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GLP-1 – a physiological incretin with pharmacological potential

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone, released from intestinal L-cells in response to food ingestion, which stimulates insulin release and improves glycaemia in type 2 diabetes. Drugs based on mimicking the action of GLP-1 or inhibiting clearance of the endogenous hormone have been licensed recently for the treatment of type 2 diabetic subjects. The idea of therapeutically targeting L-cells to enhance endogenous release of GLP-1 and Peptide YY is increasingly popular, but is hampered by current uncertainties surrounding normal L-cell function.

To enable single cell analysis of primary murine L-cells, we generated transgenic mice expressing a yellow fluorescent protein under the control of the proglucagon promoter. These mice exhibit yellow fluorescence in L-cells, pancreatic alpha cells, and populations of brainstem neurones. L-cells, purified from the transgenic mice by flow cytometry and analysed by quantitative RT-PCR, expressed high levels of K-ATP channel subunits, glucokinase and sodium glucose cotransporter 1 (SGLT1). The expression levels of Kir6.2, SUR1 and glucokinase were similar to those found in pancreatic alpha and beta cells. Single L-cells in primary culture, identified by their yellow fluorescence, were electrically active, and stimulated by glucose and tolbutamide. Identified L-cells loaded with fura-2 exhibited intracellular calcium rises in response to application of glucose, alpha-methylglucose or tolbutamide. In mixed primary cultures of upper small intestine or colon, GLP-1 secretion was glucose-dependent with an EC50 in the low millimolar range, and was also stimulated by tolbutamide or alpha-methylglucose.

The combined results from expression analysis, electrophysiological recordings, single cell fluorescence calcium imaging and GLP-1 secretion implicate important roles for K-ATP channels and SGLT1 in L-cell glucose-sensing.

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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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Adipokines: impact on carbohydrate and lipid metabolism

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During the progression from the lean to the obese state, adipose tissue undergoes hyperplasia as well as hypertrophy in an attempt to cope with the increased demand for triglyceride storage. This requires a high degree of plasticity at both the cellular and at the tissue level. Even though adipose tissue as a whole seems to be a relatively static tissue containing many adipocytes that turn over relatively slowly, these cells are embedded in an environment that can rapidly adapt to the needs of expanding and newly differentiating adipocytes. The extracellular matrix of adipose tissue faces unique challenges with respect to adjusting to the need for remodeling and expansion. In parallel, the vasculature has to adapt to altered requirements for nutrient and oxygen exchange. A decrease in the plasticity of these processes leads to metabolic dysfunction. To maintain a healthy, non-inflamed phenotype, complex regulatory mechanisms are in place to ensure adipocytes and stromal vascular cells efficiently crosstalk to allow adipose tissue to expand upon increased demand for storage of triglycerides. These changes are therefore critically dependent on local production of adipokines that include pro-angiogenic and anti-inflammatory moelcules as well as a complex set of extracellular matrix proteins.

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AMPK: a sensor of glycogen as well as AMP and ATP?

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The classical view of the AMP-activated protein kinase (AMPK) system is that it is a sensor of energy that monitors the cellular concentrations of AMP and ATP, and which regulates energy balance by stimulating catabolism and inhibiting anabolism whenever the cellular AMP:ATP ratio rises [1]. The kinase is a heterotrimeric complex of a catalytic α subunit and regulatory β and γ subunits. The γ subunits contain two tandem domains that bind the regulatory nucleotides, AMP and ATP, in a mutually exclusive manner. The kinase is only active after phosphorylation at a conserved threonine residue in the α subunit (Thr-172) by upstream kinases, of which the most important is the tumour suppressor LKB1. LKB1 appears to phosphorylate Thr-172 continually, but binding of AMP (but not ATP) to the γ subunit inhibits Thr-172 dephosphorylation. Since any fall in the cellular ATP:ADP ratio is amplified by adenylate kinase into a much larger rise in the AMP:ATP ratio, this mechanism acts as a sensitive switch that converts the kinase into its active, phosphorylated form. In addition, the phosphorylated kinase is allosterically activated 10-fold by AMP binding; the combined effects of phosphorylation and allosteric activation result in >1000-fold activation. In some cells, Thr-172 can also be phosphorylated by CaMKKβ, a calmodulin-dependent protein kinase. This occurs in response to a rise in intracellular Ca2+ and does not require any increase in AMP.

The β subunits of the AMPK heterotrimer contain a central glycogen-binding domain (GBD) that is conserved in all eukaryotic orthologues. Although this domain is known to cause binding of AMPK to glycogen in intact cells [2,3], its