We performed whole-cell recordings from hypocretin cells using brain slices taken from 13-22 day-old mice. These mice expressed enhanced green fluorescent protein under the hypocretin promoter, thus allowing us to unambiguously identify hypocretin cells as described previously (4). TRH was applied extracellularly at concentrations of 100, 250 or 500 nM while cells were recorded in voltage- or current-clamp mode.
TRH depolarized and increased the firing rate in all hypocretin cells tested (n=22) in a dose-dependent manner. Membrane depolarization persisted in the presence of tetrodotoxin (n=6 cells) or in a low Ca^{2+}/high Mg^{2+} extracellular solution (n=4 cells), suggesting a post-synaptic mechanism. When we analysed the effects of TRH on action potentials, we found that their width increased (control 1.15±0.05 ms, TRH 1.40±0.10 ms, p=0.006, n=11), whereas their firing threshold remained unchanged (control -36.1±0.9 mV, TRH -35.4±1.2 mV, p>0.1, n=11). We also measured the effects on cell excitability by quantifying input-output gain using frequency-current plots, and found that TRH significantly increased gain in 9 of 10 hypocretin cells. Finally, our voltage-clamp recordings revealed that the TRH-induced depolarization involved the activation of an inward current that depended on extracellular Na^{+}. In addition, we found that the peptide also increased post-synaptic voltage-gated K^{+} and Ca^{2+} currents.
These results show that TRH directly and potently stimulates hypocretin neurones. The actions of TRH on hypocretin cells may contribute to the physiological regulation of rhythms in feeding behaviour and cognitive arousal.

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

C5 and PC15

Characterisation of a 'non-starvation' food withdrawal response in the nematode *C. elegans*

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Food withdrawal provides a very powerful way of adapting behaviour of the model organism *C. elegans* (Hill *et al.*). Indeed, previous work has highlighted that worms are considered starved when cultivated in the absence of food for 2 hours. We have recently noted an adaptive response of the muscle organ, the pharynx, following withdrawal of food. Investigation of developmentally staged worms (L4 plus 16 hours) that were deprived of food showed differential regulation of feeding behaviour to the absence of food over time. Feeding behaviour was quantified by counting the pumping, i.e. the movement of a grinder (visible as a black line in the pharynx). This paradigm sees three phases of behaviour i. the initial cessation in pumping is followed by a slow increase in pumping until it reaches a ii. steady state pump rate (~50 pumps/min) after 70mins. After this initial plateau, which persists for approximately one hour, the worms undergo a series of erratic pumps in which they are relatively quiescent or pump at the high frequency similar to seen in the presence of food. This latter phase is quantified by the increased variation in pump rates. Although, previous investigations have defined removal from food for 5 hours as starvation (Avery *et al.*), our investigations of the nutritional status show that the worms retain their lipid stores during the initial 5 hours (Sudan Black staining), indicating that they may not be starved. However, after 10hrs there is a decrease in fat staining in the head region of the worms as measured by pixel density, from 1180862 (n=14) to 739853 (n=19) pixels (P<0.0001, t-test). The microcircuit that controls pharyngeal pumping is made up of 20 embedded neurons and a single neuron contact to the extra-pharyngeal nervous system. This circuit is underpinned by a number of fast and neuromodulatory transmitters, whose homologues regulate feeding behaviour in mammals. Analysing mutants that define key transmitters within this circuit helps define the locus for the neuroadaption and the cellular and molecular determinants of the behaviour. E.g., although serotonin is a key regulator of the worm's response to the presence of food, it does not play a role in the adaptive response to the food deprivation. This work should provide a framework to investigate microcircuits that define model feeding behaviour and the modulation to varying nutritional states of the organism.

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Funded by the Gerald Kerkut Charitable Trust. We are grateful to the *C. elegans Genetics Centre* for providing strains.

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C6 and PC16

Mechanisms of GIP secretion from primary intestinal K-cells

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Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone that promotes insulin release and coordinates the fate of dietary fat [1]. It is released from K-cells located in the duodenal and jejunal epithelia, which are thought to directly sense the presence of nutrients in the gut lumen. However, at the cellular and molecular level, little is known about how K-cells respond to stimuli. The aim of this study was to generate transgenic mice with fluorescently labelled K-cells and to use these to investigate pathways by which K-cells detect nutrients. Transgenic mice were generated in which the GIP promoter drives expression of a fluorescent protein, Venus. Fluorescent cells from intestinal tissue were purified by flow cytometry and analysed by quantitative RT-PCR. GIP secretion in response to

various stimuli was assayed in cultures of adult mouse small intestine. Our data establish for the first time that K-cells express glucose-sensing proteins, Kir6.2, SUR1, glucokinase and SGLT1, at levels 190-fold (p=0.005), 470-fold (p=0.0002) and 420-fold (p=0.007) greater than control cells respectively, but barely detectable levels of sweet taste receptor subunits. Expression of the lipid receptors, GPR40, GPR119 and GPR120, postulated to play a role in the regulation of enteroendocrine hormone release, was also enhanced in the K-cell population, 99-fold (p=0.007), 330-fold (p=0.0009) and 76-fold (p=0.002), respectively.

