unit/min (n = 6, p < 0.01). Forskolin (1 μM), an activator of adenylate cyclase, reduced the apical NHE activity to 0.05 ± 0.01 pH unit/min (n = 9, p < 0.01). The apical NHE activity in cystic fibrosis (ΔF/ΔF) ducts was 0.20 ± 0.01 pH unit/min (n = 6), which was significantly (p < 0.01) higher than that in wild type ducts and was accelerated to 0.66 ± 0.11 pH unit/min (n = 6, p < 0.01) by application of forskolin.

In interlobular duct cells from mice pancreas, the activity of apical NHE was suppressed by functional CFTR and it was stimulated by cAMP in the absence of functional CFTR. These data suggest that the inhibitory regulation of apical NHE by CFTR does not work in cystic fibrosis pancreatic duct, which may lead to acidification of pancreatic juice.

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Ishiguro H et al. (1998). J Physiol 511, 407-422.

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*Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.*

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PC28

## Human bestrophin 1 is expressed in the human pancreatic duct cell line, CFPAC-1

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Calcium-activated chloride channels (CaCC) are major targets for an alternative anion channel therapy in cystic fibrosis (CF). The molecular identity of CaCC has yet to be defined. The bestrophins are a novel candidate for the molecular origin of CaCC (Sun et al. 2001; Qu et al. 2004). In this study, we provide the first evidence for the existence of human bestrophin 1 (hBest 1) in CFPAC-1 cells.

Native CaCC were characterised in confluent monolayers of CFPAC-1 cells using a non-radioisotope, iodide efflux assay. Application of ionomycin to CFPAC-1 monolayers led to a concentration-dependent increase in iodide efflux. Maximal efflux was observed with 2 μM ionomycin, which induced an increase in efflux of 14.6 ± 1.9% (n = 4, p<0.001). Niflumic acid (200 μM) inhibited the response demonstrated with 0.5 μM ionomycin from 7.7 ± 1.3% to 1.8 ± 1.3% (n = 4, p<0.001). The purine receptor agonist, uridine 5'-triphosphate (UTP), also stimulated efflux from CFPAC-1 monolayers in a concentration dependent manner. Maximal efflux was observed at 200 μM UTP (9.0 ± 1.6%, n = 6, p<0.001). Niflumic acid (200 μM) reduced the response elicited by 100 μM UTP from 7.9 ± 2.0% to 1.99 ± 0.13% (n = 6, p<0.001) and 500 μM 4,4'-diisothiocyanatostilbene-2,2'-disulfonate (DIDS) also reduced the response from 7.9 ± 2.0% to 2.2 ± 1.5% (n = 6, p<0.001).

Total RNA was isolated from CFPAC-1 cells and RT-PCR was carried out with hBest1 specific primers corresponding to three areas of the cDNA sequence (NM 004183, Genbank database). RT-PCR yielded three bands of 489, 461 and 411 base pairs, respectively, identical in size to the predicted products. Sequence analysis of the three products demonstrated 100% sequence identity to that published in GenBank, indicating that the three products were consistent with the hBest1 gene. To assess the expression of hBest 1 at the cellular level, two commercially available antibodies were used, ab 14927 (Abcam, UK) and Bst 121 (Fabgennix, USA). Western blot analysis of CFPAC-1 protein isolates showed that both antibodies gave similar blots (n=8 and n=4, respectively), with a principal band at approximately 55 kDa and three very faint bands around 70, 75 and 78 kDa.

To further confirm the expression of hBest 1 in CFPAC-1 cells, the cells were fixed with 4% paraformaldehyde and hBest1 was labelled using either ab 14927 (n=3) or Bst 121 (n=2). Preliminary confocal image analysis of the fixed cells showed that hBest 1 is expressed to a large degree in the cytoplasm, most probably in cytoplasmic vesicles. Although not immediately obvious, some staining may be confined to the plasma membrane.

Taken together, these preliminary data suggest that hBest 1 is expressed in the CFPAC-1 cell line. Further studies are being undertaken to determine whether other bestrophin homologues are expressed in CFPAC-1 cells.

Sun H et al. (2001). Proc Nat Acad Sci 99(6), 4008-4013.

