Neuroscience

The Journal of Physiology

The regulation of K- and L-cell activity by GLUT2 and the calcium-sensing receptor CasR in rat small intestine

Oliver J. Mace, Marcus Schindler and Sonal Patel

Prosidion Limited, Windrush Court, Watlington Road, Oxford OX4 6LT, UK

Key points

- In intestine, nutrients including glucose and amino acids and non-nutrients including bile acids increase secretion of anti-diabetic gut peptides such as gluco-insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY).
- Facilitative glucose transporter pathways in addition to active electrogenic transporter pathways contribute to GIP, GLP-1 and PYY secretion; in particular, the facilitative glucose transporter 2 (GLUT2) is involved.
- Sucralose, in the presence of glucose, can strongly and acutely upregulate GIP, GLP-1 and PYY secretion in a time scale of minutes.
- Amino acid-stimulated GIP, GLP-1 and PYY secretion is acutely regulated by the calcium-sensing receptor (CasR).
- The results establish new functions for GLUT2 and CasR as regulators of gut peptide secretion that sense nutrients and provide signalling pathways for the release of GIP, GLP-1 and PYY.

Abstract Intestinal enteroendocrine cells (IECs) secrete gut peptides in response to both nutrients and non-nutrients. Glucose and amino acids both stimulate gut peptide secretion. Our hypothesis was that the facilitative glucose transporter, GLUT2, could act as a glucose sensor and the calcium-sensing receptor, CasR, could detect amino acids in the intestine to modify gut peptide secretion. We used isolated loops of rat small intestine to study the secretion of gluco-insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) secretion stimulated by luminal perfusion of nutrients or bile acid. Inhibition of the sodium-dependent glucose cotransporter 1 (SGLT1) with phloridzin partially inhibited GIP, GLP-1 and PYY secretion by 45%, suggesting another glucose sensor might be involved in modulating peptide secretion. The response was completely abolished in the presence of the GLUT2 inhibitors phloretin or cytochalasin B. Given that GLUT2 modified gut peptide secretion stimulated by glucose, we investigated whether it was involved in the secretion of gut peptide by other gut peptide secretagogues. Phloretin completely abolished gut peptide secretion stimulated by artificial sweetener (sucralose), dipeptide (glycylsarcosine), lipid (oleoylethanolamine), short chain fatty acid (propionate) and major rat bile acid (taurocholate) indicating a fundamental position for GLUT2 in the gut peptide secretory mechanism. We investigated how GLUT2 was able to influence gut peptide secretion mediated by a diverse range of stimulators and discovered that GLUT2 affected membrane depolarisation through the closure of K^+_{ATP}-sensitive channels. In the absence of SGLT1 activity (or presence of phloridzin), the secretion of GIP, GLP-1 and PYY was sensitive to K^+_{ATP}-sensitive channel modulators tolbutamide and diazoxide. L-Amino acids phenylalanine (Phe), tryptophan (Trp), asparagine (Asn), arginine (Arg) and glutamine (Gln) also stimulated GIP, GLP-1 and PYY secretion, which was completely abolished when extracellular Ca^{2+} was absent. The gut peptide response stimulated by the amino acids was also blocked by

the CasR inhibitor Calhex 231 and augmented by the CasR agonist NPS-R568. GLUT2 and CasR regulate K- and L-cell activity in response to nutrient and non-nutrient stimuli.

Abbreviations CasR, calcium-sensing receptor; CCK, cholecystokinin; GIP, gluco-insulinotropic peptide; GLP-1, glucagon-like peptide-1; GLUT2, facilitative glucose transporter 2; GPCR, G protein-coupled receptor; IEC, intestinal enteroendocrine cell; OEA, oleoylethanolamide; PYY, peptide tyrosine tyrosine; SGLT1, sodium-dependent glucose cotransporter 1; Tas1R1+Tas1R3, amino acid taste receptor; Tas1R2+Tas1R3, sweet taste receptor.

Introduction

The gut releases hormones to initiate activities throughout the body that control gut motility, nutrient absorption and disposal, and satiety in response to the arrival of food. The gut hormone gluco-insulinotropic peptide (GIP) is secreted from intestinal enteroendocrine (IEC) K-cells and the gut hormones glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) are secreted from IEC L-cells. Both IECs are distributed throughout the gastrointestinal tract and are targets for anti-diabetic therapies since GIP and GLP-1 directly stimulate insulin secretion. The secretion of these peptides has been extensively investigated and the current model of IEC sensing involves both raised intracellular Ca^{2+} and cAMP (Reimann *et al.* 2008; Tolhurst *et al.* 2009; Parker *et al.* 2010); the former through either electrogenic transport to directly depolarise the membrane and open voltage-gated Ca^{2+} channels or activation of Gq protein-coupled receptors, and the latter through Gs protein-coupled receptors.

Luminal nutrients including glucose and L-amino acids trigger GIP, GLP-1 and PYY secretion. It is well established that glucose can regulate GLP-1 secretion through electrogenic Na^{+}-coupled uptake via SGLT1. Sweet taste receptors, Tas1R2+Tas1R3, have also been demonstrated to regulate GLP-1 secretion which was impaired in mice lacking the coupling G protein Gαgustducin (Margolskee *et al.* 2007). Another glucose transporter, GLUT2, which has a well-established glucose-sensing role mediating insulin secretion from the pancreatic β-cell, also resides in the apical membrane of intestine. When rat small intestine is exposed to increasing glucose concentrations, the density and intrinsic activity of GLUT2 residing at the apical membrane transiently increases (Kellett & Helliwell, 2000) to accommodate the increased absorptive capacity required to meet increased glucose concentration. This experimental observation has been confirmed by independent laboratories in both mice (Tobin *et al.* 2008; Gorboulev *et al.* 2012), rat (Au *et al.* 2002; Wei *et al.* 2011), in intestinal cell lines (Zheng *et al.* 2012) and clinically in morbidly obese patients (Ati-Omar *et al.* 2011). GLUT2 is permanently expressed in the apical membrane of the

intestine during diabetes (Gouyon et al. 2003; Tobin *et al.* 2008) and makes for an ideal target for therapeutic intervention. Expression of GLUT2, glucokinase and subunits of K^{+}_{ATP}-sensitive channels have been detected in IECs, supporting the hypothesis that changes to apical GLUT2 expression may modulate gut peptide secretion by modulating membrane depolarisation through closure of K^{+}_{ATP}-sensitive channels (Reimann *et al.* 2008).

In addition to glucose, L-amino acids including glutamine (Gln), phenylalanine (Phe) and tryptophan (Trp) have been shown to stimulate GLP-1 secretion using intestinal epithelial cell models (Reimann *et al.* 2004; Tolhurst *et al.* 2011). Adding to the cellular mechanisms by which L-amino acids are detected, the taste receptor, Tas1R1+Tas1R3, and the calcium sensing receptor, CasR, have been shown to respond to L-amino acid stimulation (Conigrave *et al.* 2000; Oya *et al.* 2011). Given the demonstrations that L-amino acids can stimulate the secretion of the gut peptide cholecystokinin (CCK) (Koop & Buchan, 1992; Chang *et al.* 1994; Tolhurst *et al.* 2011) through CasR (Busque *et al.* 2005; Dufner *et al.* 2005; Wang *et al.* 2011), we sought to investigate whether CasR might play a role in the secretion of GIP, GLP-1 and PYY.

At present, it is technically challenging to generate time courses showing gut peptide secretion in rodent models primarily because of the small blood volume that can be sampled. In addition, the analysis of gut peptides is hampered due to the relatively high detection limits of many hormone platforms. We have employed isolated loops of rat small intestine, previously used to study the absorption of nutrients (Fisher & Gardner, 1974; Gardner & Plumb, 1979; Bronk & Hastewell, 1988; Lister *et al.* 1995) from which we were able to quantify total GIP, active GLP-1 and total PYY. The isolated loop preparation was validated using established GLP-1 secretagogues (see Supplementary information) and remained viable. Our experiments reveal that GLUT2 regulates the secretion of GIP, GLP-1 and PYY in response to a diverse set of gut peptide secretagogues. We also show that extracellular Ca^{2+} is essential for GIP, GLP-1 and PYY secretion stimulated by L-amino acids via CasR.

Table 1. The effect of glucose and inhibitors of SGLT1 and GLUT2 on glucose absorption and fluid transport

	[Glucose] (mM)	Glucose rate (mM (g dry weight)$^{-1}$ min^{-1})	Fluid rate (μl (g dry weight)$^{-1}$ min^{-1})
Glucose	5	11.8 ± 2..3	217.9 ± 5.3
	25	48.3 ± 7.5**	268.7 ± 11.9**
	50	67.4 ± 11.2***	273.1 ± 16.4**
	100	89.1 ± 28.1***	271.6 ± 14.5**
Glucose + phloridzin	25	19.7 ± 8.3	230.1 ± 13.7
	50	28.3 ± 11.7†	235.4 ± 10.2
	100	41.2 ± 10.2‡	241.2 ± 13.3
Glucose + phloridzin + phloretin	100	11.3 ± 5.1§§§	219.4 ± 21.5
Glucose + phloridzin + cytochalasin B	100	10.6 ± 4.9§§§	211.6 ± 19.7
Glucose (Na^{+}-deplete buffer) + phloretin	100	13.6 ± 7.4§§§	201.3 ± 24.8

Significance was determined using Student's paired or unpaired *t* test as appropriate *vs.* 5 mM glucose where *$P < 0.05$, **$P < 0.01$ and ***$P < 0.05$; or 5 mM glucose + phloridzin where $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$; or 100 mM glucose where $^{\S\S\S}P < 0.05$.

Methods

Animals

Male Sprague–Dawley rats (200–250 g) were obtained from Charles Rivers, UK, fed *ab libitum* and had free access to water. They were maintained on a 12 h light–dark regime. Animals were culled by Schedule 1 methods according to the UK Home Office Animals (Scientific Procedures) Act 1986. All animal procedures were approved by the local ethical committee.

Perfusion technique

Loops of small intestine (from stomach to ileal–caecal valve) were perfused in single-pass mode for 60 min as described previously (Bronk *et al.* 1987, 1988; Lister *et al.* 1995, 1997) and modified to include the entire small intestine. Viability was assessed by ability to maintain steady water flow and to actively transport D-glucose. The loop was suspended in liquid paraffin (specific gravity 0.83–0.86) at 37.5°C. The first 30 min was comprised of a control period during which Krebs–Henseleit buffer (KHB) (consisting of (in mM): 120 NaCl, 4.5 KCl, 1 MgSO$_4$, 1.8 Na$_2$HPO$_4$, 0.2 NaH$_2$PO$_4$, 25 NaHCO$_3$, 1.25 CaCl$_2$ and 5 D-glucose) was perfused at a flow rate of 1.6 ml min^{-1} to prevent distension of the preparation (Helliwell & Kellett, 2002). A phosphate- and magnesium-free buffer was used for the experiments involving high Ca^{2+} concentrations, adapted from Auchere *et al.* (1997).

Amino acid stock solutions were made up in KHB at 100 mM, except tryptophan which was made up in KHB at 50 mM. After 30 min, the loop was perfused with a second experimental perfusate (identical to the first except for the addition of test agent). As necessary, mannitol was added to the control perfusate buffer to maintain a constant osmolarity between perfusate solutions of differing glucose concentration. For dipeptide experiments where the pH of the KHB was altered to pH 6.8, the NaHCO$_3$ concentration was reduced from 25 mM to 5 mM and the NaCl concentration was increased from 120 mM to 140 mM. For those perfusions in which the luminal pH was altered, the same luminal pH was used throughout the 60 min perfusion.

Serosal secretion samples were collected every 5 min and were analysed for glucose, total GIP, active GLP-1 and total PYY using commercially available ELISAs. A measured segment of the perfused intestine was blotted dry and dried to constant weight. This provided the dry weight per centimetre used to calculate the total dry weight of the perfused segment. The volume of serosal secretion samples was also measured to determine fluid flux so that gut peptide concentrations could be corrected for changes in fluid transport.

Calculation and expression of results

All results are expressed as mean ± standard error of the mean (SEM); *n* = 8 except where noted. Data are presented as serosal appearance (e.g. pg ml^{-1} (g dry weight)$^{-1}$). Area under the curve (AUC) was calculated for control and experimental phases. Where distinct phases of peptide secretion were observed, the AUC$_{reactive}$ for each was calculated in units of pg ml^{-1} (g dry weight)$^{-1}$ min^{-1}. Statistical comparisons were carried out using 2-way analysis of variance to compare sets of concentration data, covariance analysis for cumulative serosal appearance and Student's *t* test for comparison of means.

