C11

Vasoactive intestinal peptide reduces H$^+$-coupled amino acid uptake across the apical membrane of human intestinal Caco-2 cell monolayers

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H$^+$-coupled β-alanine uptake across the apical membrane of intestinal epithelial (Caco-2) cell monolayers (when measured at apical pH 6.5) is inhibited by forskolin but not 1,9-dideoxyforskolin suggesting modulation by a cAMP-dependent pathway (Anderson *et al.* 2001). The purpose of this study was to investigate the potential (patho)physiological effects of the neuropeptide vasoactive intestinal peptide (VIP) on amino acid absorption via the H$^+$-coupled amino acid transporter hPAT1 (Thwaites *et al.* 1995; Chen *et al.* 2003).

Caco-2 cells (passage number 103–117) were cultured on permeable filters (Thwaites *et al.* 2002) and used 13–18 days post-seeding. β-[^{3}H]Alanine (0.5 μCi ml^{-1}, 100 μM) uptake was measured at apical pH 6.5 (basal pH 7.4) for 15–90 min in the presence or absence of Na$^+$, VIP (0–100 nM) or the selective NHE3 (Na$^+$/H$^+$ exchanger 3) inhibitor S1611 (3 μM) (Wiemann *et al.* 1999).

Basolateral (but not apical) VIP (5 nM) significantly reduced ($P < 0.001$, ANOVA, Bonferroni *post hoc* test) apical β-alanine uptake (15 min) from 283 ± 13 (12) to 154 ± 15 pmol cm^{-2} (11) (means ± S.E.M. (n)). This effect of VIP was concentration dependent being maximal at 5 nM. In the absence of Na$^+$, VIP had no effect (uptake being 102 ± 11 (9) and 125 ± 12 pmol cm^{-2} (12) in the absence and presence of VIP, respectively, $P > 0.05$). The VIP-induced inhibition was through a reduction in the capacity for β-alanine uptake without effect on the affinity. In the presence of Na$^+$, apical S1611 reduced β-alanine uptake to a similar level to that observed in the presence of basolateral VIP.

In conclusion, VIP reduced β-alanine uptake in a Na$^+$-dependent manner consistent with the effect being indirect through inhibition of NHE3. The lack of any apparent direct effect on hPAT1 is supported by the observations that: (i) the NHE3 inhibitor S1611 has a similar effect to VIP on β-alanine uptake; (ii) there are no potential PKA phosphorylation sites within the hPAT1 sequence (Chen *et al.* 2003); (iii) S1611 and VIP have similar indirect inhibitory effects on H$^+$-coupled dipeptide uptake (Thwaites *et al.* 2002).

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This work was supported by the BBSRC (grant 13/D17277) and MRC (grant G9801704). CMHA is supported by a BBSRC Agri-Food Committee Studentship. S1611 was obtained from H.J. Lang, Aventis Pharma Deutschland GmbH, Chemical Research, Frankfurt/Main, Germany.

C12

Transcriptional regulation of the human trefoil factor, TFF1, by gastrin

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The trefoil peptide TFF1 is expressed in surface mucous cells of the gastric epithelium. Trefoil factors are important for restitution and repair of the epithelium and are rapidly up-regulated in response to injury. TFF1 may also act as a gastric tumour suppressor (Park *et al.* 2000). We identified TFF1 as a gastrin-sensitive gene for the first time by mRNA differential display of gastric corpus from gastrin knockout (GAS-KO) mice *versus* wild-type (WT) C57/BL6 controls. Gastrin-stimulated expression of TFF1 in gastric corpus from humanely killed mice, and in the gastric cancer cell line AGS-G$_R$ (Watson *et al.* 2001), was determined by Northern blot. Regulation of TFF1 transcription in AGS-G$_R$ cells was studied using promoter–reporter assays and electrophoretic mobility shift assays (EMSA).