Qu Z et al. (2004). J Gen Physiol 123, 327-340.

*Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.*

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PC29

## Fatty acid and particle sensing mechanisms in epithelial and endocrine cells

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The murine enteroendocrine cell line STC-1 responds to fatty acids (FA) by secreting biologically active CCK, via an elevation of intracellular calcium, $[Ca^{2+}]_i$. This therefore represents a tractable model of FA sensing. Previous work has suggested that the formation of aggregates by FA in physiological media is an important factor in lipid sensing by STC-1 cells (Kazmi et al. 2003). Synthetic latex microspheres (LM) of similar size to FA aggregates (~90-300nm) also stimulate STC-1 cells causing CCK secretion, raising the possibility that these are sensed by the same cellular mechanism as FA. The cellular mechanism(s) underlying FA sensing remain largely uncharacterized, although one candidate is the newly assigned FA receptor, GPR40, first described in pancreatic beta cells, but which is also expressed by STC-1 cells. We therefore evaluated (1) if LM activate GPR40, and (2) if other cell models sensitive to LM are also FA sensitive. Real-time changes in $[Ca^{2+}]_i$ were measured using fura-2-loaded cells challenged with FA (linoleic acid, C18:2) and LM, by measuring a rise in the fura-2 340/380 ratio value which corresponds to a rise in intracellular calcium. Initially, RT-PCR confirmed expression of GPR40 in STC-1 but not in (rat) neuroendocrine PC12 cells. mGPR40 was therefore subcloned from STC-1 cells and stably overexpressed in PC12 cells. PC12 sensitivity to FA was increased 2.5-fold in PC12 mGPR40+ cells versus wild type cells (P=0.005, Mann-Whitney *U* test); PC12 mGPR40+ cells

responded to 500μM C18:2 with a rise in the fura-2 340/380 ratio value of 0.4±0.05 (n=5) compared to 0.16±0.02 (n=5) in PC12 WT cells. As with STC-1 cells, PC12-WT cells also responded to LM with a 0.23±0.06 (n=6) rise in 340/380 ratio value, but LM sensitivity was not significantly altered in mGPR40+ PC12 cells. Furthermore, the $[Ca^{2+}]_i$ response to LM was entirely abolished in the absence of extracellular calcium, whilst the FA-induced $[Ca^{2+}]_i$ response was preserved. Finally, a $[Ca^{2+}]_i$ response was also induced by LM in both MDCK (canine renal tubule epithelial model) and CaCo-2 (human intestinal epithelial model) cell lines, but not induced by FA in either. In conclusion, cell stimulation by LM is not via GPR40, nor is it a property restricted to FA-sensitive epithelial cells. This indicates that LM are not mimicking FA aggregates by activating specific FA-sensing mechanism(s). The mechanisms by which LM gate intracellular calcium entry remain to be elucidated. Nonetheless, LM induce CCK secretion in enteroendocrine cells, so may provide a novel method by which to modify upper gastrointestinal function or satiety, employing a non-nutrient signal that is retained within the lumen of the GI tract.

Kazmi S et al. (2003). J Physiol 553, 759-773.

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PC30

## Fatty acid sensing by GPR40 heterologously expressed in PC12 cells

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Fatty acid (FA) sensing mechanisms in gut epithelia remain undefined, but enteroendocrine cells (EEC) play a pivotal role. One potential candidate sensor is the recently deorphanised receptor GPR40, originally identified in pancreatic β cells (Itoh *et al.* 2003). The murine enteroendocrine cell line, STC-1, is known to express GPR40 and is responsive to FA in a chain length-dependent manner. This requires a minimum acyl chain length of 12 carbons, as is observed *in vivo* in humans. The aim of this study was therefore to express mouse GPR40 in a heterologous system, and study whether the chain length specificity of transferred fatty acid responsiveness is preserved. Initially, mGPR40, obtained by RT-PCR from the STC-1 cell line, was subcloned into the mammalian expression vector pEYFP-N1 in C-terminal fusion with the fluorescent protein EYFP, then stably expressed in the neuroendocrine cell line, PC12. Both wild type (WT) and mGPR40EYFP-expressing PC12 cells were loaded with Fura-2 to monitor $[Ca^{2+}]_i$ by fluorescence microscopy.

