results seem to exclude a significant involvement of Cl channels in the expressed Cl influx. Experiments performed in the absence and in the presence of Na show that in injected oocytes anion exchanger is operative also after Na removal, even if to a lower extent. Taken together, these results demonstrate that the expressed Cl transport mechanism has properties similar to jejunal basolateral Cl/HCO_3 exchanger.

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MECHANISMS OF NEUROTENSIN-EVOKED ELECTROGENIC CHLORIDE SECRETION IN THE MAMMALIAN COLON.

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Neurotensin is a regulatory, endocrine tridecapeptide primarily synthesized in N-cells of the ileum, but also in the jejunum and colon. The primary physiological effect of neurotensin in the large intestine appears to be the inhibition of motility. So far the interaction of neurotensin with colonic ion transport has not been characterized in detail.

Therefore the effects of neurotensin on electrogenic chloride secretion were measured in Ussing-chamber experiments with isolated guinea pig distal colon epithelial sheets. The evoked short-circuit current (Isc) was used as a measure of electrogenic chloride secretion. The interaction of neurotensin with isolated colonic crypts of human and guinea pig and with human colonic tumor cells (HT29 clone 19A) was studied by measuring the effects of neurotensin on the intracellular calcium concentration [Ca^{2+}]$_i$ with Fura-2 using a digital video-imaging system. Furthermore the action of neurotensin on cell metabolism and pH-regulation of HT29 clone 19A cells was assessed with a Cytosensor Microphysiometer (Molecular Devices).

In isolated guinea pig distal colonic epithelium neurotensin evoked a dose-dependent increase in Isc with maximal effects at 10-100 nM. The increase in Isc consisted of a rapid initial peak and then a subsequent slow decline towards an elevated plateau value, closely resembling the effects of electrical field stimulation of submucosal neurons and of carbachol application, respectively. The stimulation of Isc by neurotensin was markedly inhibited by serosal application of bumetanide (0.1 mM) and abolished by incubation of the tissues with a chloride-free solution in the serosal compartment. These observations are consistent with the stimulation of electrogenic chloride secretion by neurotensin. Neurotensin and prostaglandin E_2 (PGE_2) acted synergistically. Already at low concentrations (0.1 nM neurotensin and 0.25 µM PGE_2) a marked stimulation of Isc was elicited by the combined administration of these secretagogues.

The action of neurotensin was blocked by 50-80 % with atropine and pirenzepine, respectively, and abolished by tetrodotoxin (TTX), indicating that neurotensin exerts its effects on chloride secretion primarily through interaction with cholinergic and non-cholinergic submucosal neurons, which then secondarily evoked the secretory response. This conclusion was further supported by studies with isolated guinea pig and human colonic crypts. In these preparations, which are devoid of neuronal structures, carbachol but not neurotensin induced a characteristic biphasic increase in [Ca^{2+}]$_i$. Neurotensin, however, did induce a marked [Ca^{2+}]$_i$ response in HT29 clone 19A cells, suggesting that these cells express functional neurotensin receptors in contrast to non-transformed colonic epithelial cells. This is further strengthened by the studies with a Cytosensor Microphysiometer system, where neurotensin clearly activated HT29 clone 19A cells in a dose-dependent manner.

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DISTRIBUTION OF ALDOSTERONE RECEPTORS IN THE INTESTINE OF GUINEA-PIG.

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Little is known about the distribution of aldosterone receptors in different intestinal segments of guinea-pigs that have been fed a standard diet, and about the level of expression of these receptors in intestinal epithelial cells during differentiation from crypt to surface/villus cells.

Therefore, we sequentially isolated crypt and surface/villus cells from duodenum, ileum, caecum, proximal colon, and distal colon, by EDTA chelation and mechanical dissociation. The number of aldosterone receptors in these isolated cells was determined by performing binding studies. For this, cells were incubated in DMEM medium containing 1-20 nM [^{3}H]aldosterone and 100-fold RU28362, to inhibit binding of aldosterone to the glucocorticoid receptor. Specific binding was calculated after determining total [^{3}H]aldosterone and measuring [^{3}H]aldosterone binding in the presence and absence of 1000-fold unlabeled aldosterone. The data were normalised to cell number, and analysed by non-linear regression. (1)

At the end of each experiment, cell viability was 80-89 % for each cell fraction of each intestinal segment, as assessed by trypan blue exclusion. Maximum binding was reached after 30 min incubation at 30°C and was stable for at least 2 h.

Number of receptors per cell

	surface/villus cells	crypt cells	n
duodenum	1000 ± 200	1000 ± 300	5
ileum	1200 ± 250	1300 ± 300	5
caecum	1300 ± 400	1500 ± 250	5
proximal colon	2000 ± 350 *	1800 ± 300	5
distal colon	2200 ± 300 *	2300 ± 350 *	5

Values are means ± SE

* $p \leq 0.05$ or better vs. same cell fraction of duodenum

The dissociation constant was 5.4 ± 1.4 nM (n=5, mean ± SE) for surface cells of the distal colon, and was identical in all cell fractions of all intestinal segments.

We therefore conclude, that aldosterone receptors are present in surface/villus and crypt cells of all intestinal segments. No significant differences were observed in the number of aldosterone receptors in surface/villus and crypt cell fractions of the same intestinal segment. The number of aldosterone receptors increased from duodenum to distal colon.

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EPITHELIAL BARRIER DEFECT AND IMPAIRED ION TRANSPORT AS DIARRHEAL MECHANISMS IN ULCERATIVE COLITIS.

