C99

Iron uptake by rat proximal colon is increased in animals fed an iron deficient diet

K. Johnston[1], E. Debnam[2], D. Johnson[1], J. Marks[2], S.K. Srai[3] and P. Sharp[4]

[1]School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, [2]Department of Physiology, Royal Free & UCL Medical School, London, UK, [3]Department of Biochemistry & Molecular Biology, Royal Free & UCL Medical School, London, UK and [4]Division of Nutritional Sciences, King's College London, London, UK

The typical western diet provides 10 mg of iron each day. However only 10% of this is absorbed in the duodenum (i.e. 1mg/day), meaning that 90% of our daily intake reaches the distal small intestine and colon. It is presumed that this excess iron is simply excreted in the faeces. However, a recent report has suggested that the proximal colon might have some iron transport capacity (Bougle et al. 2002). In the present study we have investigated this possibility by measuring iron flux across the proximal colonic mucosa in animals fed either an iron replete (control) or an iron deficient (FeD) diet. In parallel studies the effect of dietary iron on the expression of the intestinal iron transporters DMT1 and IREG1 has also been determined.

All studies were carried out on male Wistar rats (250g). Rats were fed diets containing 44mg Fe/Kg (control) or <0.5mg/Kg (FeD) for 14 days prior to experimentation. For in vivo iron uptake studies, animals were anaesthetised with intraperitoneal pentobarbitone sodium (60 mg/Kg body weight) and 0.2mM $^{59}Fe^{2+}$ (complexed with 4mM ascorbate) was instilled into a tied-off segment of proximal colon. Blood samples were removed after 20, 40 and 60 min via a femoral artery cannula to determine iron transfer into the blood. Tissue iron uptake was measured at the end of each experiment by gamma counting of the colonic segments. In a separate group of animals the mucosa was isolated and used as a source of membrane protein and total RNA for analysis of iron transporter expression by Western blotting and RT-PCR respectively. Data are mean ± SEM. Statistical analysis was performed using Student's unpaired t-test.

Iron uptake was significantly increased in FeD group (162.4 ± 36.1 pmoles/mg dry wt tissue n=6) compared with control (71.4 ± 18.3 pmoles/mg dry wt tissue, $p<0.05$ n=6). This corresponded with a significant increase in DMT1 mRNA and protein in the FeD group. Interestingly, there was no difference in either iron efflux into the blood or in IREG1 expression in the two animal groups. These data indicate that the proximal colon has some capacity to absorb iron from the intestinal lumen. However, rather than transferring the iron into the blood for physiological utilisation, it is retained within the colonic mucosa. The physiological rationale for such a mechanism requires further investigation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C100

5-(N-ethyl-N-isopropyl)amiloride (EIPA) is an inhibitor of the human intestinal di/tripeptide transporter hPepT1

D.J. Kennedy and D.T. Thwaites

Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

The human intestinal di/tripeptide transporter hPepT1 (when expressed heterologously in *Xenopus laevis* oocytes or HeLa cells) functions as an H^+-coupled, pH-dependent, Na^+-independent transporter (Liang *et al.* 1995; Kennedy *et al.* 2002). In contrast, optimal dipeptide uptake in intact intestinal epithelia is partially dependent on extracellular Na^+ as a result of the functional coupling between hPepT1 and the Na^+/H^+ exchanger NHE3 (Kennedy *et al.* 2002). 5-(N-ethyl-N-isopropyl)amiloride (EIPA), which is used commonly to inhibit Na^+/H^+ exchange, reduces dipeptide uptake across the apical membrane of Caco-2 cell monolayers even in the absence of NHE3 activity (Na^+-free conditions) (Kennedy & Thwaites, 2003). At concentrations higher than those required to inhibit Na^+/H^+ exchange, amiloride and analogues interact with a number of other transporters (Kleyman & Cragoe 1988). The aim of this study was to identify whether EIPA inhibition of dipeptide uptake is direct on hPepT1 or indirect via a reduction in the transmembrane ionic driving force (the H^+-electrochemical gradient). *X. laevis* were killed humanely and oocytes removed. Gly-Sar or proline (both 100μM, 5μCi.ml^{-1}) uptake (40min, pH_o 6.0, Na^+-free conditions) was determined in oocytes 3 days after injection with 50ng hPepT1 or rat PAT2 cRNA, respectively. In hPepT1-expressing oocytes, EIPA inhibited Gly-Sar uptake [mean ± SEM (n)] in a concentration-dependent manner and at 500μM EIPA reduced uptake by 68±4 (29) % ($p<0.001$ vs. control, ANOVA, Tukey-Kramer post test). 5-(N-methyl-N-isobutyl)-amiloride (MIA) had similar effects to EIPA but amiloride and S1611 were without effect ($P>0.05$). Under voltage-clamped conditions 500μM EIPA reduced the dipeptide-induced (1mM Gly-Sar) current in hPepT1-injected oocytes. Proline uptake into oocytes expressing the H^+-coupled amino acid transporter rat PAT2 (Kennedy *et al.* 2005) was unaffected ($p>0.05$) in the presence of 500μM EIPA (98±10 (18) % of control, absence of EIPA). In conclusion, high concentrations (500μM) of EIPA inhibit dipeptide uptake via a Na^+-independent, non-NHE3 mediated pathway. This inhibition is likely through a direct effect on hPepT1 rather than an indirect effect on the H^+-electrochemical gradient as EIPA is without effect on H^+-coupled amino acid uptake via rat PAT2.