As observed in humans and STC-1 cells, FA of acyl chain length C4-C10 did not increase $[Ca^{2+}]_i$ in either PC12-WT or mGPR40EYFP+ cells. However, mGPR40EYFP+ PC12 cells displayed significantly increased $[Ca^{2+}]_i$ responsiveness to long chain FA compared to PC12-WT cells. Dodecanoic acid (C12:0) produced no increase in $[Ca^{2+}]_i$ in PC12-WT, but a large response was elicited in mGPR40EYFP+ PC12 cells, with an average ratio increase of 0.5±0.08 ratio units (n=5). Linoleic acid (C18:2) induced a small rise in $[Ca^{2+}]_i$ in PC12-WT (0.1±0.05 ratio units, n=3) which was amplified in mGPR40EYFP+ PC12 cells (0.4±0.05 ratio units, n=4; $P<0.05$, Mann-Whitney *U* test). For comparison, the maximal $[Ca^{2+}]_i$ response induced by 70mM KCl was 0.7±0.1 ratio units in PC12-WT and 0.6±0.1 ratio units in mGPR40EYFP+ PC12 cells. Responses were both reversible and reproducible. Data values are mean±SEM. In conclusion, mGPR40 heterologously expressed in the PC12 neuroendocrine cell line retains chain length-dependent FA responses entirely concordant with previous studies in both the STC-1 cell line and in humans, further supporting a functional role for GPR40 in gut FA-sensing by EEC. These data justify further detailed study into the signalling pathways and detailed molecular physiology of the GPR40 receptor in relation to EEC.

Itoh Y et al. (2003). Nature 422(6928), 173-176.

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PC31

## Roles of the N- and C-termini in the function of the urea transporter mUT-A3

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Urea transporters play an integral role in the urinary concentration process. UT-A3 is expressed basolaterally in cells lining the inner medullary collecting duct (Stewart *et al.* 2004). Although much is known about the regulation and distribution of UT-A3, we have little idea how these proteins function at the molecular level. The current study evaluates the role of the N- and C-termini in the function of the mouse urea transporter UT-A3 (mUT-A3).

In all experiments mUT-A3 and its mutations have been N-terminally tagged with eGFP. Truncation and point mutations were constructed using standard PCR techniques. All mutations were confirmed by sequencing. Oocytes were isolated from Xenopus laevis and injected with 1.5ng of cRNA encoding mUT-A3, its mutants or 50nl of water. Urea transport was assessed 3-4 days after injection by measuring uptake of $^{14}C$-labelled urea as described previously (Fenton *et al.* 2000). Statistical analysis was performed using one way ANOVA coupled with the Student-Newman-Keuls test. Results were obtained from at least 10 oocytes, isolated from 2 or more animals. Significance has been assumed at the 5% level.

The N-terminal of mUT-A3 was shortened, to start at residues M55 and M111. The M55-start mutant was functional when expressed in oocytes. M111-start, was non-functional suggesting a region between M55 and M111 is important for mUT-A3 function. A second series of truncated mutants were constructed with the N-terminal starting at S68, G94 and A103. All of these mutants were functional. In full length mUT-A3, deleting residues 103 to 113 (mUT-A3-Δ103-113) prevented urea uptake. When expressed in non-polarised MDCK cells, mUT-A3-Δ103-113 was trafficked to the plasma membrane and demonstrated

a distribution pattern similar to wild-type mUT-A3. Taken together these results suggest that the 8-aa region between residues 103 and 113 is required for normal transport function but is not linked to protein trafficking.
To investigate the role of the C-terminus, a series of truncation mutants were constructed. mUT-A3 has a predicted open reading frame of 460 amino acids. We inserted stop codons at residues 285, 315, 409, 429, 449 and 456. Urea uptake was not observed in oocytes expressing the E285X, I315X, W409X, Y429X V449X mutants. However, deleting the final 4 amino acids (K456X) did not reduce urea uptake compared to full length mUT-A3. These results suggests the 7aa between residues 450–456 are important for urea transport, although the precise role of this region in mUT-A3 function still needs to clarified.

Stewart GS *et al.* (2004). *Am J Physiol* 286, F979-F987.