TFF1 mRNA abundance in GAS-KO mice was reduced to 63.0 ± 7.0 % of that in WT controls (mean ± S.E.M., $P < 0.05$, Student's unpaired t test, $n = 5$) and increased in a hyper-gastrinaemic transgenic strain (INS-GAS) to 142.0 ± 10.0 % that of WT controls ($P < 0.05$). TFF1 mRNA abundance was acutely regulated by gastrin (10^{-8} M) in AGS-G$_R$ cells, increasing to 14.3 ± 3.6-fold above vehicle treated controls ($P < 0.02$, $n = 3$) after 3 h and 30.1 ± 6.3-fold after 15 h. A fragment of approximately 1.4 kilobases of the human TFF1 promoter was cloned into the luciferase reporter vector pXP2. Luciferase expression in AGS-G$_R$ cells was dose-dependently increased by gastrin (779 ± 137 % of that of vehicle control, $P < 0.001$, $n = 9$, with 10^{-9} M gastrin). TFF1-luciferase expression was induced both directly, and by transactivation through neighbouring cells. The response to gastrin mapped to a 16 bp GC-rich region incorporating overlapping consensus binding sites for the transcription factors SP1 and MAZ. Mutation through this region reduced gastrin-stimulated luciferase expression to between 15 and 36 % of that seen with WT constructs ($P < 0.05$, $n = 3$, ANOVA). In EMSAs a radiolabelled probe corresponding to this 16bp region of the TFF1 promoter bound to nuclear extracts from gastrin-stimulated but not unstimulated AGS-G$_R$ cells. Binding was dependent upon intact SP1 and MAZ consensus sites, and was disrupted by incubation with antibodies to MAZ or SP3.

We conclude that gastrin exerts tonic control of TFF1 expression, but also has the potential for rapid up-regulation of this trefoil factor. TFF1 is a potential candidate to counter the proliferative effects of gastrin that may occur in response to mucosal injury.

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This work is supported by the Wellcome Trust and MRC.

All procedures accord with current UK legislation.

C13

Fatty acids act directly on intracellular calcium stores in the enteroendocrine cell line STC-1

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Fatty acids with chain lengths of more than 12 carbon atoms stimulate cholecystokinin (CCK) release from enteroendocrine cells *in vivo* (McLaughlin *et al.* 1999) and *in vitro*, accompanied with an increase in $[Ca^{2+}]_i$ (McLaughlin *et al.* 1998). By monitoring $[Ca^{2+}]_i$ in an established murine CCK-producing enteroendocrine cell line STC-1, we investigated which signal pathways transduce the fatty acid signal, or whether fatty acids themselves act intracellularly to induce a Ca^{2+} signal, and hence secretion.

STC-1 cells were loaded with Ca^{2+}-sensitive fluorescent dyes and $[Ca^{2+}]_i$ measured ratiometrically, using a fluorescence-microscope imaging system. Intact cells loaded with fura-2 were exposed to 100–500 μM fatty acid (C8:0, C10:0, C12:0, C18:1) under several conditions, e.g. absence of Ca_o^{2+}, or pretreatment with drugs known to block candidate signal transduction pathways. To examine direct effects of fatty acids on the intracellular Ca^{2+} store, Ca^{2+} release was assessed by measuring intra-organellar Ca^{2+} in cells loaded with mag-fura-2 (a low affinity Ca^{2+}-sensitive dye) and permeabilized by Streptolysin O (van de Put & Elliott, 1996).

In intact cells, in the presence or absence of extracellular Ca^{2+}, C12:0 and C18:1, but not C8:0 or C10:0, induced $[Ca^{2+}]_i$ responses in a dose-dependent manner. The C12:0-induced $[Ca^{2+}]_i$ response was prevented by depletion of intracellular Ca^{2+} stores with thapsigargin. Several blockers of classical signal pathways, which may couple to Ca^{2+} release from intracellular stores (IP_3 receptor antagonists, ryanodine receptor antagonists, phospholipase inhibitors and cAMP), all failed to abolish C12:0-induced $[Ca^{2+}]_i$ responses.

In permeabilized cells, Ca^{2+} was accumulated by stores in the presence of ATP, and was released by IP_3 or thapsigargin. C12:0 (100~500 μM) released stored Ca^{2+} in a dose-dependent manner. The fatty acid chain length dependency was identical in permeabilized cells and intact cells.

Fatty acids (C12:0 and C18:1) induce Ca^{2+} release from intracellular Ca^{2+} stores, and this can occur in the absence of extracellular Ca^{2+}. The data strongly suggest that these fatty acids can act directly on an intracellular Ca^{2+} store to release Ca^{2+}, independently of the major intracellular signal pathways.

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This work was supported by the Digestive Disorders Foundation Senior Fellowship (JMcL), and the Japan Society for the Promotion of Science (TH).

C14

Luminal factors modulate colonic mucus barrier dynamics *in vivo*

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The colonic mucus barrier lines the entire colon and acts as the first line of innate protection of the body against the myriad factors within the colonic lumen. Vital to this protective role are the maximal attainable thickness of the mucus barrier, as well as the rate of mucus replenishment. This study aimed to test whether certain colonic luminal factors, namely dietary fibre type, butyrate and reactive oxygen species (ROS), could alter these measures of colonic mucus barrier functionality.