Results

Model validation

The model was extensively characterised and validated (see Supplementary information). In serosal samples, concentrations of GIP, GLP-1 and PYY to demonstrate both K- and L-cell activity could readily be detected. Glucose (5–100 mM), glycylsarcosine (0.1–5 μM), the mixed nutrient formulation Ensure plus (10–100% v/v), OEA (0.1–10 μM), propionate (5–50 mM) and
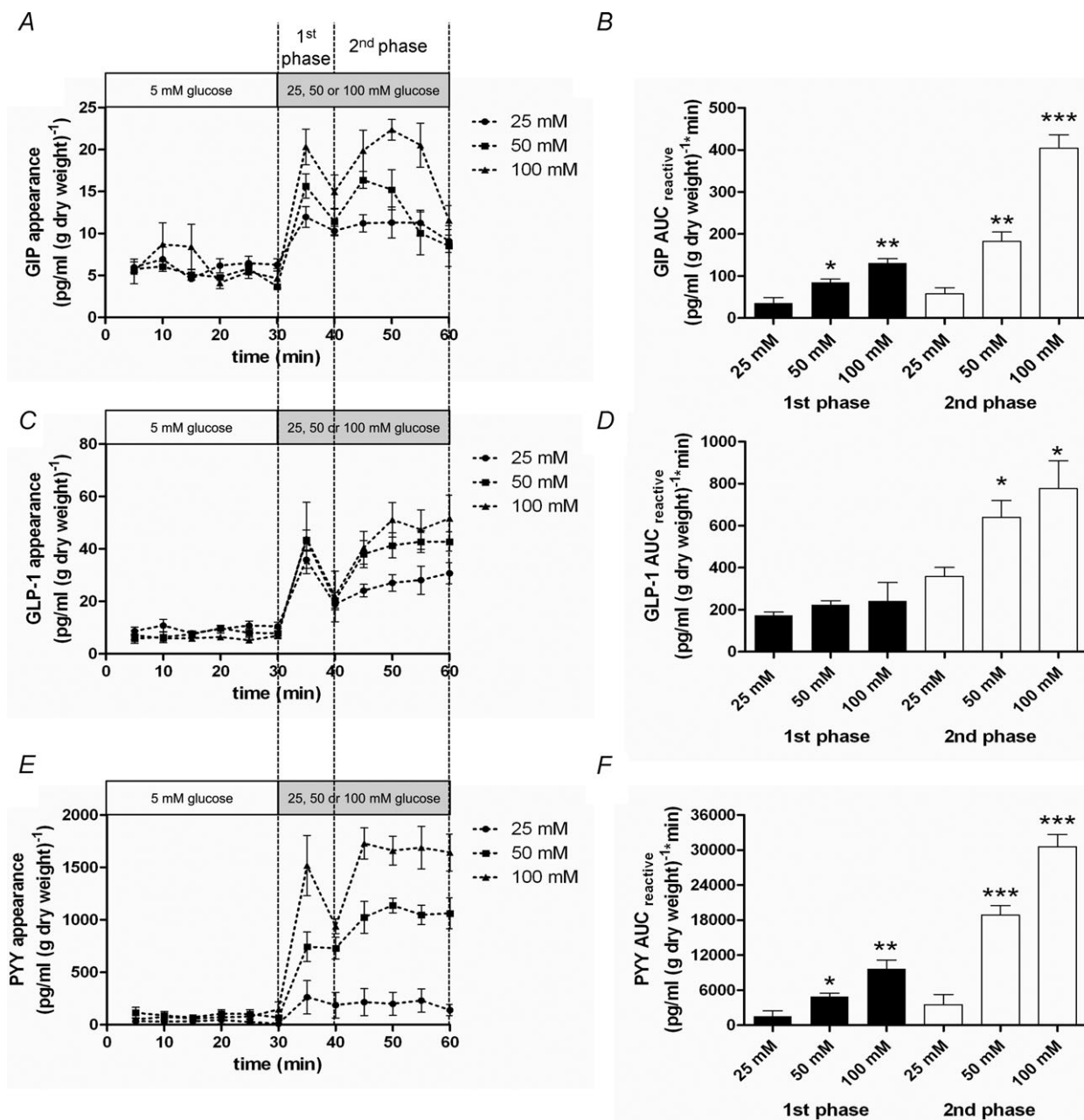

Figure 1. SGLT1 and GLUT2 regulate GIP, GLP-1 and PYY secretion
A–F, rat small intestine was perfused with 5 mM glucose in KHB. At 30 min, 25, 50 or 100 mM glucose was introduced for a further 30 min. Samples were analysed for GIP, GLP-1 and PYY. AUC$_{reactive}$ was calculated for 1st (30–40 min) and 2nd (40–60 min) phases for GIP, GLP-1 and PYY. Significance was determined using Student's unpaired *t* test *vs*. 5 mM glucose where *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. *G–L*, rat small intestine was perfused with 5 mM glucose in KHB. At 30 min, the perfusate was switched to one containing 25, 50 or 100 mM glucose (25, 50 or 100 mM) + phloridzin (0.5 mM) for a further 30 min. Samples were analysed for GIP, GLP-1 and PYY. AUC$_{reactive}$ was calculated for the 1st (30–40 min) and 2nd (40–60 min) phases for GIP, GLP-1 and PYY. Significance was determined using Student's unpaired *t* test *vs*. 25 mM glucose + phloridzin where *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

taurocholate (5–50 mM) stimulated gut peptide secretion (see Supplementary information). Full dose–response relationships were generated for each secretagogue and perfusion conditions optimised for maximal gut peptide stimulation (see Supplementary information). This included addition of the fatty acid amide hydrolase inhibitor URB579 for OEA, and changing the pH to 6.8 for glycylsarcosine, respectively.

Gut peptide secretion involves GLUT2

Challenging the small intestine with increasing glucose (5–100 mM) increased the rate of glucose absorption (Table 1). Phloridzin (0.5 mM) inhibited the rate of glucose absorption by approximately 50%. With both phloridzin and phloretin (0.5 mM) in the perfusate buffer, the rate of glucose absorption was completely abolished (Table 1).

Glucose stimulated the secretion of GIP, GLP-1 and PYY from isolated loops of rat small intestine (Fig. 1*A*–*F*). The secretion of GIP, GLP-1 and PYY by glucose was partially inhibited by phloridzin (0.5 mM) to block SGLT1 activity (Fig. 1*G*–*L*) or by using a Na^+-deplete buffer (Fig. 2), indicating that peptide secretion was not solely dependent on SGLT1 and that other mechanisms were operational. In the presence of 100 mM glucose to stimulate GIP, GLP-1 and PYY secretion and 0.5 mM phloridzin to block SGLT1, the subsequent addition of phloretin (0.5 mM) or cytochalasin B (10 μM) returned secretion to basal
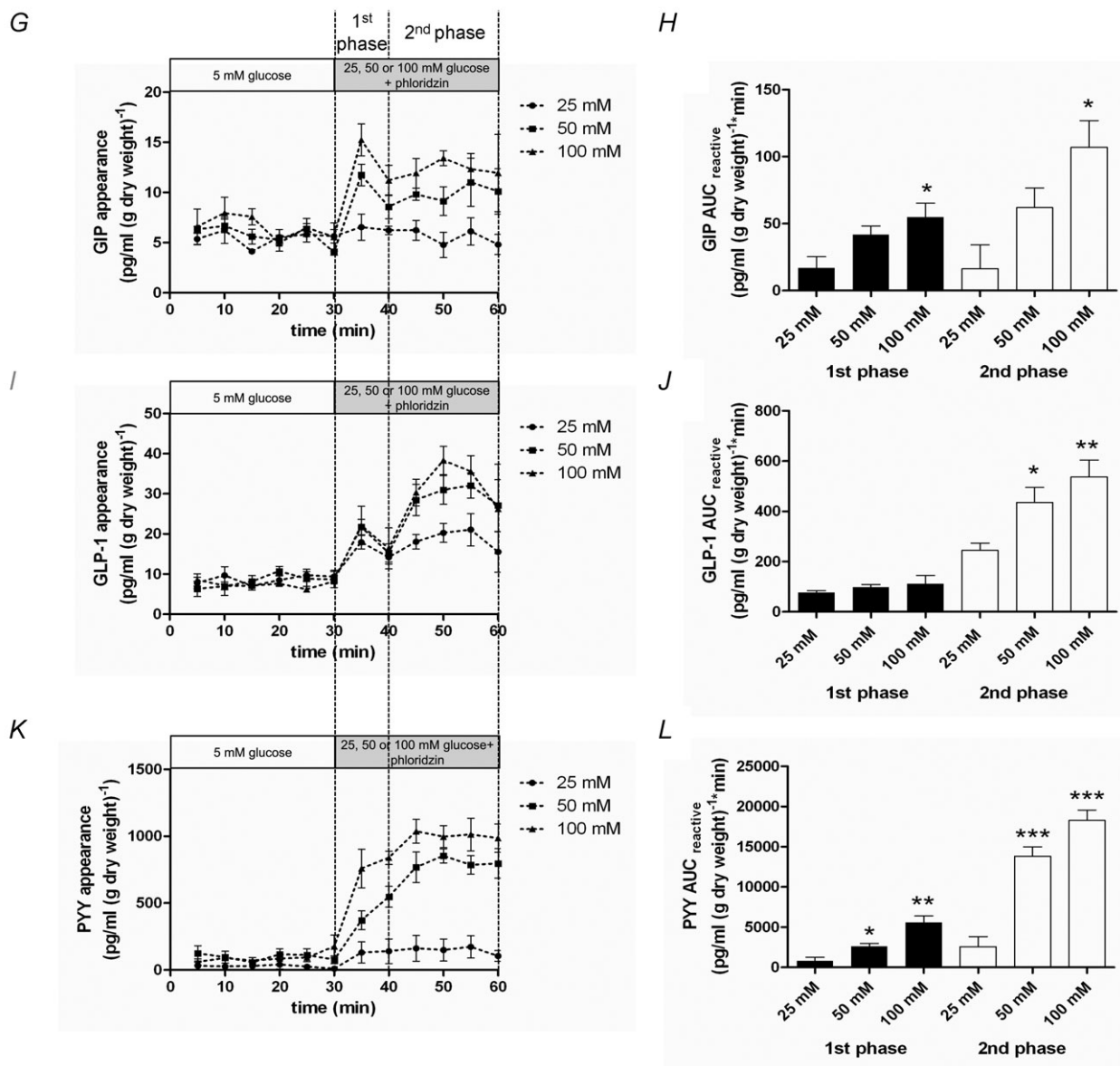

Figure 1. Continued

levels, demonstrating a significant role for GLUT2 in the secretion of GIP, GLP-1 and PYY (Fig. 2). Using two separate conditions to inhibit SGLT1, namely phloridzin and a Na$^+$-deplete buffer, we obtained very similar data suggesting that phloridzin was selective for SGLT1 only.

In the presence of 5 mM glucose, sucralose (1 mM) stimulated the secretion of GIP, GLP-1 and PYY which was rapidly reversible (Fig. 3A–F). The response

to sucralose was not changed by phloridzin but was significantly diminished by phloretin (Fig. 3G–L). At the same time, sucralose increased the rate of glucose absorption 3-fold, which was phloretin sensitive (Table 2).