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Epithelial barrier dysfunction can contribute to intestinal inflammation and diarrhea by mucosal antigen uptake and loss of ions and water, respectively. This study aimed to characterize epithelial barrier and transport function of the inflamed mucosa in ulcerative colitis (UC).

Ussing experiments were performed on surgically removed sigmoid colon of UC patients and, for controls, of patients with sigmoidal or rectal cancer. Conventional histology showed no gross defects of the epithelial layer. Alternating current impedance analysis was applied in order to discriminate between the two components of total resistance (R^t), epithelial resistance (R^e) and subepithelial resistance (R^{sub}). The functionally important barrier between between lumen and circulation is given by R^e. However, in UC subepithelial inflammation may affect R^{sub} and thus conventional R^t recordings may not represent a valid measure of R^e. In addition, barrier function was investigated using the paracellular permeability marker ^{3}H-mannitol.

In UC the total tissue resistance (R^t) was only 61% of the respective control value (68±6 $\Omega \cdot cm^2$ versus 111±5, $p<0.01$), which was paralleled by an enhanced ^{3}H-mannitol flux in UC. However, the decrease in R^t was not accompanied by a proportional loss of R^e and R^{sub}. Alternating current impedance analysis uncovered a fourfold decrease of R^e to 22±3 $\Omega \cdot cm^2$ in UC compared with 97±7 $\Omega \cdot cm^2$ in controls ($p<0.001$). The epithelial barrier was affected more seriously in severe than in moderate chronic inflammation (R^e 14±5 versus 32±5 $\Omega \cdot cm^2$; $p<0.05$). In contrast, R^{sub} even increased to 46±5 $\Omega \cdot cm^2$ in UC compared to 14±2 $\Omega \cdot cm^2$ in controls ($p<0.001$) most probably due to submucosal inflammation. Thus, the decrease in barrier function in UC is more dramatic than so far indicated by total resistance measurements.

Aldosterone (3 nM) stimulated Na^+ absorption in controls by 6.1±1.7 $\mu mol \cdot h^{-1} \cdot cm^{-2}$ (given as amiloride-sensitive ΔI_{SC} 8 hours after in vitro steroid addition). In contrast, UC tissues did not exhibit significant Na^+ absorption after aldosterone (ΔI_{SC} = 0.1±0.1 $\mu mol \cdot h^{-1} \cdot cm^{-2}$). Electrogenic Cl^- secretion was stimulated in controls by PGE_2 by 2.3±0.7 $\mu mol \cdot h^{-1} \cdot cm^{-2}$, while in UC tissues there was no significant spontaneous or secretagogue-stimulated Cl^- secretion.

Conclusion: The inflamed colonic mucosa in ulcerative colitis (UC) is characterized by a dramatic loss of epithelial barrier function, mainly due to altered permeability of the paracellular pathway. This might lead to enhanced antigen uptake and contribute to diarrhea by a leak-flux mechanism. In addition, active transport mechanisms of Na^+ absorption and of Cl^- secretion are abolished. Thus, diarrhea in UC is not caused by secretory mechanisms but by a severe barrier dysfunction as well as by malabsorptive effects.

CELLULAR MECHANISM OF SEROTONIN ACTION ON L-LEUCINE INTESTINAL TRANSPORT IN RABBIT.

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Serotonin (5-Hydroxytryptamine; 5-HT) is found in high concentrations throughout the gastrointestinal tract of a variety of mammalian species. In the gut, the amine is contained predominantly within the mucosal enterochromaffin cells, but it is also found in the myenteric plexus, suggesting a neurotransmitter role for 5-HT in the gut. Serotonin stimulates fluid electrolyte secretion and motility in the gastrointestinal tract. Previous studies from our laboratory have demonstrated that this hormone inhibits L-leucine intestinal absorption, mainly by acting on the Na^+-dependent system of amino acid and this effect does not seem to be due to a direct action of the hormone on the tissue. The regulation of cellular function by hormones which bind to cell surface receptors is initiated by signal transduction mechanisms at the plasma membrane that lead to the generation of intracellular signals, o second messengers. In order to study the possible intracellular mechanisms of serotonin effect in the absorption of nutrients, we carried out experiments to determine which cellular messengers are implicated in this action. The experimental work was carried out *in vitro* by the tissue accumulation method (incubation was for 3 or 15 min, at 37°C, under 95% O_2 - 5% CO_2 bubbling). To study the effect of intracellular Ca^{2+} in serotonin action on L-leucine intestinal transport, we carried out the experiments with 0.1 mM trifluoroperazine, a calmodulin antagonist. The results showed that this hormone has no effect on the amino acid accumulation (15 min.) in presence of this antagonist. When we added TMB-8, an inhibitor of intracellular Ca^{2+} release, to the medium, we obtained the same results.In order to investigate the intracellular mechanim of serotonin in more depth, the L-leucine uptake (3 min. of incubation) was measured in the presence of the PKC inhibitor, staurosporine at concentration 10^{-7}M. The results indicate that in presence of this inhibitor, the serotonin effect disappears. The role of PKC was corroborated with an activator of PKC, phorbolmyristate acetate (PMA) at concentration 10^{-7}M. It inhibited the L-leucine uptake in the presence or absence of serotonin. This effect disappeared when the medium simultaneously contained PMA and staurosporine 10^{-7}M. To complete these results, we carried out experiments with an inhibitor (Protein Kinase Inhibitor) and an activator (8-bromoadenosine cAMP) of cAMP, descibed as a second messenger in many physiological processes. The results showed that the addition of 10^{-7}M Protein Kinase Inhibitor or $3x10^{-4}$M 8-Br-cAMP to the medium did not alter either the control conditions or the serotonin effect. In conclusion, the serotonin action on the intestinal absorption of nutrients is probably related to intracellular processes mediated by PKC.