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C101

Expression studies in *Xenopus* oocytes show that the SLC36 (PAT)-related *Drosophila* gene *path* encodes the high-affinity, low capacity amino acid transporter PATH

D. Meredith, D. Goberdhan, C. Wilson and C. Boyd

Human Anatomy & Genetics, University of Oxford, Oxford, UK

The members of the PAT (SLC36) family of transporters characterized to date have been shown to be proton-coupled amino acid transporters, with alanine, glycine and proline as the major physiological substrates (Boll *et al.* 2004). Recently we reported that the *Drosophila melanogaster* protein CG1139, with ~30% identity to the mammalian PATs, is also a proton-coupled alanine, glycine and proline transporter (Meredith *et al.* 2005). The gene *path* from *Drosophila melanogaster* encodes a protein (PATH) with a predicted open reading frame of 471 amino acids which also has ~30% amino acid identity to mammalian PATs, and a 38% identity to CG1139. PATH (short for Pathetic), was so-named because disruption of the *path* gene in *Drosophila* results in a small fly phenotype, implicating this transporter in a nutrient sensing role in the control of growth (Goberdhan *et al.* 2005). Here we provide evidence that although *path* does encode an amino acid transporter, it has different functional characteristics to the mammalian PAT and CG1139 transporters.

The *path* clone (*CG3424*) was obtained from the Drosophila Genomics Resource Centre and expressed in *Xenopus laevis* oocytes. Uptake of [^{3}H]-Ala (75nM) was measured using the method described in Goberdhan *et al.* (2005).

The uptake of alanine was fastest when the external pHout was 7.4, with uptake abolished by extracellular acidification (Fig. 1A, 79.1 ± 17.9 versus 11.8 ± 11.9 fmoles/oocyte/h, mean ± S.E.M., pHout 7.4 versus 5.5, respectively, n=5 oocytes, $p<0.001$ Student's t test). The affinity for alanine was very high (apparent Km 2.7 ± 1.0μM at pHout 7.4 (Fig. 1B), and uptake was inhibited by glycine (by 76 ± 5%, $p<0.001$) but not proline (13 ± 8%, $p=0.31$) (both 50μM, data not shown).

In conclusion, PATH is an amino acid transporter with much higher affinity than the mammalian PATs (mouse PAT1 and PAT2 Km for alanine of 7.5 and 0.26 mM, respectively, when expressed in oocytes; Boll *et al.* 2002) and the recently characterized *Drosophila* protein CG1139 (1.2mM, Meredith *et al.* 2005; Goberdhan *et al.* 2005). In addition, transport through PATH is not stimulated by extracellular protons, and indeed at pHout 5.5 there was virtually no uptake. These very high affinity/very low capacity transport characteristics, taken together with the finding that disrupting the *path* gene results in a small fly phenotype, suggest a role for PATH as an amino acid sensor involved in the control of growth.