The secretion of GIP, GLP-1 and PYY stimulated by either lipid, OEA (10 μM in the presence of URB597 for maximal stimulation), dipeptide glycylsarcosine (pH 6.8
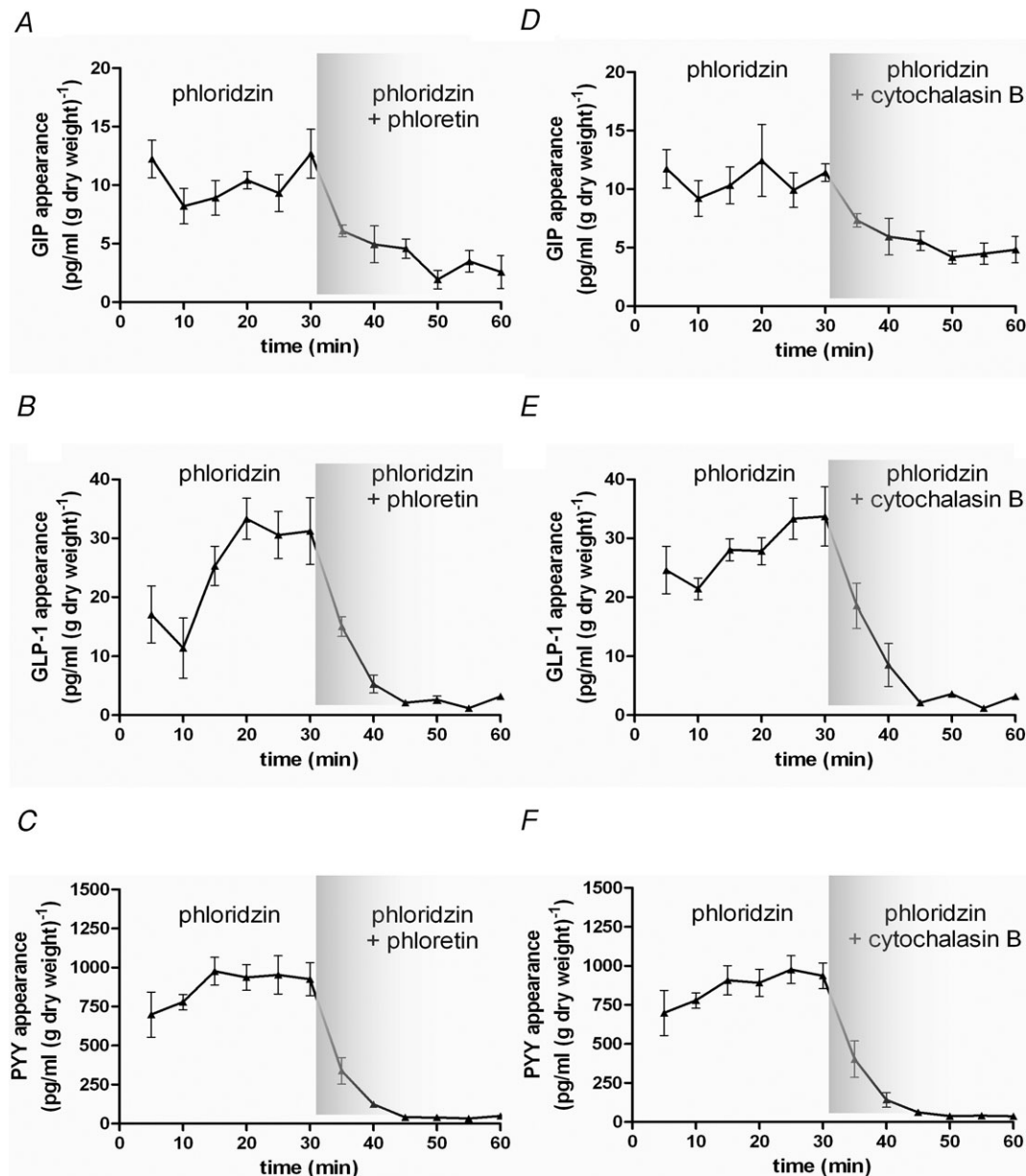

Figure 2. SGLT1 and GLUT2 inhibitors reduce GIP, GLP-1 and PYY secretion

A–F, rat small intestine was perfused with glucose (100 mM) + phloridzin (0.5 mM) in KHB. Phloretin (0.5 mM) or cytochalasin B (10 μM) was added at 30 min. Control perfusions with 100 mM glucose were also conducted and phloretin (0.5 mM) added from 30 min. Samples were analysed for GIP, GLP-1 and PYY. *G–I*, rat small intestine was perfused with glucose (100 mM) in KHB in which Na$^+$ was replaced with choline. At *t* = 30 min, phloridzin was added. Samples were analysed for GIP, GLP-1 and PYY. *J–L*, AUC was calculated for *t* = 0–30 min and *t* = 30–60 min for GIP, GLP-1 and PYY. Significance was determined using Student's paired *t* test *vs.*. *t* = 0–30 min where *** $P < 0.001$.

for maximal stimulation) or the bile acid taurocholate was also significantly inhibited by phloretin (Fig. 4). In parallel, the rate of glucose absorption in the presence of lipid, dipeptide or bile acid was increased 2-fold, which again was phloretin sensitive (Table 3–5).

Intestinal K^+_{ATP}-sensitive channels modulate gut peptide secretion

To prevent membrane depolarisation by SGLT1, we included 0.5 mM phloridzin in the perfusate buffer. Under basal conditions, closure of K^+_{ATP} channels by
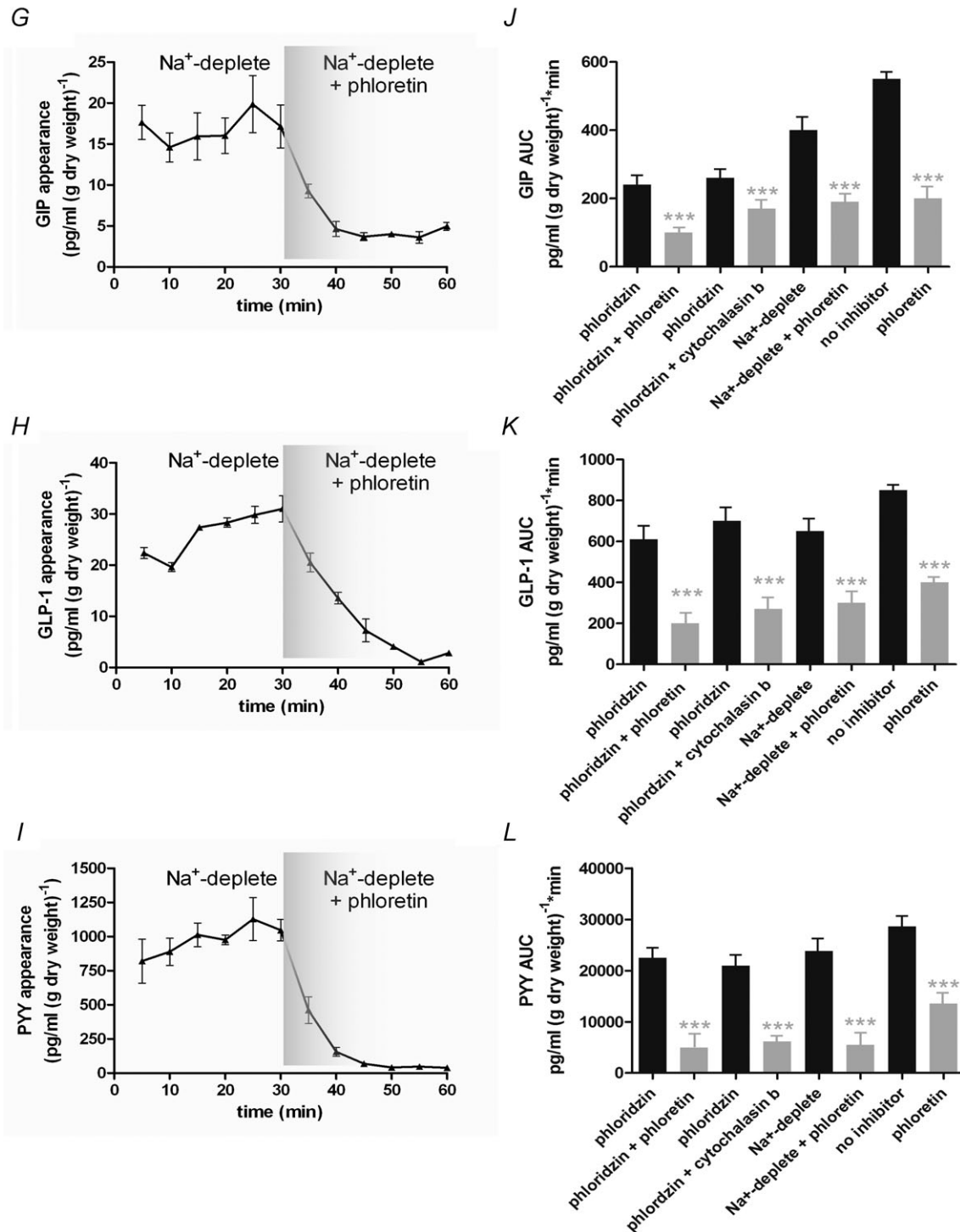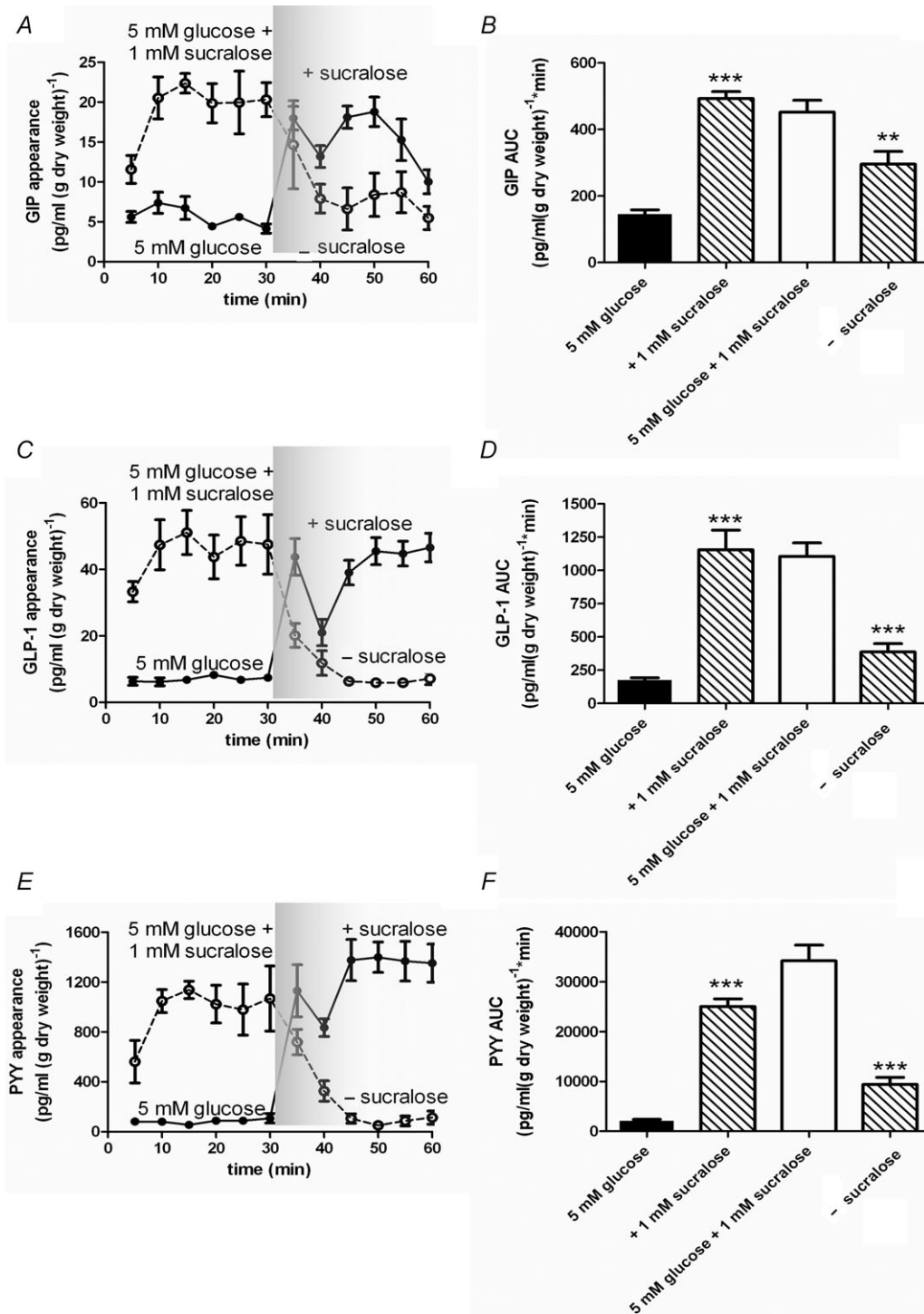

Figure 2. Continued

Figure 3. Gut peptide secretion stimulated by sucralose is blocked by GLUT2 inhibition
A–F, rat small intestine was perfused with glucose (5 mM; ●) in KHB. At $t = 30$ min, sucralose (1 mM) was introduced. In a separate series of experiments rat small intestine was perfused with glucose (5 mM) containing sucralose (1 mM; ○) in KHB. At $t = 30$ min, sucralose was removed. Samples were analysed for GIP, GLP-1 and PYY. AUC was calculated for $t = 0$–30 min and $t = 30$–60 min for GIP, GLP-1 and PYY. Significance was determined using Student's paired t test *vs.* the control period ($t = 0$–30 min) where **$P < 0.01$ and ***$P < 0.001$. *G–L*, rat small intestine was perfused with glucose (5 mM) + sucralose (1 mM) in KHB. At $t = 30$ min, phlorein (0.5 mM; ○) or phloridzin (0.5 mM; ●) was added. Samples were analysed for GIP, GLP-1 and PYY. AUC was calculated for $t = 0$–30 min and $t = 30$–60 min for GIP, GLP-1 and PYY. Significance was determined using Student's paired t test *vs.* the control period ($t = 0$–30 min) where ***$P < 0.001$.

tolbutamide (500 μM) stimulated GIP, GLP-1 and PYY secretion (Fig. 5). In a separate set of experiments, the perfusate was switched to one containing both 100 mM glucose (+0.5 mM phloridzin) and 340 μM diazoxide, a K^+_{ATP}-channel opener. Diazoxide significantly impaired the secretion of GIP, GLP-1 and PYY stimulated by glucose (Fig. 5).