CA-SENSITIVE ELECTROGENIC NA-TRANSPORT ACROSS THE SHEEP OMASUM.

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The moderate tight epithelium of sheep omasum exhibits a positive short-circuit current (I_{SC}) which is sensitive to divalent cations. Elimination of mucosal Ca^{2+} causes a steep increase of I_{SC} (overshoot) due to an enhanced mucosal to serosal Na^+-transport followed by a decline to a stable plateau [1]. The Ussing-chamber technique was applied to investigate the influence of different mucosal Na^+-concentrations (Na_M) on the calciumsensitive I_{SC}. The electromotive force of Na^+ (E_{Na}) which determines the mucosal entry of this ion was calculated by applaying the methode of YONATH and CIVAN (1971) which allows the transepithelial determination of E_{Na}.

The results obtained are:

1. The total increase of the calciumsensitive I_{SC} is dependent on Na_M. An overshoot is only observed at high Na_M.
2. Increasing Na_M up to 115 mM does not lead to a saturation of the current passing across this Ca^{2+}-sensitive conductance ($r = 0.99$).
3. The caculated E_{Na} is 61 9 ± 0.9 mV.
4. The alteration of Na_M, i.e. 10 → 115 mM, leads to a corresponding increase of the cellular Na^+-concentration from 2.97 ± 0.03 to 9.20 ± 090 mM respectively.

Conclusion:

The change in I_{SC} after the omission of Ca^{2+} from the mucosal solution is correlated to the added Na_M and requires the presence of this ion. The E_{Na} estimated is in good agreement with other epithelia such as rabbit colon [2].

DISTENSION-INDUCED SECRETION IN RAT RECTUM: INTERNEURONS AND AFFERENT NEURONS.

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Distension of rat rectum induces chloride secretion mediated by the submucosal plexus involving VIP as a neurotransmitter of the secretomotor neurons (Am. J. Physiol. 1995; 268: G725-31). This study aimed to identify afferent neurons and contributing nicotinergic interneurons.

Cl^- secretion was measured as increase in short circuit current (I_{SC}) after distension (±100 µl, 2 Hz, 20 s) in partially stripped rat rectum in Ussing-chambers. Starting at a baseline I_{SC} of 2.7±0.2 $\mu mol \cdot h^{-1} \cdot cm^{-2}$, I_{SC} increased after distension and slowly declined again within 30 min. The increase in I_{SC} 10 min after distension was measured as ΔI_{SC}^{10}. Nicotine itself at 10^{-4} M did not affect ΔI_{SC}^{10} (n.s. versus control). Then, the nicotinergic blockers lobeline, dimethylphenylpiperazine (DMPP), and mecamylamine were tested:

($\mu mol \cdot h^{-1} \cdot cm^{-2}$)	decrease in baseline I_{SC}	ΔI_{SC}^{10}	n
control	–	2.1 ± 0.3	7
lobeline (10^{-6} M)	-0.5 ± 0.2 (p<0.05)	1.8 ± 0.5 (n.s.)	6
lobeline (10^{-5} M)	-1.0 ± 0.4 (p<0.05)	0.3 ± 0.1 (p<0.001)	7
lobeline (10^{-4} M)	-1.2 ± 0.2 (p<0.001)	0.1 ± 0.1 (p<0.001)	9
DMPP (10^{-4} M)	-0.7 ± 0.2 (p<0.01)	1.1 ± 0.3 (p<0.05)	10
mecamylamine (10^{-3} M)	-0.6 ± 0.2 (p<0.01)	0.3 ± 0.1 (p<0.01)	7

The nicotinergic blockers lobeline, DMPP, and mecamylamine reduced ΔI_{SC}^{10}, indicating that nicotinergic neurons contribute to this reflex pathway. Baseline I_{SC} was also reduced by all 3 antagonists, indicating that nicotinergic inputs contribute to tonic stimulation of basic transport rates.

Finally, capsaicin was tested, a substance which in short term experiments can affect afferent neurons. Capsaicin (1 µM, added 30 min prior to distension) did not affect baseline I_{SC} but increased ΔI_{SC}^{10} to 4.2±0.8 $\mu mol \cdot h^{-1} \cdot cm^{-2}$ (p<0.05).

Conclusion: Distension-induced chloride secretion is mediated by a submucosal neuronal reflex pathway which includes – in addition to efferent VIP-ergic secretomotor neurons – also nicotinergic interneurons and afferent neurons.

Na^+-INDEPENDENT TRANSPORT OF L-METHIONINE IN BRUSH-BORDER MEMBRANE VESICLES OF CHICKEN JEJUNUM.

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We have previously shown that L-methionine is taken up by the chicken jejunum by both Na^+-dependent and Na^+-independent transport mechanisms (1). Since systems $b^{0,+}$ and y^+ have been demonstrated in this intestinal region (2), we have now investigated the contribution of both systems to L-methionine uptake as well as the possible participation of other Na^+-independent mechanisms. Brush-border membrane vesicles from the jejunum of 6-week-old Label chickens were incubated with 50 µmol/L L-[^{14}C]-methionine under a *zero-trans* KSCN gradient (100 mmol/L). The inhibitory effect of N-ethylmaleimide on L-methionine uptake was consistent with the involvement of system y^+ in apical L-methionine transport. *Cis*-inhibition experiments using 10 mmol/L , of unlabelled amino acids showed that the inhibition exerted by L-lysine and L-arginine on L-methionine uptake was only partial. However, 2-amino-2-norbornanecarboxylic acid (BCH), a specific substrate of system L, fully inhibited the L-lysine-insensitive component of L-methionine uptake. Kinetic analysis of L-methionine transport in Na^+-free conditions gave futher support to the involvement of $b^{0,+}$ and y^+ transport systems in neutral amino acid absorption. In conclusion, L-methionine is transported in the brush-border of the chicken jejunum by at least three pathways: two ($b^{0,+}$ and y^+) shared with cationic amino acids and a third one which is sensitive to BCH.