Male Wistar rats were terminally anaesthetised by intraperitoneal administration of 0.26 ml (g body weight)$^{-1}$ Inactin. Colonic mucus layer dynamics were measured *in vivo* using the methods of Atuma *et al.* (2001). For dietary fibre studies, rats ($n = 10$ for each diet) were fed diets containing approximately 14 % w/w fibre, except the fibre-deficient diet. Various saline-based ROS-generating solutions (0.5 mM EDTA/Fe^{2+} and 5–50 mM H_2O_2) and 7 mM butyrate were applied to the colonic lumen of separate animals ($n = 5$). The effects of these luminal agents on colonic mucus barrier thickness and mucus replenishment rate were then assessed. One-way analysis of variance was used for statistical analyses (significant difference taken as $P < 0.05$). Butyrate at 7 mM trebled the rate of mucus replenishment (compared to saline controls).

ROS-generating solutions containing 17.5 mM H_2O_2 or higher significantly reduced maximal mucus thickness linearly over time, by as much as 50 % of the initial thickness (in the case of 50 mM peroxide). The mean effects of dietary fibre types on colonic mucus dynamics are summarized in Table 1. High levels of ROS in the colon reduce the protective capacity of the mucus layer. Low ROS (5 mM) and 7 mM butyrate increase mucus barrier functionality. Feeding rats on diets containing wheat bran and ispaghula husk increased mucus barrier function compared to cellulose and pectin. The latter two diets showed similar trends on mucus barrier dynamics to a fibre-deficient diet.

Table 1

Dietary fibre type	Maximal mucus thickness (μm)	Mucus secretion in 5 h (μm)
Wheat bran	578 ± 9^{a}	541 ± 111^{a}
Ispaghula husk	517 ± 7^{b}	679 ± 129^{a}
Fibre-deficient	429 ± 19^{d}	270 ± 70^{b}
Cellulose	440 ± 16^{c}	175 ± 26^{c}
Pectin	476 ± 25^{bc}	300 ± 81^{b}

Data are means ± S.E.M. Values in each column with same superscript are not significantly different.

This study suggests that the protective action of the colonic mucus barrier, and therefore the potential for mucosal damage, is altered by colonic luminal factors of dietary and bacterial origin.

Atuma C *et al* (2001). *Am J Physiol* **280**, G922–929.

All procedures accord with current UK legislation.

PC22

EIPA inhibition of dipeptide uptake across the apical membrane of human Caco-2 cell monolayers

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The cloned intestinal di/tripeptide transporter hPepT1 is a H^+-coupled, Na^+-independent carrier. However, for optimal dipeptide uptake to occur in intact intestinal epithelia extracellular Na^+ is required to allow functional coupling between the Na^+/H^+ exchanger NHE3 and hPepT1 (Kennedy *et al.* 2002; Thwaites *et al.* 2002). The Na^+-dependent component of dipeptide uptake in Caco-2 cell monolayers is reduced in the presence of the NHE3 inhibitor S1611 whereas S1611 has no effect on dipeptide uptake in hPepT1-expressing oocytes (Kennedy *et al.* 2002). The aim of the present study was to investigate whether or not other pharmacological NHE inhibitors are able to modulate dipeptide uptake via the same mechanism as S1611.

Caco-2 cells (passage 102–118) were cultured on permeable supports and used 14–17 days post seeding (Thwaites *et al.* 1999). Apical uptake (37 °C) of Gly-Sar, leucine or arginine (all 10–100 μM, 0.5 μCi ml^{-1}) was determined using Na^+ and Na^+-free (choline chloride) modified Krebs–Ringer solution (pH 5.0–7.4). *Xenopus laevis* were killed humanely and oocytes removed. Gly-Sar (88 μM, 5 μCi ml^{-1}) uptake (40 min, pH 6.5 in the presence of Na^+) was determined in *X. laevis* oocytes 3 days after injection with 50 ng hPepT1 cRNA.