L-Amino acids modulate K- and L-cell activity

The L-amino acids glutamine (Gln), phenylalanine (Phe), tryptophan (Trp), asparagine (Asn) and arginine (Arg) (10 mM) stimulated GIP, GLP-1 and PYY secretion from isolated loops of rat small intestine (Fig. 6). Importantly, the KHB used throughout our experiments contained 1.25 mM Ca^{2+}.
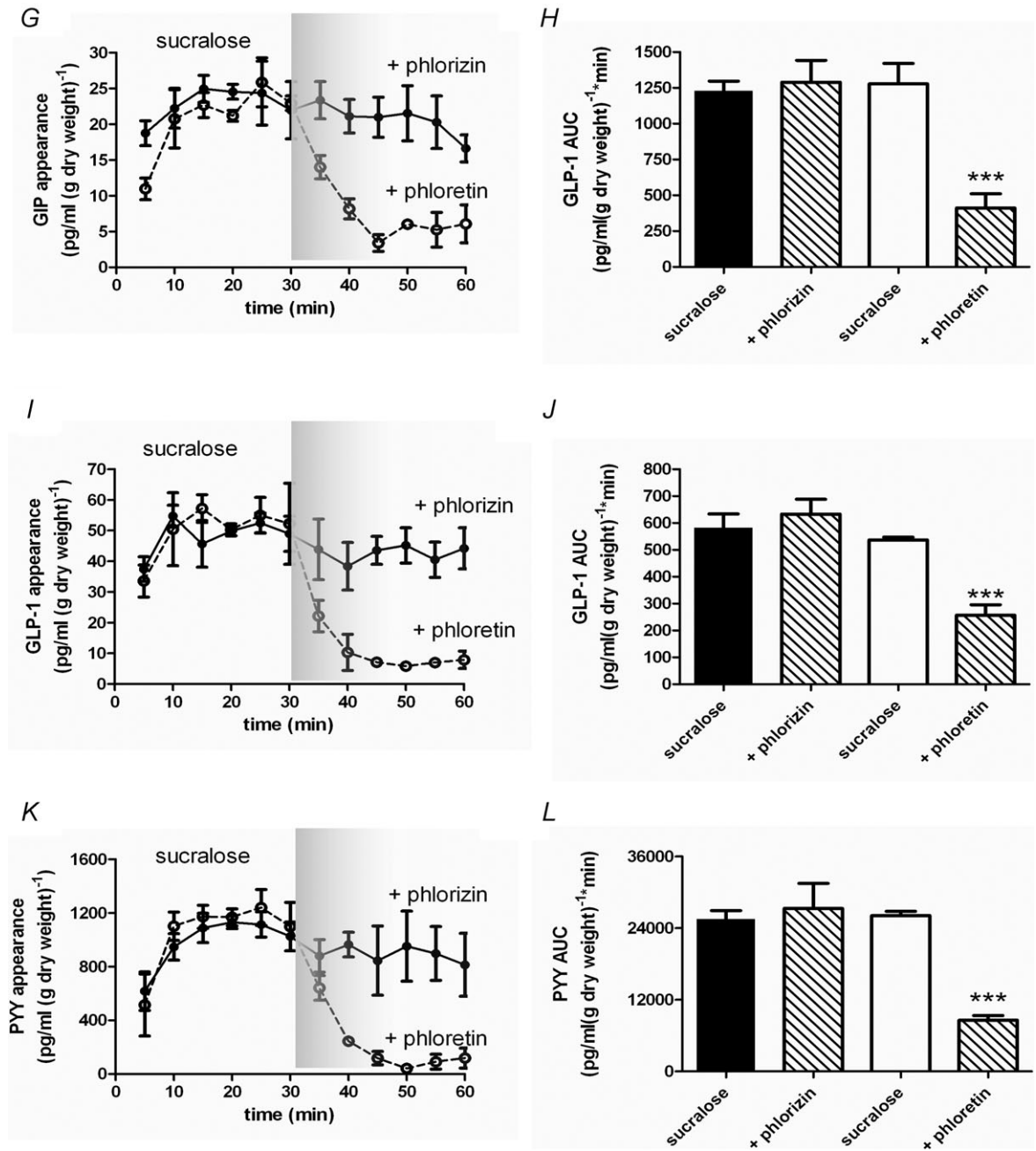

Figure 3. Continued

Table 2. The effect of sucralose and inhibitors of SGLT1 and GLUT2 on glucose absorption and fluid transport

	[Glucose] (mM)	Glucose rate (mM (g dry weight)$^{-1}$ min^{-1})	Fluid rate (μl (g dry weight)$^{-1}$ min^{-1})
Glucose	5	11.8 $\pm$ 2..3	217.9 $\pm$ 5.3
Sucralose	5	31.9 $\pm$ 6.8***	259.7 $\pm$ 34.8
Sucralose + phloretin	50	15.8 $\pm$ 5.4§§§	216.5 $\pm$ 19.3
Sucralose + phloridzin	100	35.8 $\pm$ 7.6	264.8 $\pm$ 26.5

Significance was determined using paired or unpaired Students *t* test as appropriate *vs.* 5 mM glucose where ***$P < 0.05$; or sucralose where $^{\S\S\S}P < 0.05$.

L-Amino acids modulate K- and L-cell activity by CasR

Within 5 min, GIP, GLP-1 and PYY secretion was observed in response to L-amino acids. There appeared no significant difference between the ability of each amino acid to stimulate total GIP secretion at 10 mM. In the absence of extracellular Ca^{2+}, GIP secretion was completely abolished (data not shown). The CasR antagonist Calhex 231 was used to determine whether CasR contributed to GIP secretion mediated by L-amino acids. In the absence of extracellular Ca^{2+}, Calhex 231 did not have any effect on basal GIP secretion (data not shown). However, in the presence of extracellular Ca^{2+}, Calhex 231 was able to inhibit L-amino acid-stimulated GIP secretion (Fig. 6A). Calhex 231 had the largest inhibitory effect on GIP secretion mediated by Arg. In contrast, Calhex 231 inhibited GIP secretion by Gln just 37%. The potency of the L-amino acids to stimulate active GLP-1 secretion was Phe > Arg > Gln ~ Trp > Asn. There was no amino acid-induced stimulation of GLP-1 in the absence of extracellular Ca^{2+}, and no effect of the CasR antagonist Calhex 231 (data not shown). Calhex 231 significantly inhibited Phe-, Trp- and Arg-stimulated GLP-1 secretion by ~70% in the presence of extracellular Ca^{2+} (Fig. 6B). CasR activation did not appear to contribute to Gln-stimulated GLP-1 secretion (Fig. 6B). Again, Calhex 231 had no effect on total PYY secretion in the absence of extracellular Ca^{2+} (data not shown), but inhibited amino acid-induced total PYY secretion in the presence of extracellular Ca^{2+} (Fig. 6C). Since Phe and Trp were the most sensitive to inhibition of CasR with Calhex 231, we investigated the effect of the specific CasR agonist, NPS R-685, on Phe- and Trp-stimulated gut peptide release (Fig. 7). Treatment with NPS R-685 (20 μM), following elevation of Phe-, Trp- and Gln-stimulated gut peptide secretion,

augmented secretion to a maximum level implying that under physiological extracellular Ca^{2+} levels, CasR could contribute towards generation of GIP, GLP-1 and PYY secretion.

Extracellular Ca^{2+} increases the potency of phenylalanine to stimulate K- and L-cell activity

We went on to investigate the effect of extracellular Ca^{2+} on Phe-stimulated gut peptide release because Phe was deemed to be most sensitive to CasR activity. We perfused isolated loops of rat small intestine with 10 mM Phe and without Phe (control) for 90 min (Fig. 8) in a Ca^{2+}-deplete buffer. We took advantage of the fact that CasR is relatively insensitive to desensitisation following repeated exposure to agonist (Huang & Miller, 2007) and cumulatively increased the extracellular Ca^{2+} concentration in 0.1, 0.3, 1, 3 to 10 mM steps every 15 min. In the absence of extracellular Ca^{2+}, gut peptide secretion could not be stimulated by Phe (data not shown). Cumulative additions of extracellular Ca^{2+} stimulated GIP, GLP-1 and PYY secretion. The EC$_{50}$ values generated for extracellular Ca^{2+} were 3.5, 2.5 and 2.7 mM for GIP, GLP-1 and PYY, respectively. In the presence of 10 mM Phe, peptide secretion by extracellular Ca^{2+} was significantly increased. Above a threshold concentration >0.3 mM, Phe-induced secretion of GIP, GLP-1 and PYY was augmented by increasing the extracellular Ca^{2+} concentration. The EC$_{50}$ values for extracellular Ca^{2+} in the presence of 10 mM Phe were decreased to 2.3, 1.8 and 1.3 mM for GIP, GLP-1 and PYY, respectively. This implies that extracellular Ca^{2+} increased the potency of L-Phe for gut peptide secretion.

Discussion

Measurement of K- and L-cell activity in isolated loops of rat small intestine

For the first time, we show that K- and L-cell activity can be examined in isolated loops of rat small intestine to obtain measurable quantities of GIP, GLP-1 and PYY. The profile of GLP-1 and PYY release parallels studies performed using alternative vascular intestinal perfusion and *in vivo* perfusion techniques (Dumoulin *et al.* 1995). In the presence of 100 mM glucose, phloridzin inhibited GIP, GLP-1 and PYY secretion by approximately 45%; the remainder was sensitive to the GLUT2 inhibitors phloretin and cytochalasin B, indicating that in addition to SGLT1, GLUT2 has an equally important role. Fluid absorption increased in response to higher glucose concentrations. However, elevated fluid absorption was not significantly inhibited in the presence of phloridzin, phloretin or cytochalasin B. This effect has previously been reported in rabbit small intestine, where there was no effect of phloridzin on water

transport when the tissue had previously been exposed to glucose (Naftalin *et al.* 1986), most probably due to the residual glucose present in the submucosal fluid. Similarly, in our intestinal preparation there is no clearance from the serosal side where residual glucose is likely to accumulate.