(1) Soriano-García et al. Ital. J. Gastroenterol. 168, 27:1995
(2) Torras-Llort et al. 14th Meeting of the European Intestinal Transport Group, Mikolajki, Poland, 1996.

Subsidized by DGICYT PB91/0274 from Ministerio de Educación y Ciencia, Spain.

MOTILITY-ACTIVATED RELEASE OF SECRETORY IMMUNOGLOBULIN A FROM THE CRYPTS – A NEW "SECRETORY COMPONENT" OF THE MIGRATING MOTOR COMPLEX?

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The migrating motor complex is a cyclic motor programme that is activated in the fasting state. Three phases are defined: phase I with motor quiescence, phase II with intermittent motor activity and phase III with maximal motor activity. During late phase II, an electrogenic mucosal secretory process is activated, and this phenomenon is abolished in cystic fibrosis patients, suggesting that the underlying mechanism is active CFTR-mediated chloride secretion. The link between motility and PD is blocked by TTX but is resistant to atropine, selective destruction of the myenteric plexus and inflammatory atrophy of the villi. The mechanism consequently seems to be a non-cholinergic reflex mechanism relayed in the submucous plexus that activates electrogenic chloride secretion from the intestinal crypts. The physiological role of this phenomenon is unknown. The aim of the present tudy was to test the hypothesis that activation of fluid secretion in late phase II is associated with an increased release of secretory IgA (sIgA) into the lumen.

Methods: Duodenal and jejunal sIgA production was measured by a triple lumen perfusion technique in 17 healthy volunteers. Secretory data were related to the current phase of the migrating motor complex (MMC), as recorded by manometry.

Results: Within both segments, luminal sIgA concentration changed cyclically, with peak concentrations occurring at the end of the cycle ("late phase II"). These cycles were due to two factors: a cyclic inflow of sIgA from more proximal segments, and a cyclic increase in the production of sIgA within the test segment that correlated in time with an increase in epithelial fluid secretion.

Conclusion: The data are compatible with the hypothesis that the volume flow generated by motility-activated active chloride secretion may carry sIgA produced by the crypt epithelium into the luminal phase. This phenomenon may be a previously not described "secretory component" of the MMC programme that reduces the risk for bacterial adherence and penetration in the interdigestive state.

METABOLISM OF DANTHRON, A DIPHENOLIC LAXATIVE.

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Danthron (= 1,8-dihydroxyantraquinone) shows a complex pattern of biotransformation *in vivo* (1, 2), but metabolites other than the simple conjugates danthron monosulphate and -monoglucuronide have so far not been identified. However, there is clear evidence also of phase I metabolites, which in conjugated form are excreted predominantly in the bile. In the present study, a suspension of micronised danthron was instilled intraperitoneally in the rat, and the bile continuously sampled for 6-8 hours afterwards. The bile specimens were subjected to enzymatic hydrolysis followed by acid extraction into ethyl acetate, whereafter the products in the extract were separated and purified by various methods. TLC and HPLC demonstrated several phase I metabolites. The main one was a monohydroxylated danthron, which according to NMR spectroscopy of its triacetate was 1,2,8-trihydroxyanthraquinone. Evidence was obtained that some of the others represented further hydroxylated products of danthron.

Biliary drug conjugates seem generally to pass largely unabsorbed to the colon to become more or less extensively hydrolysed by the microflora. The question thus arises to which extent the above phase I metabolites are pharmacologically active and upon their hydrolytic release in the colon may contribute to the laxative action of danthron.

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2) Sund et al. Pharmacology 47: suppl. 1, 134-137, 1993.

CHOLECYSTOKININ INHIBITION OF INTESTINAL GLUCOSE TRANSPORT *IN VITRO*.

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Events early in digestion could actutely regulate the intestinal absorption and distribution of non-electrolytes such as glucose. Immediately after a meal the pH at the gastric fundic mucosal surface stimulates the release of a novel peptide that reduces intestinal glucose absorption (1). Cholecystokinin is released from the proximal small intestine early after ingestion (2) and delays rises in blood glucose concentration by prolonging gastric retention (3) and by inducing insulin release (4). We are interested in the action of cholecystokinin on intestinal glucose absorption and the functional/structural relationships between cholecystokinin and the novel gastric fundic peptide (1). The methods used have been described previously (1).

A series of paired sheets of mucosal tissue from guinea pig proximal ileum were equilibrated in glucose-free buffer for 30 minutes then incubated with 5mM glucose on the luminal side. The serosal buffer contained 1μM peptide in 0.1% buffered bovine serum albumin; the paired control sheet from the same 2cm segment of gut was bathed with serosal 0.1% buffered albumin. The serosal gain in glucose was measured enzymically after 45 minutes incubation at 37°C, sheets judged to be leaky because of excessive permeation by ^{3}H polyethylene glycol were discarded.

CCK-8 sulphate decreased glucose transport by 41 ± 13% (n = 22 pairs of sheets). The non-sulphated CCK-8 was also effective in that glucose serosal gain was inhibited by 45.8 ± 8.2% (n = 16 pairs of sheets).