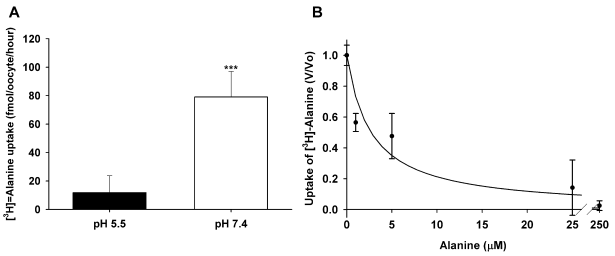

Figure 1. A, pH dependence of 75nM [^{3}H]-alanine uptake into Xenopus oocytes expressing CG3424 (n=5 oocytes, *** $p<0.001$ versus pH 5.5, Student's t test). B, self inhibition of 75nM [^{3}H]-alanine uptake at pHout 7.4 (n=5 oocytes per data point, $R^2=0.95$).

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C102

Regulation of a Cl^- conductance in cultured mouse collecting duct cells by pH

H. Taylor and L. Robson

Biomedical Science, University of Sheffield, Sheffield, UK

The renal collecting duct is composed of principal and intercalated cells, which serve to control the extracellular fluid volume and play an important role in acid base balance. Previous studies have shown pH sensitive Cl^- currents in a variety of mammalian epithelia, including the collecting duct (Estevez et al, 2001, Nobles et al, 2004 and Yoshikawa el at, 1999). The aim of the following study was to examine the effect of pH on a Cl^- conductance in a cultured mouse collecting duct cell line (M8).

Cl^- currents were examined in whole cell patches with symmetrical CsCl solutions, with a low pipette Ca^{2+} concentration. Clamp potential was stepped from a holding value of -40mV, to between +100 and -100 mV in 20mV steps. The current sensitive to 100 μM DIDS was measured in the presence of intracellular and extracellular solutions of varying pH (pH 7.4, 6.2 or 8.2). All values are expressed as means ± SEM. Statistical significance was tested using ANOVAs and assumed at the 5% level. At pH 7.4 the outward conductance (Gout) was 305 ± 105 μS/cm^2 (n=18) and the inward conductance (Gin) was 54.8 ± 25.9 μS/cm^2 (n=18). In unpaired cells, changing extracellular pH to 6.2 increased both Gout and Gin, 903 ± 204 μS/cm^2 (n=16) and 189 ± 58.8 μS/cm^2 (n=16), respectively. Increasing bath pH to 8.2 was without effect. In a separate series of experiments increasing intracellular pH to 8.2 from 7.4 decreased Gout from 572 ± 113 μS/cm^2 (n=28) to 192 ± 95.6 μS/cm^2 (n=12) and Gin from 97.2 ± 17.1 μS/cm^2 (n=28) to 15.73 ± 4.66 μS/cm^2 (n=12), at pH

7.4 and 8.2, respectively. Decreasing intracellular pH to 6.2 was without effect.
These data indicate that cultured mouse collecting duct cells contain an outwardly rectifying Cl^- conductance, which is sensitive to both extracellular and intracellular pH. Given the role of the collecting duct in acid base balance this pH sensitivity could be important in collecting duct function.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA37

Surprising Versatility of Na/Glucose Cotransporters

E.M. Wright

Physiology, UCLA, Los Angeles, CA, USA

The primary function of SGLT1 is to transport glucose across the brush border membrane of the human intestine. The clearest evidence for this is that subjects with mutations in SGLT1 are unable to absorb significant amounts of glucose. For example, adult subjects with Glucose-Galactose-Malabsorption caused by SGLT1 mutations are unable to absorb glucose as judged by oral tolerance and hydrogen breath tests (Wright et al. 2003). Members of the SLC5 gene family including SGLT1 (Wright & Turk, 2004) have other important functions as Na-uniporters, water and urea channels, water cotransporters, and glucosensors (Wright et al 2004). For example, SGLT1 behaves as a water channel and pump and so the 250,000 copies in the intestinal brush border of each enterocyte can account for the absorption of up to 6 liters of fluid a day across the brush border (Loo et al. 2001). Human SGLT3 is not a Na/glucose cotransporter but a glucosensor (Wright et al 2004). In response to an increase in glucose concentration this protein transports Na into the cell and this causes a depolarization of the membrane potential. In those cells where SGLT3 is expressed, cholinergic neurons in the enteric nervous system and neuroendocrine cells, this depolarization results in an increase in frequency of action potentials and, at least in neuroendocrine cells, an increase in peptide secretion. Thus SGLT3 in the gut may play an important role in the response of the GI tract to a meal. SGLT genes are expressed brain, heart, lung and muscle and so our challenge is to determine the physiological significance of their diverse functions in these organs and tissues. Careful study of human subjects with mutations in SGLT genes may provide some clues to resolving this challenge.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA38