One hundred micromolar EIPA inhibits NHE3 (Orlowski, 1993; Thwaites *et al.* 1999) and has the same effect as S1611 (3 μM) on Gly-Sar uptake across the apical membrane of Caco-2 cell monolayers ($P > 0.05$, ANOVA). However, at higher (e.g. 500 μM) EIPA concentrations there was an additional S1611-insensitive effect ($P < 0.05$ *versus* S1611). In the presence of Na^+, Gly-Sar uptake (pH 6.5) was reduced ($P < 0.001$) by EIPA (500 μM) from 32.6 ± 2.6 (10) to 12.4 ± 1.0 pmol cm^{-2} (15 min)$^{-1}$ (11) (means ± S.E.M. (n)). Even in the absence of extracellular Na^+ (where NHE3 is inactive) Gly-Sar uptake was reduced ($P < 0.05$) by EIPA (500 μM) (from 16.8 ± 0.8 (11) to 11.3 ± 0.7 pmol cm^{-2} (15 min)$^{-1}$ (11)). EIPA (500 μM) had no effect ($P > 0.05$) on either arginine or leucine uptake. In the absence of Na^+, EIPA (500 μM) inhibited Gly-Sar uptake in a pH-dependent manner being maximal at pH 5.0–6.0. In addition, EIPA (500 μM) inhibited ($P < 0.001$) Gly-Sar uptake into hPepT1-expressing oocytes whereas S1611 was without effect.

In conclusion, high concentrations of EIPA (e.g. 500 μM) are able to inhibit dipeptide uptake via a Na^+-independent, non-NHE3 mediated pathway. This pharmacological inhibition may be either direct on hPepT1 or indirect by a reduction in the H^+-driving force for hPepT1 activity.

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Orlowski J (1993). *J Biol Chem* **268**, 16369–16377.

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This work was supported by the BBSRC (grant 13/D17277).

All procedures accord with current UK legislation.

PC23

The effect of modified fatty acids on CCK secretion in murine STC-1 cells

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Previous studies showed that fatty acid-induced CCK secretion is chain-length dependent with fatty acids shorter than dodecanoic acid (C12) having no effect on plasma CCK (McLaughlin *et al.* 1999). Chain-length dependence is also observed in the established CCK secreting enteroendocrine cell line, STC-1. Recently, we demonstrated that STC-1 cells respond to hydrophobic fatty acids as aggregates, since the cellular response to fatty acid filtrates was significantly decreased (Benson *et al.* 2002). In the light of this, we have investigated the structural components of the fatty acid molecule that are necessary for inducing CCK secretion.

Several fatty acid analogues were examined and compared to C12 in their ability to induce CCK secretion and $[Ca^{2+}]_i$ mobilisation in STC-1 cells. $[Ca^{2+}]_i$ was measured using Fura-2 and dual excitation ratiometric fluorescence microscopy. CCK secretion was measured by radioimmunoassay.

The fatty acid analogue 1,10-decanedicarboxylic acid, which replaces the terminal methyl group with a second carboxylic group, was unable to stimulate CCK secretion or a rise in $[Ca^{2+}]_i$ (1,10-decanedicarboxylic acid, 0.78 ± 0.08 pmol mg^{-1}; C12, 1.68 ± 0.23 pmol mg^{-1}; means ± S.E.M., $P < 0.05$, Student's paired t test). This suggests that the amphipathic nature of fatty acids is necessary for their detection by STC-1 cells. The non-metabolisable fatty acid 2-bromododecanoic acid induced CCK secretion (2-bromododecanoic acid, 1.59 ± 0.15 pmol mg^{-1}; control, 0.22 ± 0.08 pmol mg^{-1}; means ± S.E.M., $P < 0.05$, Student's paired t test) and a rise in $[Ca^{2+}]_i$, suggesting that fatty acid metabolism is not necessary. Interestingly, more major changes to the fatty acid molecule (e.g. replacing each hydrogen by a fluorine) did not perturb the STC-1 cellular response. To investigate the possibility that insoluble fatty acid aggregates of a certain size were responsible for CCK secretion (rather than fatty acid monomers in solution), the fatty acid analogue emulsions were sized, using enhanced laser diffraction and polarization intensity differential scattering. Generally, there was a poor correlation between particle size and CCK secretion, since some hydrophilic fatty acid analogues which did not form particles, such as 1-undecanesulfonic acid and the fluorine-containing analogues, were able to induce CCK secretion.

These data add a cautionary note to our previous finding that fatty acid-induced CCK secretion is mediated by particles of a certain size independent of chemical composition. However, this study remains consistent with the hypothesis that the signal transduction pathway involved in fatty acid recognition is not mediated by a receptor that specifically responds to saturated carboxylic acids, since extensive modification of the fatty acid molecule still results in agents which are able to induce CCK secretion and calcium mobilisation in STC-1 cells.

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