CCK-33 appeared to be inactive against glucose transport in preliminary experiments but it is not possible yet to distinguish between true inactivity and the failure of the larger peptide to permeate the sheets from the serosal buffer.

An analogue of CCK-8 sulphate, also used on the serosal side at 1μM, inhibited glucose transport by 41.8 ± 11.6% (n = 9 pairs of sheets). That analogue is an agonist for CCK type A and type B receptors. Experiments are in hand with receptor specific agonists and antagonists. Time-course experiments over 90 minutes using each sheet as its own control indicate that CCK-8 suphate added after 45 minutes acts more slowly than the gastric fundic peptide (1). The fundic origin of the novel peptide and the largely pyloric distribution of gastric cholecystokinin also support the idea that the gastric fundic peptide is not cholecystokinin but do not rule out a structural relationship between the two peptides.

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2. Silver, A.J. and J.E. Morley (1991) Progr in Neurobiol. 36 23-34
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CALCIUM CHANNELS AND INTESTINAL FLUID SECRETION: SITES OF ACTION.

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Background/aims: The enteric nervous system (ENS) is of importance in explaining the intestinal fluid secretion evoked by several secretagogues among them cholera toxin (CT). Several mechanisms involved in the enteric nervous secretory reflex(es) (e.g. action potentials, neurotransmitter release) may be dependent on the flux of calcium across the plasma membrane, which, in turn, may be controlled by voltage gated calcium channels. In our experiments we have investigated the importance of plasma membrane Ca-channels for intestinal fluid secretion and tried to determine the site of action of Ca-channel blockers in the nervous reflex arch.

Methods: Studies were performed, in which intestinal net fluid transport (NFT), potential difference (PD) and luminal serotonin release were measured in anaesthetized rats in vivo and short circuit current (SCC) in vitro. Three series of experiments was performed. (1) The fluid secretion evoked by cholera toxin, bile acid or A23187 (calcium ionophore) was studied on line with a gravimetric method. The influence of various calcium channel and nerve blockers was tested with regard to the induced fluid secretion (2) The effects of nifedipine (L-type channel antagonist) on CT and A23187 calcium ionophore induced 5-HT release, PD changes and fluid secretion were tested. (3) Experiments were performed to study how nifedipine affects the changes of SCC evoked by electrical field stimulation (EFS) in an Ussing chamber. The stimulation activates the efferent nerves influencing the secretory epithelium of the crypts.

Results: CT, sodium deoxycholate (4 mM) and A23187 evoked a fluid secretion that was almost totally abolished by nifedipine (2 mg/kg b.wt i.v.), hexamethonium (10 mg/kg b.wt.i.v.) or placing lidocaine (1%) on the intestinal serosa (Karlström et al.1986). Other L-type calcium channel antagonists like felodipine, R-felodipine, or N-type antagonist gadolinium chloride markedly decreased the CT induced fluid secretion. Similarly, CT and A23187 induced a release of 5-HT and increase of PD that was reduced to the control level by the administration of nifedipine i.v.. Nifedipine had no significant effect on SCC evoked by electrical field stimulation (EFS) as has been shown earlier (Hubel et al. 1980).

Discussion and conclusion: The present study shows that voltage-sensitive calcium channels of a neuronal L-type, particularly sensitive to dihydropyridine derivatives, appear to play an important role in the CT evoked fluid secretion. This may be explained by an effect on the stimulus-secretion coupling of 5-HT release from enterochromaffin (EC) cells. Since blockade of these channels also inhibited the bile salt induced secretion, it seems likely that the Ca-channel blockade also influences the function the afferent limb of the secretory nervous reflex. Moreover, the failure to reduce the SCC caused by EFS strongly suggest that L channels are not located in the efferent part of the reflex arch. Hence Ca-channel blockade may interfere with the secretory reflexes of the ENS at the EC cell lever and on the afferent limb of the reflex.

STRUCTURE FUNCTION STUDIES ON EPITHELIAL PEPTIDE TRANSPORT: TRANSLOCATION OF SYNTHETIC SUBSTRATES LACKING A PEPTIDE BOND.

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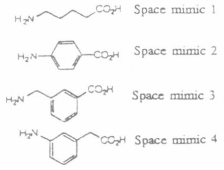
A series of four dipeptide-like geometrical 'space' mimics have been synthesised which retain the amino and carboxy terminals typical of a dipeptide but do not contain the peptide bond (figure). In this study we have investigated if these molecules are substrates for the renal brush border membrane proton-peptide cotransporter(s).



Brush border membrane vesicles (BBMV) were prepared from rat renal cortex using the double magnesium precipitation technique (1). The influx and efflux of a model, hydrolysis resistant radiolabelled dipeptide D-Phe-L-Glu (0.42μM) was measured under initial rate conditions by rapid filtration as described previously (2). The ability of each of the 'space mimics' to cis-inhibit influx pHout=5.5, pHin=7.4, E_m=-ve) and trans-stimulate efflux (pHout=pHin=7.4, E_m=0) of D-Phe-L-Glu was studied.

Cis-inhibition of influx of D-Phe-L-Glu by the 'space' mimics was investigated over the concentration range 0-10 mM. The data was well described by inhibition kinetics for a single system and half-saturation constants (K_i) were calculated. The K_m for D-Phe-L-Glu was 0.26 mM and the K_i's for the 'space' mimics 1-4 were; 23.73, 13.59, 65.04 and 7.93 mM respectively. The rate of D-Phe-L-Glu efflux from BBMV loaded with [^{3}H]D-Phe-L-Glu was also studied in the absence or presence of external (trans) substrate (10 mM). The rate (k) of control efflux (no external substrate) was 0.052 s^{-1} and this was increased in the presence of external D-Phe-L-Glu to 0.134 s^{-1} and by the 'space-mimics' 1-4 to 0.097, 0.063, 0.070 and 0.093 s^{-1} respectively implying that all these molecules are being translocated by the peptide transporter.