SGLT1 and GLUT2

Oral glucose stimulates GLP-1 secretion whilst elevated blood glucose does not, suggesting that detection originates from the apical side. GIP, GLP-1 and PYY secretion was sensitive to phloridzin which inhibited
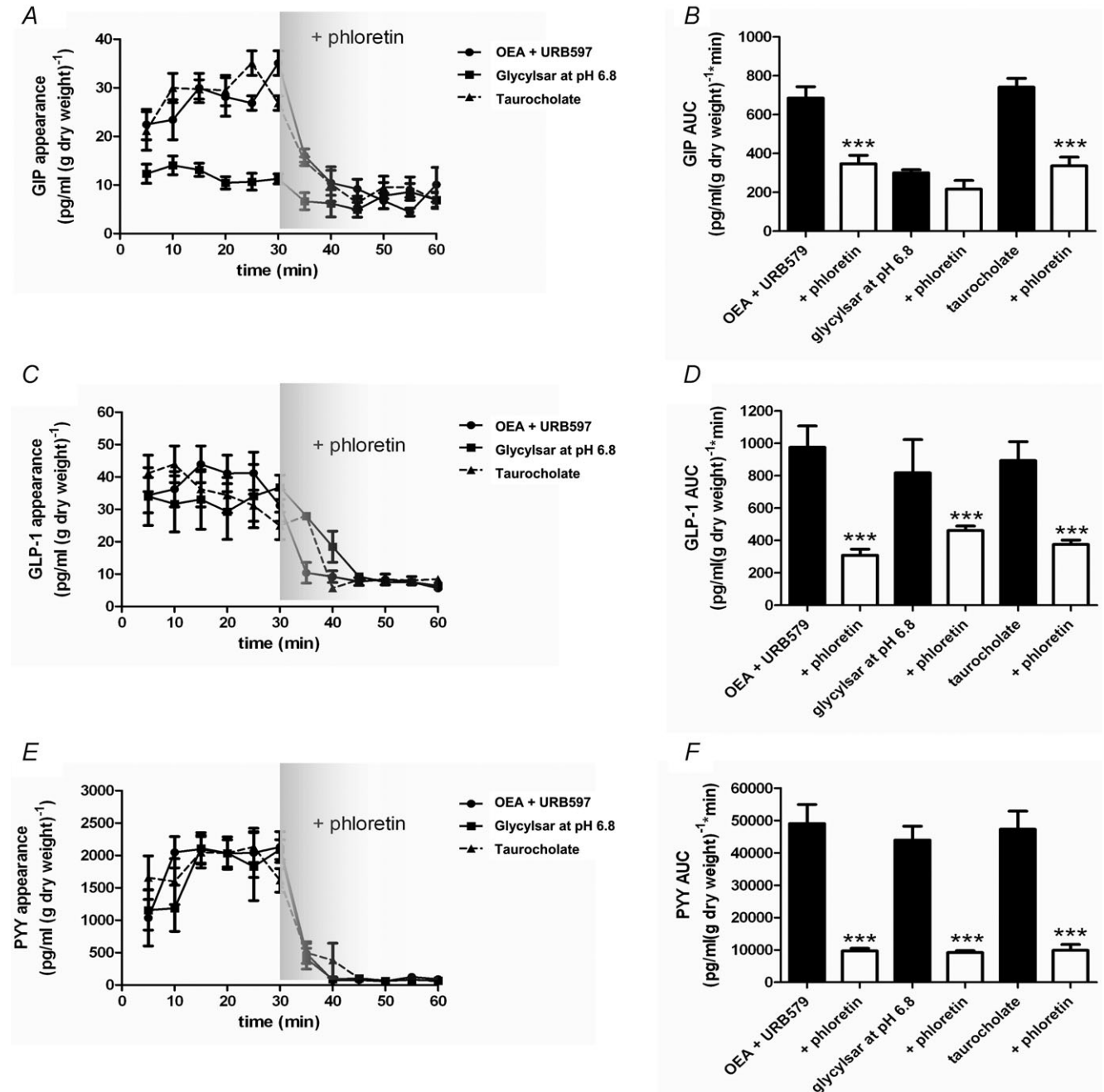

Figure 4. Gut peptide secretion stimulated by OEA, glycylsarcosine or taurocholate is blocked by inhibition of GLUT2
Rat small intestine was perfused with glucose (5 mM) and OEA (10 μM) + URB597 (10 μM; ●), or glycylsarcosine (5 mM) at pH 6.8 (■) or taurocholate (50 mM; ▲) in KHB. Phloretin (0.5 mM) was added at 30 min. Samples were analysed for GIP, GLP-1 and PYY. AUC was calculated for $t = 0$–30 min and $t = 30$–60 min for GIP, GLP-1 and PYY. Significance was determined using Student's paired t test *vs.* the control period ($t = 0$–30 min) where ***$P < 0.001$.

Table 3. The effect of OEA and GLUT2 inhibition on glucose absorption and fluid transport

	[Glucose] (mM)	Glucose rate (mM (g dry weight)$^{-1}$ min^{-1})	Fluid rate (μl (g dry weight)$^{-1}$ min^{-1})
Glucose	5	12.4 ± 3.7	223.7 ± 4.7
OEA	5	25.9 ± 13.7**	267.5 ± 32.5
OEA + phloretin	50	11.5 ± 4.8§§§	216.5 ± 19.3

Experiments were performed in the presence of URB597. Significance was determined using Student's paired or unpaired *t* test as appropriate *vs.* 5 mM glucose where **$P < 0.01$; or OEA where $^{§§§}P < 0.001$.

Table 4. The effect of glycylsarcosine and GLUT2 inhibition on glucose absorption and fluid transport

	[Glucose] (mM)	Glucose rate (mM (g dry weight)$^{-1}$ min^{-1})	Fluid rate (μl (g dry weight)$^{-1}$ min^{-1})
Glucose	5	13.7 ± 5.9	209.7 ± 6.1
Glycylsarcosine	5	26.7 ± 5.6**	267.5 ± 32.5
Glycylsarcosine + phloretin	50	11.5 ± 4.8§§§	216.5 ± 19.3

Experiments were performed at pH 6.8 to achieve maximum glycylsarcosine stimulation of gut peptide secretion. Significance was determined using Student's paired or unpaired *t* test as appropriate *vs.* 5 mM glucose where **$P < 0.01$; or glycylsarconsine where $^{§§§}P < 0.001$.

Table 5. The effect of taurocholate and GLUT2 inhibition on glucose absorption and fluid transport

	[Glucose] (mM)	Glucose rate (mM (g dry weight)$^{-1}$ min^{-1})	Fluid rate (μl (g dry weight)$^{-1}$ min^{-1})
Glucose	5	12.1 ± 2.8	226.8 ± 13.7
Taurocholate	5	26.8 ± 6.7**	267.5 ± 32.5
Taurocholate + phloretin	50	11.3 ± 4.9§§§	201.5 ± 13.7

Significance was determined using Student's paired or unpaired *t* test as appropriate *vs.* 5 mM glucose where **$P < 0.01$; or taurocholate where $^{§§§}P < 0.001$.

stimulation of GIP and GLP-1 secretion in response to glucose (Gorboulev *et al.* 2012). It is difficult to draw firm conclusions towards the contribution that SGLT1 makes towards gut peptide secretion and whether the absence of SGLT1 directly caused the reduction in GIP and GLP-1 observed in the SGLT1$^{-/-}$ mice, particularly when the effect of SGLT1 knockout on the total amount of gut peptide was not measured and when in the supplementary information of the same paper, isolated primary epithelial cell cultures from the SGLT1$^{+/-}$ and SGLT1$^{-/-}$ mice show equivalent GIP and GLP-1 secretion when challenged with glucose (Gorboulev *et al.* 2012). Without a full time course, one can only take a snap shot of the gut peptide response which could potentially mask any effect of GLUT2. For example, if one were to sample from Fig. 1*C* at 10 min post glucose challenge ($t = 40$ min), the erroneous conclusion that glucose was without effect would be drawn. It is interesting to note that in the same SGLT1$^{-/-}$ mouse study, glucose did not increase active GLP-1 in either wild type or knockout mice 13 min post gavage presumably because of the short half-life of GLP-1 and that only total plasma GLP-1 at 5 min post glucose was elevated, in which the proportion of active GLP-1 was unknown (Gorboulev *et al.* 2012).

The ability of GLUT2 to act as a sugar sensor is not a new idea (Thorens, 2001). Pancreatic β-cells fail to secrete insulin when GLUT2 is replaced with other GLUT proteins that afford equivalent glucose flux kinetics (Hughes *et al.* 1993; Newgard & McGarry, 1995). The time scale of gut peptide secretion observed in the studies presented here is in keeping with those of apical GLUT2 insertion (e.g. the $t_{1/2}$ being in the order of minutes) (Gouyon *et al.* 2003; Shepherd *et al.* 2004; Kellett & Brot-Laroche, 2005; Mace *et al.* 2007*a*,*b*; Morgan *et al.* 2007). Sugar transporters detected in taste receptor cells of the lingual epithelium that are morphologically similar to IECs include GLUT2 where GLUT2-containing vesicles have been observed below the apical membrane (Merigo *et al.* 2011; Yee *et al.*
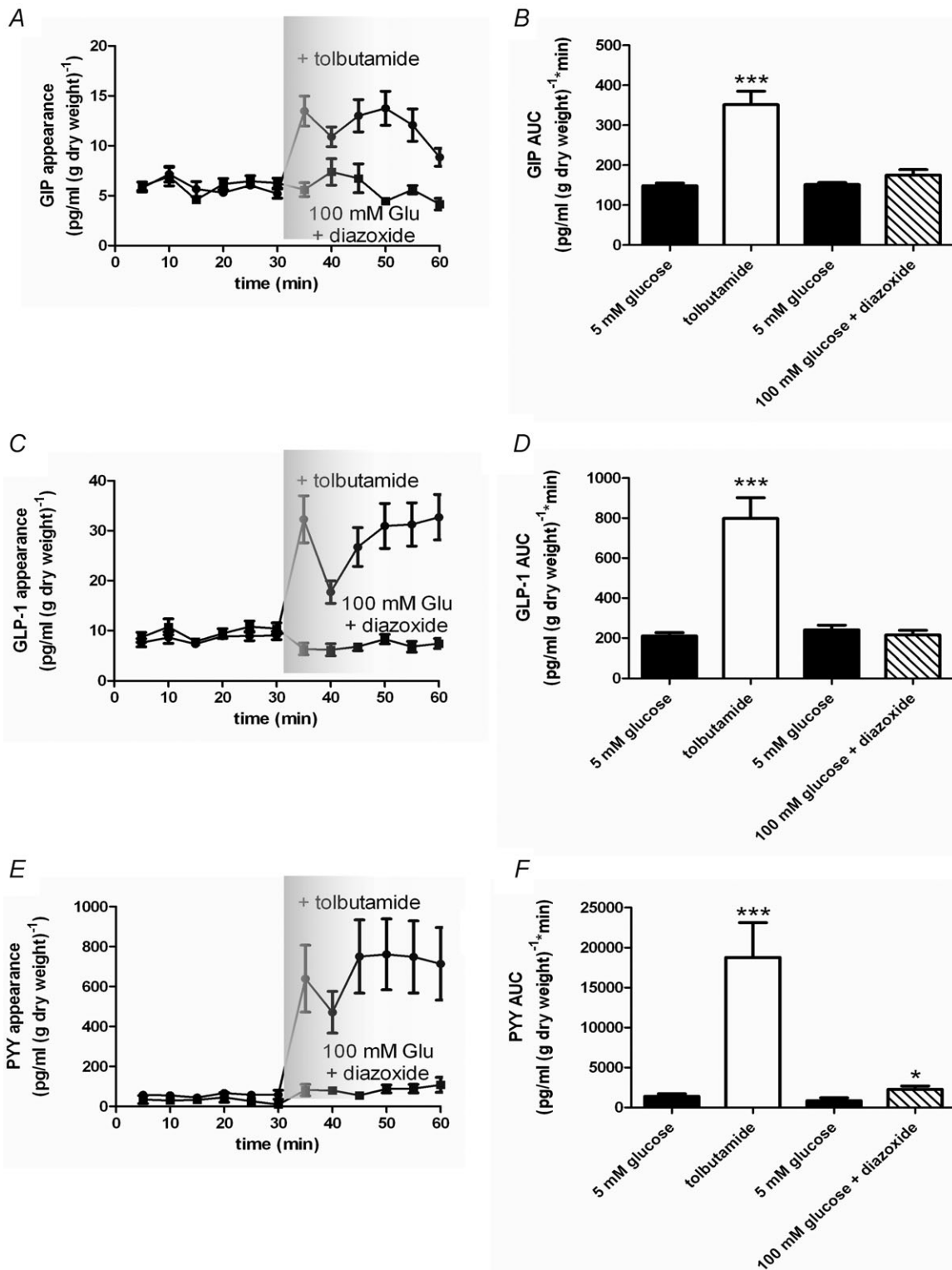
~45% of the secretory response. The remaining secretory response was completely abolished by phloretin or cytochalasin B suggesting that a significant component is regulated by GLUT2. We are confident that apical GLUT2 is targeted by luminal phloretin and luminal cytochalasin B. We were unable to detect phloretin, or its conjugated metabolites, in the samples obtained from the serosal side, indicating that during the time scale of our perfusion experiments, phloretin did not access the basolateral side. Furthermore, we are not aware that luminal cytochalasin B can traverse the epithelium.

GIP and GLP-1 are stimulators of insulin release. Insulin secretion is characterised by two distinct phases, the first occurring within minutes of β-cell stimulation and a second prolonged release. This phenomenon was also observed for glucose stimulation of GIP, GLP-1 and PYY in this study (Fig. 1), with the first peak occurring within 5 min. It is interesting to note that mice lacking SGLT1 still exhibit significant elevation of insulin 13 min post gavage with glucose (2 mg (kg body weight)$^{-1}$) without

Figure 5. K$^+$$_{ATP}$-sensitive channels regulate GIP, GLP-1 and PYY secretion
Rat small intestine was perfused with glucose (5 mM) + phloridzin (0.5 mM) in KHB. At $t = 30$ min, tobutamide (500 μM; ●) or diazoxide (340 μM; ■) was added. Samples were analysed for GIP, GLP-1 and PYY. AUC was calculated for $t = 0$–30 min and $t = 30$–60 min for GIP, GLP-1 and PYY. Significance was determined using Student's paired t test $vs.$ $t = 0$–30 min where *$P < 0.05$ and ***$P < 0.001$.