Apical GLUT2, calcium and intestinal nutrient sensing

G.L. Kellett

Biology, University of York, York, UK

High luminal concentrations of glucose in vivo cause the activation and insertion of GLUT2 into the apical membrane of rat jejunum. Activation and insertion are dependent in part on transport of glucose through SGLT1, which both activates the Ca^{++}-requiring enzyme PKC βII and causes cytoskeletal rearrangement through Ca^{++}-dependent contraction of the perijunctional actomyosin ring. Recent experiments reveal that luminal Ca^{++} is essential for GLUT2 insertion and enters the enterocyte by an L-type channel, most likely $Ca_v1.3$; both apical GLUT2 insertion and Ca^{++} absorption are regulated by L-type effectors. Contraction of the perijunctional actomyosin ring is also essential for apical GLUT2 insertion; inhibition of myosin II phosphorylation with the myosin light chain kinase inhibitor, ML7, inhibits insertion and GLUT2-mediated absorption, but has no effect on Ca^{++} absorption. Paracellular flow of glucose is negligible when assessed using ^{14C}-mannitol as a tracer. Glucose and other sugars cause the rapid turnover and degradation of PKC βII by a pathway involving cleavage, dephosphorylation and ubiquitylation. The data are consistent with the view that SGLT1, PKC βII and Ca^{++} are among key elements in an intestinal sugar sensing system, which targets the rapid upregulation of glucose absorption through apical GLUT2 to match dietary intake.

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SA39

GLUT isoforms expressed in the intestine: their characteristics and possible functions

C. Cheeseman

Dept. of Physiology, University of Alberta, Edmonton, AB, Canada

Hexose absorption in the small intestine has long been understood to be mediated by at least three proteins. Uptake across the apical membrane (BBM) involves a sodium-coupled active transporter (SGLT1) and two facilitated transporters, GLUT5 and GLUT2. Exit across the basolateral membrane occurs via GLUT2. All three are expressed predominately in the duodenum and jejunum and at a much lower level in the ileum and not at all in the colon. A reserve capacity for the absorption of hexoses is ensured by changes in the expression of these proteins in parallel with the dietary content of carbohydrate. This adaptive response takes several days to be fully realized and appears to be programmed as enterocytes leave the crypts and migrate onto the villi. The sequencing of the human genome has led to the identification of several additional members of the GLUT protein family (SLC2A genes), which are now grouped into three sub-classes (1). Recently, this laboratory cloned GLUT7 (SLC2A7), a class II GLUT most closely related to GLUT5 (2). It is a high affinity glucose and fructose transporter isoform which is expressed predominantly in the BBM of the ileum and colon. Unlike the class I GLUTs (1-4), which have a Km in the mM range, the class II proteins are high affinity with Km in the range of 100-300 μM. The majority appear to transport fructose as well as glucose, but not galactose or 2-deoxyglucose, which would explain why expression cloning using the latter substrate failed to identify these proteins.
We have examined the effect of altered dietary carbohydrate content on the expression of intestinal GLUT7 to determine if it is changed in a similar manner to the other intestinal hexose transporters, which could indicate that this protein also plays a role

in the absorption of nutritional hexoses. In addition, we investigated if GLUT9 (3), another class II member known to be expressed in the kidney, is found in the intestine. Rats were maintained for 7 days on one of three diets, normal rat chow, a low carbohydrate diet, LC (23% glucose & 7.7% corn starch) or a high carbohydrate diet, HC (44 % glucose & 14.75% corn starch). The LC and HC diets were formulated to be isocaloric by changing the carbohydrate and fat content reciprocally. After 1 week on the diets, the animals were terminally anesthetized and the jejunum, ileum and colon excised for mRNA preparation, immunohistochemistry and Western blotting of isolated membranes. We found that ileal brush-border membranes from HC-fed rats had significantly greater expression of GLUT7 than those on the LC diet. GLUT9 was found to be expressed in the ileal and colonic apical membranes, but the level of expression of this protein in the colon was decreased by the HC diet.