This study allows the following conclusions to be made; (1) All the space mimics can bind and be translocated by the D-Phe-L-Glu transporter although the affinity for the transporter is at least an order of magnitude lower than for the peptide substrate. This suggests that the peptide bond aids, but is not essential, for substrate recognition; (2) Inclusion of an aromatic ring into the 'space' mimic (generally) increases the affinity but reduces the rate of translocation of the substrate and (3) Addition of a methyl group between the aromatic ring and the carboxy terminus increases binding affinity in comparison to addition of a methyl group between the aromatic ring and the amino terminus which markedly decreases binding affinity.

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(2) Temple *et al.* Pflugers Arch. **430**: 825-829 (1995)

ADAPTATION OF L-LYSINE TRANSPORT *VIA* SYSTEM $b^{0,+}$ TO AN L-LYSINE-ENRICHED DIET.

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L-lysine uptake by the brush-border membrane of the chicken jejunum is mediated by two Na^+-independent transport systems: $b^{0,+}$ and y^+. We have now studied the effect of diet supplementation with L-lysine on the kinetic properties of both transport systems. The vesicles were prepared from the jejunum of 6-week-old Label chickens fed a control diet (48 g L-lysine/kg dietary protein) or an L-lysine-enriched diet (LYS diet: 68 g L-lysine/kg d.p.). The kinetic parameters were estimated from L-lysine relative transport rates (1), in the presence or in the absence of a *zero-trans* Na^+ gradient (100 mmol/L NaSCN or KSCN).

	KSCN		NaSCN	
	CONTROL	LYS Diet	CONTROL	LYS Diet
$K_i b^{0,+}$ (μmol/L)	55.0 ± 8.4	29.4 ± 5.1	21.0 ± 8.7	13.0 ± 1.9
$K_i y^+$ (mmol/L)	3.4 ± 0.09	4.8 ± 1.7	1.7 ± 0.3	0.877 ± 0.13
F	1.6 ± 0.12	1.9 ± 0.3	0.6 ± 0.1	0.99 ± 0.09
$K_m b^{0,+}$ (μmol/L)	2.7 ± 0.62	17.7 ± 2.0	2.4 ± 0.7	28.9 ± 7.4
$K_m y^+$ (mmol/L)	0.130 ± 0.020	0.165 ± 0.034	0.164 ± 0.013	0.2 ± 0.055
$V_{max}b^{0,+}/V_{max}y^+$	0.033	0.206	0.0088	0.144

For K_i calculations L-methionine was used as inhibitor. F is the "permeability ratio" defined as $(V_{max}b^{0,+}/K_m b^{0,+})/(V_{max}y^+/K_m y^+)$. Mean ± SE, n = 4.

In the absence of Na^+, LYS chickens showed a 6.6-fold increase in $K_m b^{0,+}$ whereas no modification of $K_m y^+$ was observed. Furthermore, F values remained unchanged, indicating no effect of the diet on the relative contribution of the two systems. This suggests that LYS-fed animals show an increase in $V_{max}b^{0,+}$ consistent with the 6.4-fold increment in the V_{max} ratio. In the presence of Na^+, there was a 12-fold increase in $K_m b^{0,+}$ while $K_m y^+$ again remained unchanged. The rise in F observed in the presence of Na^+ (1.7-fold), indicates a greater relative participation of system $b^{0,+}$ in the LYS-fed chickens, which may lead to a strong increase in $V_{max}b^{0,+}$ (16.4-fold increase in V_{max} ratio). Results support the view that the higher L-lysine uptake observed in birds fed a lysine-enriched diet can be explained by an increase in the $b^{0,+}$ transport capacity. (1) Devés et al. J. Physiol. 454:491-501, 1992.

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EVIDENCE FOR CL/HCO_3 ANTIPORT IN ABSORPTIVE EPITHELIAL CELLS ISOLATED FROM RAT JEJUNUM. M. Tosco, M.N. Orsenigo, A. Faelli, *Dipartimento di Fisiologia e Biochimica Generali, Universita di Milano, Milano, Italy.*

Recent studies performed with membrane vesicles (1) indicate that at the basolateral pole of rat jejunal enterocyte a Cl/HCO_3 exchanger could account for HCO_3 efflux from the cell during HCO_3 absorption. The presence of Na positively affects the rate of anion exchanger, but neither HCO_3 nor Cl gradients can drive the countertransport of Na (2). Aim of the present work was to isolate intact and viable jejunal enterocytes in which Cl/HCO_3 exchanger is operative. Enterocytes were isolated using a modification of the method described by Weiser (3) that involves a citrate and EDTA treatment in the absence of calcium. The plasma membrane integrity was assessed by determining both Trypan blue exclusion and lactate dehydrogenase released to the medium. The ability of cells to perform biochemical functions was checked by measuring lactate formation from glucose. Moreover the transport capability was ascertained by evaluating 3-O-methylglucose uptake in the presence of a sodium gradient. Cells were found to retain their functional integrity long enough to allow further investigation. To test for the existence of Cl/HCO_3 exchanger in the plasma membrane of the isolated cells, both Cl efflux and Cl uptake studies were performed. In both experimental conditions Cl flux was found to be drastically reduced by DIDS. To exclude the possibility that Cl moves through DIDS-sensitive channels, in subsequent experiments Cl gradient was inverted by removal of extracellular Cl. Also in this condition Cl is accumulated in the cells in a DIDS-sensitive mode, suggesting an antiport activity energetically driven by Cl efflux. Finally, to evaluate the Na-sensitivity of Cl/HCO_3 exchanger, experiments were carried out with Na-depleted enterocytes incubated in the absence of Na: in agreement with previous studies, it seems that anion exchanger is operative also after Na removal, even if to a lesser extent. In summary, these results suggest that a Na-sensitive Cl/HCO_3 antiport, inhibited by DIDS, is functional in isolated enterocytes.