2011). In addition, transcripts for K^+_{ATP} channel subunits Kir6.2 and SUR1, and glucokinase, are also present in GLUTag cells and purified L-cells (Reimann & Gribble, 2002), implying that glucose entry through GLUT2 and its

subsequent metabolism in IECs can influence membrane depolarisation.

GLUT2-null mice exhibit diminished GLP-1 secretion in response to a glucose challenge (Cani *et al.* 2007). In
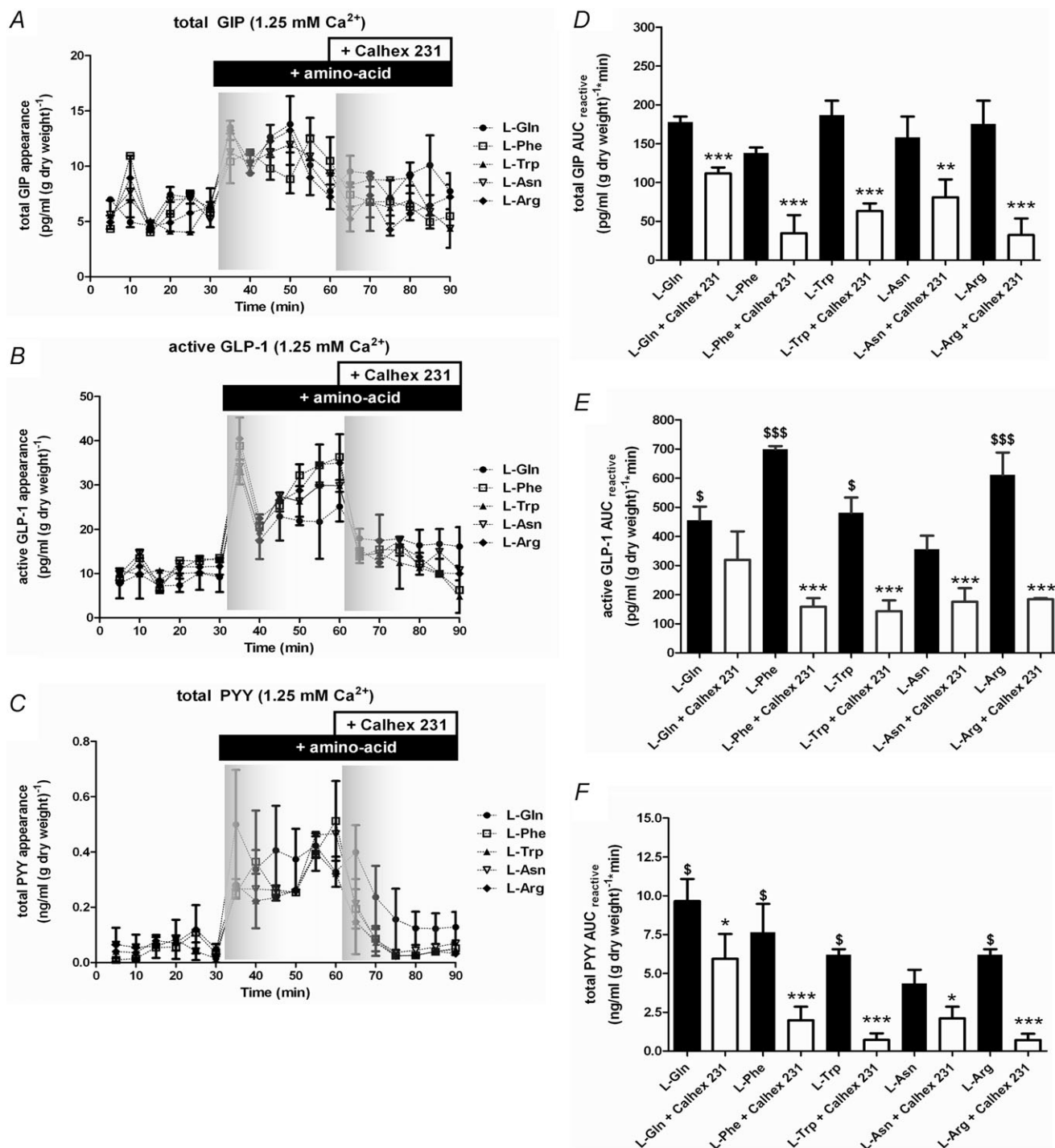

Figure 6. Calhex 231 antagonises gut peptide secretion stimulated by ʟ-amino acids
Rat small intestine was perfused with KHB $\pm$ 1.25 mM Ca^{2+}. At 30 min, 10 mM Gln (●), Phe (□), Trp (▲), Asn (▽) or Arg (◆) was introduced. At 60 min, Calhex 231 (10 μM) was introduced. Samples were analysed for GIP, GLP-1 and PYY content. The $AUC_{reactive}$ was calculated for $t = 30$–60 and 60–90 min periods. Student's paired t tests were used to determine significance between the experimental periods where $**P < 0.01$ and $***P < 0.001$.
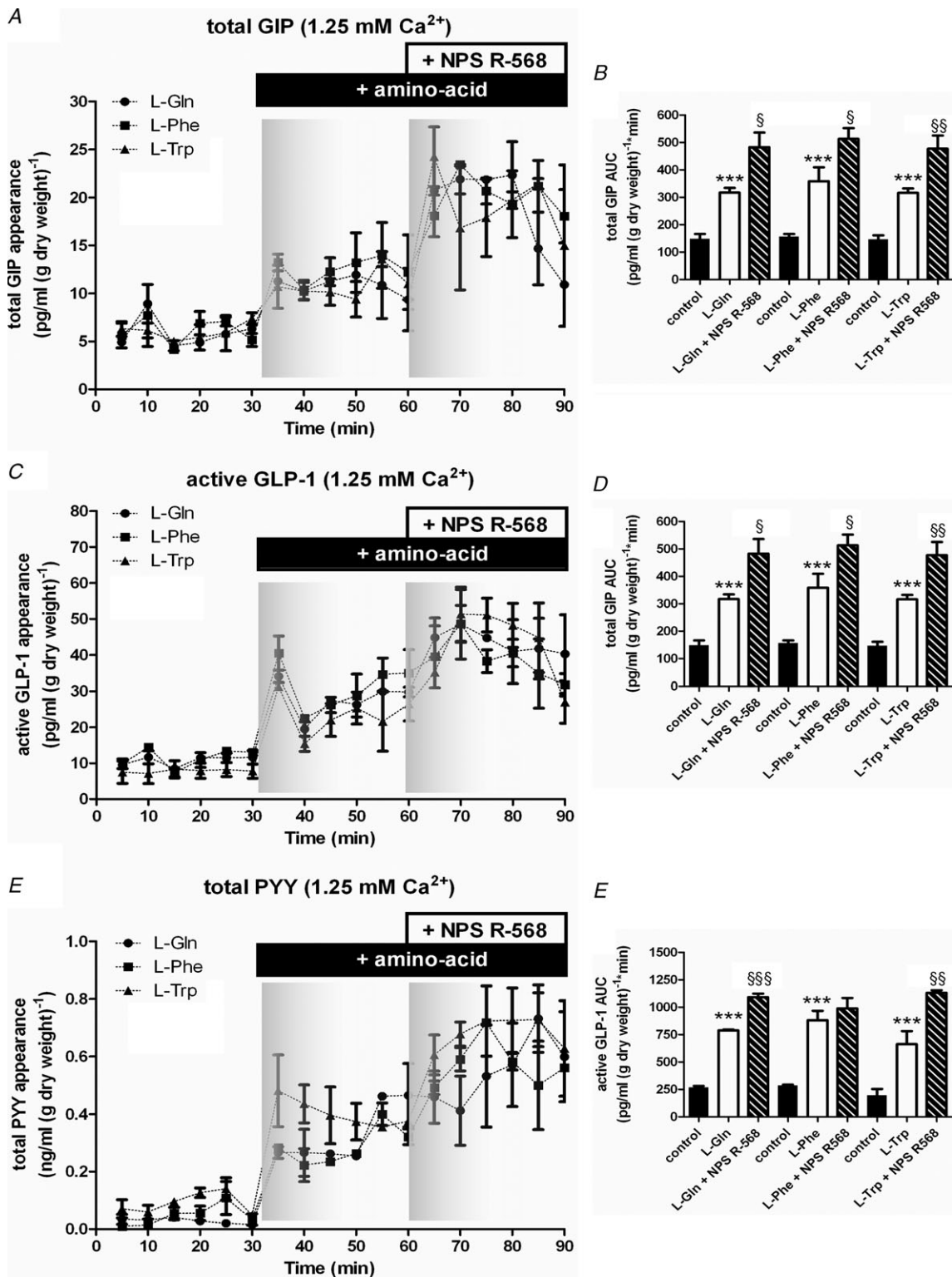
Figure 7. NPS R-568 agonism of gut peptide secretion stimulated by L-amino acids

Rat small intestine was perfused with KHB containing KHB ± 1.25 mM Ca²⁺. At 30 min, 10 mM Gln (●), Phe (■) or Trp (▲) was introduced. At 60 min, NPS R-568 was added into the perfusate. Samples were analysed for GIP, GLP-1 and PYY content. The AUC for $t = 0–30$, $30–60$ and $60–90$ min periods were calculated. Student's paired t tests were used to determine significance between the $t = 0–30$ and $t = 30–60$ min where $**P < 0.01$ and $***P < 0.001$, and the $t = 30–60$ and $t = 60–90$ min periods where $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ and $^{\S\S\S}P < 0.001$

addition, intestinal GLP-1 content in those GLUT2-null mice was significantly reduced (Cani *et al.* 2007). In the present study, inhibition of GLUT2 inhibited GIP, GLP-1 and PYY secretion mediated by a diverse range

of secretagogues indicating a fundamental role in the gut peptide secretory mechanism. In the presence of the SGLT1 inhibitor phloridzin, we observed that the small intestine was sensitive to tolbutamide, suggesting
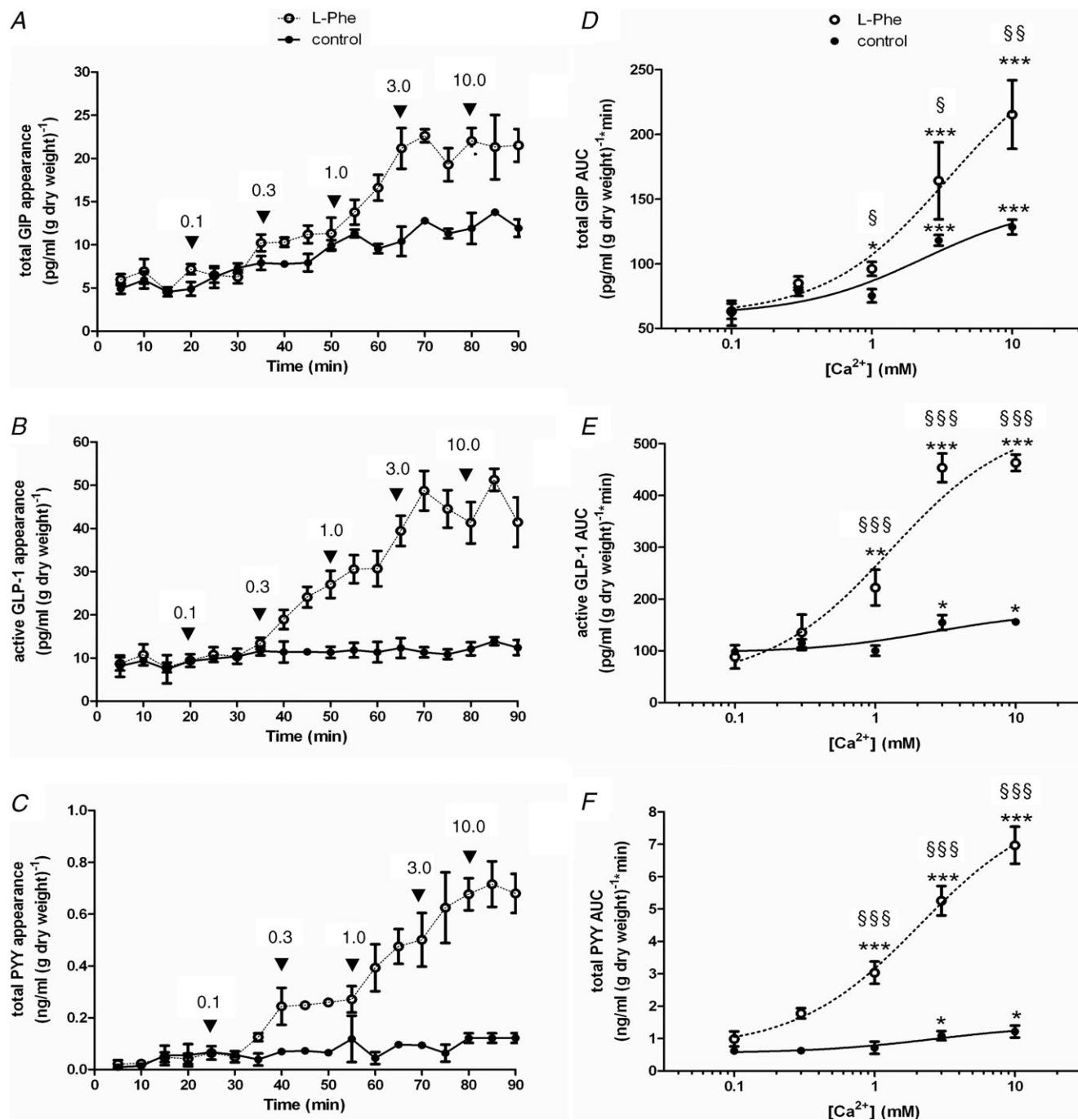