These data indicate that GLUT7 may play a role in hexose absorption during the later stages of a meal. This is also the first demonstration that GLUT9 is expressed in the intestine, primarily in the apical membrane of the colon, a tissue which is not believed to be normally involved in hexose absorption. However, its expression increased when the fat content of the diet was raised suggesting that perhaps, like a related isoform HMIT (SLC2A13), its primary substrates are not hexoses (4).

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SA40

Transcriptional and post-transcriptional regulation of SGLT1 by RS1

H. Koepsell

Institute of Anatomy and Cell Biology, Wuerzburg, Germany

The intronless human gene *RSC1A1* on chromosome 1p36.1 encodes a 70 kDa protein called RS1 that is expressed in various tissues (1). RS1 contains several consensus sequences for protein kinase C, a consensus sequence for casein kinase II, and a C-terminal ubiquitin-associated domain. Upon confluence of renal epithelial LLC-PK1 cells, the expression of RS1 protein is down-regulated posttranscriptionally, and its intracellular distribution changes dramatically (2). In subconfluent LLC-PK1 cells, RS1 is located at the plasma membrane, at vesicles below the plasma membrane, at the *trans*-Golgi network, and within the nucleus. In confluent LLC-PK1 cells, RS1 was observed at the *trans*-Golgi but could not be detected at the plasma membrane or within the nucleus. Because the Na^+-D-glucose cotransporter SGLT1 is - opposite to RS1 - up-regulated by confluence, and because up-regulation of SGLT1 was similarly observed after reducing the expression of RS1 by an antisense strategy, we investigated the role of RS1 in the transcriptional and posttranscriptional regulation of SGLT1.

Run-off assays with nuclei from confluent LLC-PK1 cells showed that transcription of SGLT1 mRNA was increased when the expression of RS1 was reduced (2). This suggests that nuclear RS1 inhibits transcription of SGLT1. Posttranscriptional regulation of SGLT1 by RS1 was investigated in oocytes of *Xenopus laevis* (3). SGLT1 was expressed by cRNA injection and the transport activity of SGLT1 was determined as phlorizin-inhibitable uptake of radioactively labeled α-methyl-D-glucoside (AMG). Effects of RS1 on the expression of SGLT1 were tested in two ways: Either the cRNAs of RS1 and SGLT1 were injected together and oocytes were incubated 3 days for expression, or SGLT1 cRNA was injected, oocytes were incubated for 3 days, and purified RS1 protein was injected into the oocytes shortly before uptake of AMG was measured. Co-expression of cRNAs (human RS1 with human SGLT1) resulted in a 50-80% decrease of the expressed AMG uptake. This decrease was due to a decrease of SGLT1 expression because the V_{max} for AMG uptake was reduced whereas the functional characteristics of glucose transport were not changed (K_m value, sodium acitivation, and sugar specificity). Interestingly, injection of human RS1 protein produced a similar decrease of AMG uptake (50-80%). For the effect of RS1 protein a time period of 30 min was required. A functional characterization of the posttranscriptional inhibition of SGLT1 by RS1 revealed (*i*) that the effect of RS1 was dependent on the function of dynamin, (*ii*) that the effect of RS1 was stimulated by protein kinase C, (*iii*) that it persisted in the presence of inhibitors of endocytosis, and (*iv*) that the effect of RS1 was abolished after inhibition of exocytosis by botulinus toxin. The data strongly suggest that the posttranscriptional regulation of SGLT1 by RS1 involves modulation of fission of SGLT1 containing vesicles from the *trans*-Golgi network.

RS1 is more broadly expressed compared to SGLT1 and inhibits a variety of other plasma membrane transporters in addition to SGLT1 (3). To elucidate the physiological importance of RS1, we removed the *Rsc1A1* gene in mice and analyzed phenotype and expression of SGLT1 (4). In small intestine of *Rsc1A1* knockout-mice a sevenfold posttranscriptional up-regulation of SGLT1 was observed that resulted in a twofold increase of small intestinal glucose reabsorption. Several months after birth, the mice developed obesity of the intestinal type. The data indicate that RS1 participates in the regulation of SGLT1 expression and plays a critical role for the regulation of glucose uptake in small intestine.

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