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FLUOXETINE IMPAIRS L-LEUCINE ABSORPTION BY THE HUMAN INTESTINAL CELL LINE CACO-2. E. Urdaneta, I. Idoate, J. Larralde, *Departamento de Fisiología y Nutricion, Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain.*

To date, fluoxetine is the most clinically useful antidepressant. This oral bicyclic drug selectively inhibits serotonine reuptake at the presinaptyc level. Compared to other tri- and tetra-cyclic antidepressants has more gastrointestinal side effects, like nausea, soft-stools and diarrhoea. Because of its oral administration intestinal interaction with several food components might occur. In fact we have reported that fluoxetine interferes with intestinal sugar absorption in the rat (1).We also have found, in the same animal model, an interaction between fluoxetine and amino acid absorption due to its effect on the basolateral Na^+/K^+ ATPase activity and on the brush border membrane sodium dependent transport system.
The human intestinal cell line Caco-2 has proven to be a good model to study nutrient absorption. Here we investigate the effect of fluoxetine on leucine absorption using this human model. Cells were grown on microporous membrane inserts, cellular confluence being checked by monitoring the transepitelial electrical resistance. Leucine uptake was assayed at different times at both the apical and basal sides of the cells. 90% of the leucine uptake at the apical side and 35% percent of the leucine uptake at the basal side took place by a sodium dependent system. Sodium independent system and diffusion accounted for the remaining uptake. Fluoxetine inhibited the sodium dependent uptake at both sides, the inhibition was time dependent at the apical side, (reaching a maximum value of 45%), and remained constant at the basal side (25% inhibition). The drug did not affect sodium independent uptake or diffusion. Kinetic assays performed with different drug concentrations showed a non-competitive inhibition. Apical-basal and basal-apical fluxes were also diminished by fluoxetine.
These results are similar to the previously obtained in rat intestine and confirm that fluoxetine impairs intestinal leucine absorption.

(1) Monteiro el al. J Clin Nutr Gastroenterol 8:13-20, 1993

BASOLATERAL POTASSIUM CHANNELS OF COLON EPITHELIUM: PROPERTIES AND FUNCTION. K. Turnheim, C. Wachter, H. Plass, *Pharmakologisches Institut, Universität Wien, Vienna, Austria.*

The basolateral membrane of epithelial cells contains K^+ channels which are primarily responsible for the conductance of this cell membrane. It is generally accepted that the regulation of the basolateral K^+ conductance is of central importance for the function of epithelial cells, but two questions remain unresolved: 1) Which class(es) of K^+ channels are responsible for the basolateral K^+ conductance; and 2) which intracellular signals regulate these channels?

Single-channel properties of K^+ channels from the basolateral membrane of rabbit distal colon epithelium were studied by incorporating these channels into planar phospholipid bilayers. The functional role that different classes of K^+ channels play in transepithelial electrolyte transport was assessed in intact colonic epithelia mounted in the short-circuit apparatus.

Following fusion of basolateral membrane vesicles from rabbit colon epithelium with planar lipid bilayers, K^+ and Cl^- channels are observed. Among the K^+ channels we can distinguish two classes according to their conductance. The high conductance (~200 pS) K^+ channel is activated by Ca^{2+} and depolarizing membrane voltages, the selectivity for K^+ over Na^+ is high (>20). In addition to Ca^{2+} and voltage, this channel is regulated by Mg^{2+}, nucleotides, the pH, and the phospholipid composition of the ambient bilayer. The venom of the scorpion *Leiurus quinquestriatus* (LQV) and charybdotoxin block the high conductance K^+ channel from the extracellular side. The low conductance (~20 pS) K^+ channel, on the other hand, is insensitive to Ca^{2+} and it is not inhibitable by LQV or charybdotoxin. The activity of the low conductance K^+ channel is not voltage-dependent, the selectivity for K^+ over Na^+ is ~7.

In isolated rabbit colon epithelia under short-circuit conditions, we examined the effects of a number of K^+ channel blockers, added to the serosal side of the epithelium, on transepithelial Cl^- secretion (induced by the adenosine derivative NECA) and Na^+ absorption. If the blocked K^+ channels play a role in basolateral K^+ recycling, a decrease in Cl^- and/or Na^+ transport is expected. Ba^{2+} blocks both Cl^- and Na^+ transport. Cl^- secretion is partially inhibited by LQV, but LQV does not alter Na^+ absorption. Lidocain (an inhibitor of high-conductance K^+ channels) and tolbutamide (a blocker of ATP-inhibtable K^+ channels) reduce Na^+ absorption.

It is concluded that high-conductance, Ca^{2+}-activated K^+ channels in the basolateral membrane of colon epithelium are involved in Cl^- secretion but not in Na^+ absorption. The nature of the K^+ channels responsible for basolateral K^+ recycling during Na^+ absorption remains obscure, but is appears that different classes of K^+ channels participate.