In primary cultures, GIP secretion was enhanced 1.34-fold by 10mM glucose (n=27, p=0.003), 1.25-fold by 500μM tolbutamide (n=19, p=0.01) and 1.21-fold by 10mM fructose (n=9, p=0.03) confirming that K_{ATP} channels in K-cells are functional. Secretion was also enhanced 2.0-fold by PMA (n=3, p=0.005) and 3.8-fold by a combination of forskolin/IBMX (n=44, p=10^{-13}) highlighting an important role for pathways coupled to the activation of adenylate cyclase and/or PKC. In the presence of forskolin/IBMX, we observed a 1.7-fold stimulation of GIP release by 10mM α-methylglucose (αMG; n=18, p=0.0001), demonstrating that glucose metabolism is not required for monosaccharide triggered secretion. The measured ED_{50} for glucose of 0.6mM is close to the K_m for glucose and αMG of SGLT1 (~0.3mM [2]), suggesting that SGLT1 plays a key role in glucose-triggered GIP release. Forskolin/IBMX potentiated the response to 10mM glucose by 3.1-fold (n=17, p=$2x10^{-6}$) indicating a synergistic interaction between these two pathways. GIP release was unaffected by the artificial sweetener, 1mM sucralose.

In conclusion, glucose-dependent GIP secretion was found to be SGLT1-dependent, and modulated by K_{ATP} channels but not determined by sweet taste receptors. Synergistic stimulation by elevated cAMP and glucose suggests that targeting appropriate G-protein coupled receptors may provide opportunities to modulate GIP release in vivo.

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Wellcome Trust, MRC, Lister Institute and St John's College, Cambridge

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C7 and PC17

Electrophysiological characterisation of murine primary L-cells

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Glucagon-like peptide-1 (GLP-1) is an enteric hormone released by L-cells in response to nutrient ingestion. Although such cells are present throughout the gastrointestinal tract, they are most abundant in the ileum and colon. GLP-1 is a potent insulinotropic agent that also inhibits beta-cell apoptosis, making it a particularly attractive therapeutic target for the treatment of type 2 diabetes. Pharmacological GLP-1 mimetics and inhibitors of GLP-1 degradation are currently employed to activate the GLP-1 axis therapeutically. An alternative approach would be to enhance the secretion of endogenous GLP-1. However, this has been hampered by a poor understanding of stimulus-secretion coupling in this cell type due largely to difficulties in identifying and culturing primary L-cells. The generation of transgenic mice that express YFP under the control of the proglucagon promoter enables the identification of L-cells, making functional characterisation of such cells possible.

The purpose of this study was to characterise the electrical properties of L-cells and their expression of voltage-gated ion channels. This was achieved by performing standard and perforated-patch whole-cell patch-clamp experiments on primary cultured murine colonic L-cells identified by their expression of YFP. Immunocytochemistry confirmed that glucagon and peptide YY (PYY) expression was restricted to YFP-expressing cells and that the cells maintain their differentiated phenotype in culture. L-cells were electrically active, with a resting membrane potential of ~-50 mV and a threshold for firing action potentials of -35 ± 2 mV (n=9). Whole cell voltage-clamp recordings revealed large rapidly-inactivating, tetrodotoxin (TTX)-sensitive sodium currents (-850 ± 123pA cell-1 at 0mV, n=9), which exhibited half maximal activation at -17 ± 1 mV (n=9), and half-maximal inactivation at -46 ± 1 mV (n=10). FACs-sorted L-cells predominantly expressed scn3a, as assessed by quantitative RT-PCR. In the presence of TTX (0.3μM), the residual inward current was abolished by 5mM Co^{2+}, strongly suggesting that this is a voltage-dependent Ca^{2+} current (n=3). The use of pharmacological blockers such as isradipine (10μM) and NNC 55-0396 (1μM) partially ablated the TTX-resistant current confirming the presence of L- and T-type calcium channels, respectively.

We believe that this is the first demonstration and characterisation of Na^+-dependent electrical activity in a primary enteroendocrine cell type. Improving our understanding of L-cell function may have potential implications for the development of new anti-diabetic therapeutics targeting the enteroendocrine axis.

We would like to thank the Wellcome Trust, MRC, Lister Institute and St John's College, Cambridge for funding.

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