Figure 8. L-Phe increases the potency of extracellular Ca^{2+}-stimulated gut peptide secretion
A, rat small intestine was perfused with KHB deplete of Ca^{2+} ± 10 mM Phe (+Phe, ○, and −Phe, ●). At 20 min, Ca^{2+} was re-introduced cumulatively into the perfusate every 15 min. Samples were analysed for GIP, GLP-1 and PYY content. *A–C* depict the time courses for the perfusions; *D–F* show extracellular Ca^{2+}–gut peptide response relationships using the calculated AUC following each Ca^{2+} addition. Student's paired *t* tests were used to determine significance between additions where *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Student's unpaired *t* tests were used to determine significance between control and L-Phe where §$P < 0.05$, §§$P < 0.01$ and §§§$P < 0.001$

that GIP, GLP-1 and PYY secretion is modulated by K^+_{ATP}-sensitive channels and consistent with reports showing K^+_{ATP}-sensitive channels are expressed in purified L-cells (Reimann & Gribble, 2002).

Phloretin has been shown to affect both Ca^{2+} and K^+_{ATP}-sensitive channels (Olsen *et al.* 2007). We found similar data with cytochalasin B (Fig. 1*C*), which to our knowledge, does not affect either Ca^{2+} or K^+_{ATP}-sensitive channels so we are confident that phloretin is selectively targeting GLUT2. Neither phloretin, nor cytochalasin B inhibited L-amino acid-induced gut peptide secretion.

A gut peptide response to sucralose in the presence of glucose

Artificial sweeteners have been shown to increase gut peptide release (Steinert *et al.* 2011). They have also been shown to increase glucose absorption across the rat small intestine through the insertion of GLUT2 into

the apical membrane (Mace *et al.* 2007*a*, 2009). In the present study, sweet taste receptor proteins expressed in absorptive enterocytes and enteroendocrine cells (Mace *et al.* 2007*a*, 2009; Kaji *et al.* 2009) responded to sucralose with GIP, GLP-1 and PYY secretion and enhanced glucose absorption. Gut peptide secretion stimulated by sucralose is consistent with recent evidence showing activation of the sweet taste receptor leads to gut peptide secretion (Gerspach *et al.* 2011; Steinert *et al.* 2011). In support, knockout mice for Tas1R3 or the G protein αgustducin also exhibit impaired glucose-stimulated GLP-1 secretion *in vivo* (Margolskee *et al.* 2007). Indeed, one possible alternative explanation of the Tas1R3 or the αgustducin protein knockout mice data could be that reductions in taste receptor proteins would compromise apical GLUT2 levels to consequently impair GLP-1 secretion (Margolskee *et al.* 2007).

It appears evident from the literature that intestinal responses to sucralose emerge when sucralose is co-dosed
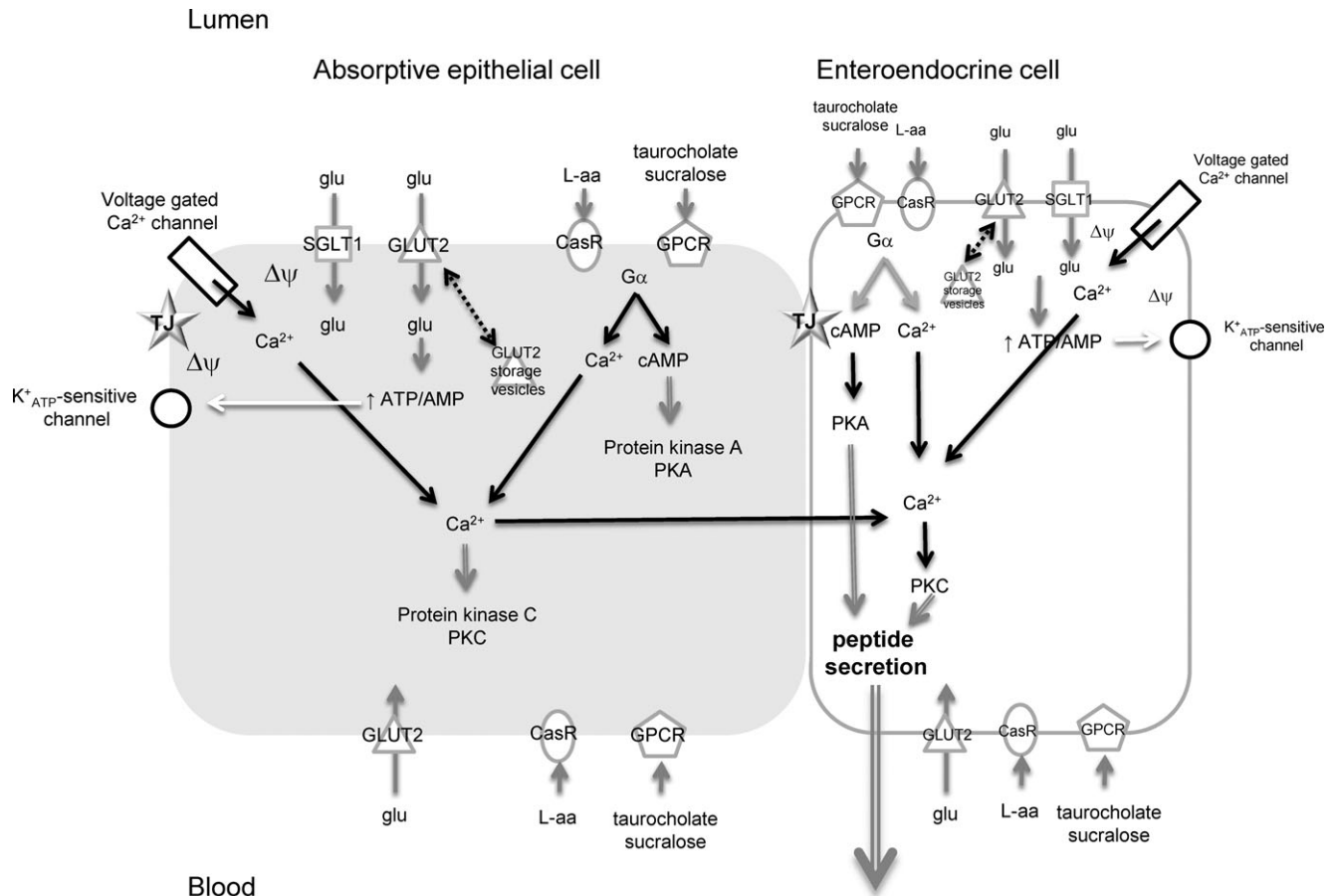

Figure 9. Proposed working model
Absorptive epithelial cells and EECs share common signalling elements initiating gut peptide secretion, namely SGLT1, GLUT2, CasR, GPCRs, voltage-gated Ca^{2+} and K^+_{ATP}-sensitive channels. Glucose (glu) can stimulate gut peptide secretion by (1) Na^+-coupled glucose transport via SGLT1 to directly depolarise the membrane ($\Delta\Psi$), opening voltage-gated Ca^{2+} channels or (2) transport of glucose into cells via GLUT2 leads to increased metabolism and closure of K^+_{ATP}-sensitive channels located close to the tight junction (TJ). This generates depolarisation of the membrane. A rise in intracellular Ca^{2+} results with gut peptide secretion from EECs.

with glucose (Mace *et al.* 2007*a*). In primary rodent intestinal cells, sucralose induced secretion of GLP-1 in the presence of 10 mM glucose (Reimann *et al.* 2008). Higher concentrations of sucralose (20 mM) were required to elicit a response, most probably due to desensitisation of the sweet taste receptor following culture in medium containing 25 mM glucose (Reimann *et al.* 2008). Since GLUT2 and therefore glucose is necessary to observe a response to sucralose, it appears that gut peptide secretion in response to sucralose is glucose dependent. Potentially, activation of sweet taste receptors with sucralose synergises with glucose.

Conditions that impede GLUT2 targeting to the apical membrane including stress (Shepherd *et al.* 2004; Boudry *et al.* 2007), Ca^{2+} depletion (Morgan *et al.* 2007; Mace *et al.* 2007*b*, 2009), glucose concentration (Shepherd *et al.* 2004; Mace *et al.* 2007*a*,*b*; Grefner *et al.* 2010) and SGLT1 knockout (Gorboulev *et al.* 2012) will hinder attempts to observe glucose-dependent gut peptide secretion, which may have contributed to the current conflict in the literature regarding the ability of intestinal sweet taste receptor activity to modulate gut peptide secretion.

CasR is involved in L-amino acid stimulation of K- and L-cell activity

Depletion of extracellular Ca^{2+} diminished secretion of GIP, GLP-1 and PYY in response to L-amino acids. The specific CasR agonist, R-476, potentiated amino acid-dependent gut peptide secretion, whilst the inhibitor, Calhex, inhibited gut peptide responses. Our data show that enteroendocrine-expressed CasR is functionally active and physiologically important in mediating a response to L-amino acids. The partial inhibition by Calhex of the Gln-mediated response is not surprising since it has previously been reported that Gln stimulates GLP-1 secretion from primary murine L-cells via elevating intracellular Ca^{2+}, which is augmented further by increasing cAMP levels.

These data show that CasR provides a link between amino acids in the lumen of the gastrointestinal tract and the stimulation of gut peptides, GIP, GLP-1 and PYY. Consistent with this hypothesis, purified murine L-cells contain transcripts for CasR (Reimann *et al.* 2004). Whether apical or basolateral CasR activity mediates the effects of L-amino acids is unknown. However, activation of CasR in enteroendocrine G-cells and I-cells stimulates the secretion of gastrin and cholecystokinin (CCK), respectively (Ray *et al.* 1997; Buchan *et al.* 2001; Bevilacqua *et al.* 2005; Ceglia *et al.* 2009; Feng *et al.* 2010; MacLeod, 2011). The data presented here show that CasR detects L-amino acids within the concentration ranges found in the diet (Adibi & Mercer, 1973; Adibi *et al.* 1973; Feldman & Grossman, 1980).

EC_{50} values for extracellular Ca^{2+} in the presence of Phe were close to the reported level of free ionised and bioavailable Ca^{2+} in human plasma (1.5 mM) so that Phe-induced gut peptide secretion is likely to be sensitive to variations in extracellular Ca^{2+} that correspond to physiological levels observed under normal conditions. Furthermore, Phe increased the sensitivity of this response, and the EC_{50} decreased for peptide secretion. These data indicate that L-amino acids can affect CasR activity and suggest that the prevailing extracellular Ca^{2+} concentration may determine the magnitude of a gut peptide secretory response. It was evident, at least for Gln, that the interaction between L-amino acids and CasR was not solely responsible for mediating secretion of the gut peptides examined. *In vitro*, Gln-induced GLP-1 secretion is mediated by both Ca^{2+} and cAMP signalling pathways (Reimann *et al.* 2004; Tolhurst *et al.* 2011).

Conclusion

In conclusion, we propose that GLUT2 regulates K- and L-cell gut peptide responses to a diverse set of secretagogues. Our current working model is described in Fig. 9. It is clear that CasR participates in K- and L-cell gut peptide responses to L-amino acids. Other receptor proteins including Tas1R2+Tas1R3, Tas1R1+Tas1R3 and GPRc6A receptors are likely to coordinate a vast network of signal integration to culminate in a coordinated gut peptide response.

References

Adibi SA & Mercer DW (1973). Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. *J Clin Invest* **52**, 1586–1594.