EFFECT OF HEAVY METALS ON D-GLUCOSE UPTAKE IN BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM EEL INTESTINE. S. Vilella, L. Ingrosso, M.G. Lionetto, V. Zonno, T. Schettino, C. Storelli, *Dipartimento di Biologia, Laboratorio di Fisiologia, Universita di Lecce, Italy.*

Heavy metals are natural components of seawater and only their excess in the environment is to be considered as pollution. They are usually classified, with respect to their biological activity, in essential (such as zinc) or non-essential (such as cadmium).
The purpose of the present study has been to verify the possible effect of heavy metals on the D-glucose uptake measured in brush border membrane vesicles isolated from the intestine of seawater adapted eels (*Anguilla anguilla*).
The experiments have been performed by using: 1) radioactive tracer (^{14}C-D-glucose) to measure the effect of the two metals on the substrate uptake into the vesicular space and 2) the membrane potential sensitive dye Dis-C_2(5), to measure the effect of the heavy metals on the glucose-dependent Na^+ influx.
Results obtained with the radioactive tracer, indicate that: Na-dependent ^{14}C-D-glucose uptake (measured under short circuited membrane potential conditions) was significantly ($P<0.05$) enhanced when the brush border membrane vesicles were preincubated with cadmium, while no effect was detected on the equilibrium uptake values. This effect was dose-dependent, with a maximum of stimulation (in the tested range) at 100 µM. Furthermore, the stimulatory effect was time dependent with a maximum at 60 minutes of preincubation time. Of interest was the observation that the preincubation of BBMV with cadmium had no effect on the Na-independent (diffusive) glucose movement into the vesicular space. At the opposite of cadmium, zinc had no effect either on the the Na-dependent D-glucose uptake or on the Na-independent mechanism.
The potential sensitive dye Dis-C_2(5) has been used to evaluate the effect of cadmium on the dissipation rate of an inside-negative membrane potential difference, under different experimental conditions. Results suggest that the preincubation of the vesicles with cadmium affected only the D-glucose-dependent Na influx transport rate, while the D-glucose independent Na influx was not affected. Taken together all these results demonstrate a stimulatory (activatory ?) influence of cadmium on Na-dependent D-glucose uptake and suggest that the site of action of cadmium should be located at the internal side of the plasma membranes.

THE ANTIMYCOTIC DRUG CLOTRIMAZOL ACTS AN MDR-1-REVERSING AGENT IN CACO-2 AND T84 CELLS. U. Wenzel, H.C. Korte, H. Daniel, *Institute of Nutritional Sciences, University of Giessen, Giessen, Germany.*

The MDR-1 gene product (pgp) acts as an ATP-dependent export pump for a large variety of xenobiotics in many mammalian cells, including the intestinal cell lines Caco-2 and T84. Its overexpression in cancer cells increases efflux of cytotoxic drugs below their effective concentration. One of the goals in cancer therapy therefore is to overcome multidrug resistance by targeting pgp-activity with specific inhibitors acting as reversing agents. Using Caco-2 and T84 cells we investigated the effects of the antifungal agent clotrimazol, recently described to inhibit cell proliferation in vitro and in vivo (1), on pgp transport function assessed by flux studies with the anticancer drug ^{3}H-daunomycin (^{3}HD). **METHODS:** Caco-2 and T84 cells were grown as monolayers on permeable filter supports and secretory fluxes were determined by applying ^{3}HD from the basal or apical compartment. Cellular accumulation and transepithelial secretory fluxes of ^{3}HD were measured for up to 3hrs using ^{14}C-mannitol as an extracellular marker. **RESULTS:** Net secretion of ^{3}HD in Caco-2 as well as in T84 cells was demonstrated basal to apical fluxes exceeding apical to basal fluxes 4-5 times. Net daunomycin transport could be inhibited completely by 100 µM of the reversing agents verapamil or cyclosporine A. Clotrimazol in a concentration of 20 µM also inhibited the net secretion ^{3}HD almost completely but increased the cellular accumulation of ^{3}HD by 160 ± 5 % in Caco-2 cells and by 145 ± 6 % in T84 cells over control values. Since clotrimazol has been shown to prevent mitogen induced elevation of intracellular Ca^{2+} ion concentration in quiescent Swiss 3T3 cells (1) we assumed that the inhibition of ^{3}HD secretion was a consequence of altered intracellular signalling cascades. We therefore studied whether compounds known to affect intracellular [Ca^{2+}] were also able to block daunomycin secretion. Thapsigargin that prevents Ca^{2+} to be released from intracellular stores after mitogen stimulation did not show any effect on ^{3}HD secretion. An elevation of intracellular [Ca^{2+}] by the ionophores A 23187 or ionomycin did neither influence ^{3}HD secretion nor did they prevent inhibition of pgp activity by clotrimazol. BHQ, which mobilizes Ca^{2+} specifically from IP_3 sensitive Ca^{2+}-stores and PMA which activates directly protein kinase C showed no effect on ^{3}HD transport. In addition, a number of physiological ligands acting via the Ca^{2+}-dependent signalling cascades (acetylcholine) or acting mainly via adenylyl cyclase dependent pathways, including gastrin, VIP or epinephrine, were without any effects on ^{3}HD secretion. **CONCLUSIONS:** Besides the ability of clotrimazol to inhibit mitogen induced cell proliferation our data suggest that this drug is also able to reverse MDR-1 mediated drug resistance by blocking pgp transport function in the apical membranes of Caco-2 and T84 cells. This feature could be an important additional advantage of using this drug in cancer therapy.

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Notes