Fenton RA *et al.* (2000). *Am J Physiol* 279, C1425-C1431.

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PC32

## Lack of evidence *in vivo* for vagally mediated remote effects of *Escherichia coli* heat stable (STa) enterotoxin on jejunal fluid absorption

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Heat stable (STa) enterotoxin from *E. coli* reduces absorption from the jejunum of the anaesthetised rat. STa is claimed (1) remotely to reduce fluid uptake since ileal perfusion with STa apparently reduces jejunal fluid absorption but not after cervical vagotomy. In view of likely cardiovascular effects of vagotomy on uptake and of reflexes caused by ileal perfusion, remote effects of STa were re-examined. Fluid uptake from jejunal loops was measured in anaesthetised (70 mg/kg i.p. Sagatal) unfasted Sprague Dawley rats (2). Twenty five cm loops were perfused with bicarbonate solution (150 mM). The vagus was intact, or sectioned at the neck or below the diaphragm. Completeness of sub-diaphragmatic section was determined by histology. Adequacy of function of isolated vagi were shown by effects of vagal stimulation on respiration and gastric secretion. Results are expressed as the mean and standard error, with the number of animals given. One loop was used per experiment. Significance was calculated after Dunnet's correction for multiple comparisons; $P < 0.01$.
Normal absorption experiments were intercalated with the vagotomy series, to confirm the activity of the STa. In these experiments, STA reduced fluid absorption in the jejunum from 81.6 ± 12.0 (8) µl/cm/h to 23.4 ± 4.2 (7) µl/cm/h. Control net jejunal fluid absorption of 79.5 ± 14.0 (7) µl/cm/h did not differ from absorption after cervical vagotomy of 75.6 ± 6.0 (6) µl/cm/h or after abdominal vagotomy of 76.1 ± 6.6 (8) µl/cm/h. The creation and perfusion of ileal loops without STa did nothing to net jejunal fluid absorption. *E. coli* STa added to ileal loops had no remote effect on jejunal fluid absorption as absorption was 94.4 ± 3.4 (7) µl/cm/h with saline and 89.3 ± 8.4 (6) µl/cm/h when STa (80 ng/ml) was included in the ileal loop. There was therefore no evidence for the remote effect of STa that has been claimed. The lack of a remote effect of STa on fluid movement confirms earlier observations (3) and supports the concept of a lack of vagally mediated fluid secretion being a factor in *E. coli* STa-mediated alterations in net fluid absorption.

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Lucas ML, Thom MMM, Bradley JM, O'Reilly NF, McIlvenny TJ & Nelson YB (2005). J Membr Biol 206, 29–42.

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PC33

## Vectorial transport by Capan-1 cells: a model for human pancreatic ductal bicarbonate secretion

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Human pancreatic ducts secrete a bicarbonate-rich fluid but our knowledge of the secretory process is based mainly on animal models. Our aim was to determine whether the bicarbonate transport mechanisms in Capan-1, a human pancreatic ductal cell line are similar to those previously identified in guinea-pig pancreatic ducts.
The expression of potential key transporters and receptors was compared in normal human pancreas and cultured Capan-1 cells by RT-PCR. Monolayers of Capan-1 cells were grown on Transwell-COL PTFE membranes. To estimate transmembrane and transcellular bicarbonate movements, intracellular pH ($pH_i$) was monitored by microfluorometry using BCECF, a pH-sensitive fluoroprobe. Bicarbonate secretion was estimated from the initial rate of decrease in $pH_i$ following inhibition of basolateral bicarbonate uptake by EIPA and H2DIDS.
By RT-PCR we found that pNBC1, NHE1, AE2, AE3, SLC26A6 transporters, CFTR channel and secretin, VPAC1, P2Y1,2,4,6 receptors were expressed both in normal human pancreas and in Capan-1 cells. Capan-1 cells grown on permeable supports formed confluent, polarized monolayers with well developed tight junctions. The recovery of $pH_i$ from an acid load, induced by a short $NH4^+$ pulse, was mediated by $Na^+$-dependent transporters located exclusively at the basolateral membrane. One was independent of bicarbonate and blocked by EIPA (probably NHE1) while the other was bicarbonate-dependent and blocked by H2DIDS (probably pNBC1). Simultaneous administration