Adibi SA, Modesto TA, Morse EL & Amin PM (1973). Amino acid levels in plasma, liver, and skeletal muscle during protein deprivation. *Am J Physiol* **225**, 408–414.

Ait-Omar A, Monteiro-Sepulveda M, Poitou C, Le Gall M, Cotillard A, Gilet J *et al.* (2011). GLUT2 accumulation in enterocyte apical and intracellular membranes: a study in morbidly obese human subjects and ob/ob and high fat-fed mice. *Diabetes* **60**, 2598–2607.

Au A, Gupta A, Schembri P & Cheeseman CI (2002). Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochem J* **367**, 247–254.

Auchere D, Tardivel S, Gounelle JC & Lacour B (1997). Stimulation of ileal transport of calcium by sorbitol in in situ perfused loop in rats (in French). *Gastroenterol Clin Biol* **21**, 960–966.

Bevilacqua M, Dominguez LJ, Righini V, Valdes V, Toscano R, Sangaletti O *et al.* (2005). Increased gastrin and calcitonin secretion after oral calcium or peptones administration in patients with hypercalciuria: a clue to an alteration in calcium-sensing receptor activity. *J Clin Endocrinol Metab* **90**, 1489–1494.

Boudry G, Cheeseman CI & Perdue MH (2007). Psychological stress impairs Na^+-dependent glucose absorption and increases GLUT2 expression in the rat jejunal brush-border membrane. *Am J Physiol Regul Integr Comp Physiol* **292**, R862–R867.

Bronk JR & Hastewell JG (1988). The transport and metabolism of naturally occurring pyrimidine nucleosides by isolated rat jejunum. *J Physiol* **395**, 349–361.

Bronk JR, Lister N & Lynch S (1987). Absorption of 5-fluorouracil and related pyrimidines in rat small intestine. *Clin Sci (Lond)* **72**, 705–716.

Bronk JR, Lister N & Shaw MI (1988). Transport and metabolism of 6-thioguanine and 6-mercaptopurine in mouse small intestine. *Clin Sci (Lond)* **74**, 629–638.

Buchan AM, Squires PE, Ring M & Meloche RM (2001). Mechanism of action of the calcium-sensing receptor in human antral gastrin cells. *Gastroenterology* **120**, 1128–1139.

Busque SM, Kerstetter JE, Geibel JP & Insogna K (2005). L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. *Am J Physiol Gastrointest Liver Physiol* **289**, G664–G669.

Cani PD, Holst JJ, Drucker DJ, Delzenne NM, Thorens B, Burcelin R & Knauf C (2007). GLUT2 and the incretin receptors are involved in glucose induced incretin secretion. *Mol Cell Endocrinol* **276**, 18–23.

Ceglia L, Harris SS, Rasmussen HM & Dawson-Hughes B (2009). Activation of the calcium sensing receptor stimulates gastrin and gastric acid secretion in healthy participants. *Osteoporos Int* **20**, 71–78.

Chang CH, Chey WY, Sun Q, Leiter A & Chang TM (1994). Characterization of the release of cholecystokinin from a murine neuroendocrine tumor cell line, STC-1. *Biochim Biophys Acta* **1221**, 339–347.

Conigrave AD, Quinn SJ & Brown EM (2000). L-amino acid sensing by the extracellular Ca^{2+}-sensing receptor. *Proc Natl Acad Sci U S A* **97**, 4814–4819.

Dufner MM, Kirchhoff P, Remy C, Hafner P, Muller MK, Cheng SX *et al.* (2005). The calcium-sensing receptor acts as a modulator of gastric acid secretion in freshly isolated human gastric glands. *Am J Physiol Gastrointest Liver Physiol* **289**, G1084–G1090.

Dumoulin V, Dakka T, Plaisancie P, Chayvialle JA & Cuber JC (1995). Regulation of glucagon-like peptide-1-(7–36) amide, peptide YY, and neurotensin secretion by neurotransmitters and gut hormones in the isolated vascularly perfused rat ileum. *Endocrinology* **136**, 5182–5188.

Feldman EJ & Grossman MI (1980). Liver extract and its free amino acids equally stimulate gastric acid secretion. *Am J Physiol Gastrointest Liver Physiol* **239**, G493–G496.

Feng J, Petersen CD, Coy DH, Jiang JK, Thomas CJ, Pollak MR & Wank SA (2010). Calcium-sensing receptor is a physiologic multimodal chemosensor regulating gastric G-cell growth and gastrin secretion. *Proc Natl Acad Sci U S A* **107**, 17791–17796.

Fisher RB & Gardner ML (1974). A kinetic approach to the study of absorption of solutes by isolated perfused small intestine. *J Physiol* **241**, 211–234.

Gardner ML & Plumb JA (1979). Release of dipeptide hydrolase activities from rat small intestine perfused *in vitro* and *in vivo*. *Clin Sci (Lond)* **57**, 529–534.

Gerspach AC, Steinert RE, Schonenberger L, Graber-Maier A & Beglinger C (2011). The role of the gut sweet taste receptor in regulating GLP-1, PYY, and CCK release in humans. *Am J Physiol Endocrinol Metab* **301**, E317–E325.

Gorboulev V, Schurmann A, Vallon V, Kipp H, Jaschke A, Klessen D *et al.* (2012). Na^+-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes* **61**, 187–196

Gouyon F, Caillaud L, Carriere V, Klein C, Dalet V, Citadelle D *et al.* (2003). Simple-sugar meals target GLUT2 at enterocyte apical membranes to improve sugar absorption: a study in GLUT2-null mice. *J Physiol* **552**, 823–832.

Grefner NM, Gromova LV, Gruzdkov AA & Komissarchik I (2010). Comparative analysis of SGLT1 and GLUT2 transporter distribution in rat small intestine enterocytes and Caco2 cells during hexose absorption (in Russian). *Tsitologiia* **52**, 580–587.

Helliwell PA & Kellett GL (2002). The active and passive components of glucose absorption in rat jejunum under low and high perfusion stress. *J Physiol* **544**, 579–589.

Huang C & Miller RT (2007). The calcium-sensing receptor and its interacting proteins. *J Cell Mol Med.* **11**, 923–34.

Hughes SD, Quaade C, Johnson JH, Ferber S & Newgard CB (1993). Transfection of AtT-20ins cells with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion. Relationship to glucose metabolism. *J Biol Chem* **268**, 15205–15212.

Kaji I, Karaki S, Fukami Y, Terasaki M & Kuwahara A (2009). Secretory effects of a luminal bitter tastant and expressions of bitter taste receptors, T2Rs, in the human and rat large intestine. *Am J Physiol Gastrointest Liver Physiol* **296**, G971–G981.

Kellett GL & Brot-Laroche E (2005). Apical GLUT2: a major pathway of intestinal sugar absorption. *Diabetes* **54**, 3056–3062.

Kellett GL & Helliwell (2000). The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. *Biochem J* **2000**, 155–162

Koop I & Buchan AM (1992). Cholecystokinin release from isolated canine epithelial cells in short-term culture. *Gastroenterology* **102**, 28–34.

Lister N, Bailey PD, Collier ID, Boyd CA & Bronk JR (1997). The influence of luminal pH on transport of neutral and charged dipeptides by rat small intestine, in vitro. *Biochim Biophys Acta* **1324**, 245–250.

Lister N, Sykes AP, Bailey PD, Boyd CA & Bronk JR (1995). Dipeptide transport and hydrolysis in isolated loops of rat small intestine: effects of stereospecificity. *J Physiol* **484**, 173–182.

Mace OJ, Affleck J, Patel N & Kellett GL (2007*a*). Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* **582**, 379–392.

Mace OJ, Lister N, Morgan E, Shepherd E, Affleck J, Helliwell P *et al.* (2009). An energy supply network of nutrient

absorption coordinated by calcium and T1R taste receptors in rat small intestine. *J Physiol* **587**, 195–210.

Mace OJ, Morgan EL, Affleck JA, Lister N & Kellett GL (2007*b*). Calcium absorption by Cav1.3 induces terminal web myosin II phosphorylation and apical GLUT2 insertion in rat intestine. *J Physiol* **580**, 605–616.

MacLeod RJ (2011). A new role for the extracellular calcium-sensing receptor demonstrated by using CCK-eGFP BAC mice. *Am J Physiol Gastrointest Liver Physiol* **300**, G526–G527.

Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K *et al.* (2007). T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci U S A* **104**, 15075–15080.

Merigo F, Benati D, Cristofoletti M, Osculati F & Sbarbati A (2011). Glucose transporters are expressed in taste receptor cells. *J Anat* **219**, 243–252.

Morgan EL, Mace OJ, Affleck J & Kellett GL (2007). Apical GLUT2 and Cav1.3: regulation of rat intestinal glucose and calcium absorption. *J Physiol* **580**, 593–604.

Naftalin RJ & Tripathi S (1986). The roles of paracellular and transcellular pathways and submuncosal space in isotonic water absorption by rabbit ileum. *J Physiol* **370**, 409–432

Newgard CB & McGarry JD (1995). Metabolic coupling factors in pancreatic beta-cell signal transduction. *Annu Rev Biochem* **64**, 689–719.

Olson ML, Kargacin ME, Ward CA & Kargacin GJ (2007). Effects of phloretin and phloridzin on Ca^{2+} handling, the action potential, and ion currents in rat ventricular myocytes. *J PHarmacol Exp Ther* **321**, 921–9

Oya M, Suzuki H, Watanabe Y, Sato M & Tsuboi T (2011). Amino acid taste receptor regulates insulin secretion in pancreatic beta-cell line MIN6 cells. *Genes Cells* **16**, 608–616.

Parker HE, Reimann F & Gribble FM (2010). Molecular mechanisms underlying nutrient-stimulated incretin secretion. *Expert Rev Mol Med* **12**, e1.

Ray JM, Squires PE, Curtis SB, Meloche MR & Buchan AM (1997). Expression of the calcium-sensing receptor on human antral gastrin cells in culture. *J Clin Invest* **99**, 2328–2333.

Reimann F & Gribble FM (2002). Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* **51**, 2757–2763.

Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ & Gribble FM (2008). Glucose sensing in L cells: a primary cell study. *Cell Metab* **8**, 532–539.

Reimann F, Williams L, da Silva Xavier G, Rutter GA & Gribble FM (2004). Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells. *Diabetologia* **47**, 1592–1601.

Shepherd EJ, Helliwell PA, Mace OJ, Morgan EL, Patel N & Kellett GL (2004). Stress and glucocorticoid inhibit apical GLUT2-trafficking and intestinal glucose absorption in rat small intestine. *J Physiol* **560**, 281–290.

Steinert RE, Gerspach AC, Gutmann H, Asarian L, Drewe J & Beglinger C (2011). The functional involvement of gut-expressed sweet taste receptors in glucose- stimulated secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). *Clin Nutr* **30**, 524–532.

Thorens B (2001). GLUT2 in pancreatic and extra-pancreatic gluco-detection (review). *Mol Membr Biol* **18**, 265–273.

Tobin V, Le Gall M, Fioramonti X, Stolarczyk E, Blazquez AG, Klein C *et al.* (2008). Insulin internalizes GLUT2 in the enterocytes of healthy but not insulin-resistant mice. *Diabetes* **57**, 555–562.

Tolhurst G, Reimann F & Gribble FM (2009). Nutritional regulation of glucagon-like peptide-1 secretion. *J Physiol* **587**, 27–32.

Tolhurst G, Zheng Y, Parker HE, Habib AM, Reimann F & Gribble FM (2011). Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca^{2+} and cAMP. *Endocrinology* **152**, 405–413.

Wang Y, Chandra R, Samsa LA, Gooch B, Fee BE, Cook JM, Vigna SR, Grant AO & Liddle RA (2011). Amino acids stimulate cholechstokinin release through the Ca2+-sensing receptor *Am J Physiol Gastrointest Liver Physiol*. **300**, G528–37.

Wei N, Liu R, Ou Y, Li X, Qiang O, Guo W & Tang CW (2011). Effects of octreotide of glucose transporter type 2 expression in obese rat small intestine. *World J Gastro* **17**, 4434–4439

Yee KK, Sukumaran SK, Kotha R, Gilbertson TA & Margolskee RF (2011). Glucose transporters and ATP-gated K^+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells. *Proc Natl Acad Sci U S A* **108**, 5431–5436.

Zheng Y, Scow JS, Duenes JA & Sarr MG (2012). Mechanisms of glucose uptake in intestinal cell lines: role of GLUT2. *Surgery* **151**, 13–25.

Author contributions

O.J.M., M.S. and S.P. were responsible for the conception & design of the experiments, collection, analysis & interpretation of the data and writing the article. All authors approved the final version.

Acknowledgements