SA8

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.

SA9

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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SA10

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

physiological function has been unclear. We have now shown that it is a regulatory domain and that binding of glycogen to it causes allosteric inhibition of AMPK. Glycogen is a polymer of glucose units joined by α1-4 linkages, with occasional branches formed by α1-6 linkages. A major problem with the study of glycogen as a regulatory molecule is that it does not have a defined structure, but varies both in size and branching content. Based on findings suggesting that the degree of branching affected its inhibitory potency, we have synthesized small α1-4 linked glucose oligosaccharides containing single α1-6 branches, and have shown that these are potent allosteric inhibitors of AMPK. The most potent oligosaccharide gives half-maximal inhibition at 90 μM, and also markedly inhibits phosphorylation of Thr-172 by LKB1 and CaMKKβ. Inhibition is due to binding to the GBD, because point mutations in the latter than abolish glycogen binding also abolish inhibition.

One of the physiological targets of AMPK is muscle glycogen synthase (mGS), which is inactivated by phosphorylation at Ser-7 [4]. A curious paradox is that although AMPK is activated by ATP depletion during exercise, mGS is usually found to be dephosphorylated and activated following exercise, as long as the exercise had been sufficiently prolonged to cause significant glycogen depletion. We believe that the regulation of AMPK via the GBD may explain this paradox. The outer tier of glycogen (representing the glucose that can be released by glycogen phosphorylase without the need for the action of debranching enzyme) contains up to one third of all of the glucose units in a single molecule. Theoretical studies suggest that in a fully synthesized molecule of glycogen the outer chains are so tightly packed that the branch points would not be accessible. We suspect that under these conditions AMPK binds to glycogen but is not inhibited, so that it would phosphorylate mGS, thus exerting a feedback inhibition of further extension of the outer chains of glycogen. We also propose that when exercise commences, phosphorylase removes some of the outer chains, exposing the branch points which would then bind AMPK and cause inhibition. mGS would now be dephosphorylated and activated by the glycogen-bound forms of protein phosphatase-1, so that it was ready to replenish glycogen stores as soon as exercise ceased. This hypothesis may also explain why insulin-stimulated glucose uptake and glycogen synthesis is enhanced following a single bout of exercise, as long as the exercise bout was sufficient to cause significant glycogen depletion. It can also answer another unsolved question: how do cells "know" when their glycogen stores are sufficient, and conversely how do they "know" when they are insufficient and need replenishing? These are important questions because insulin resistance, the primary cause of type 2 diabetes and the metabolic syndrome, can be viewed as a mechanism to limit the amount of nutrient that cells can store.

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SA11

The carotid body; a chemosensitive neurogenic center

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The carotid body (CB), a small neural crest-derived paired organ located at the carotid bifurcation, is a principal component of the homeostatic acute oxygen (O_2) sensing system required to activate the brainstem respiratory center to produce hyperventilation during hypoxemia (e.g. in high altitude residents or in patients with chronic obstructive pulmonary diseases) (Weir *et al. 2005*). The CB parenchyma is organized in glomeruli, clusters of cells in close contact with a profuse network of capillaries and afferent sensory fibers joining the glossopharyngeal nerve. The most abundant cell types in the CB glomeruli are the neuron-like, glomus or type I cells, which are enveloped by processes of glia-like, sustentacular type II cells. Glomus cells are physiologically complex, as they are electrically excitable and express a broad variety of voltage- and ligand-gated ion channels, as well as TRP and background K^+ channels. These cells behave as presynaptic-like elements that establish contact with the postsynaptic sensory nerve fibers.

Glomus cells are arterial chemoreceptors, activated by hypoxia, hypercapnia and extracellular acidosis. Recently, it has been shown that, as suggested by experiments *in vivo* (Alvarez-Buylla & Alvarez-Buylla, 1988; Koyama *et al*. 2000), glomus cells *in vitro* are glucose sensors, releasing transmitters in response to hypoglycemia (Pardal & Lopez-Barneo, 2002; Garcia-Fernandez *et al*. 2007). Hypoxia and hypoglycemia are additive stimuli that appear to activate cell secretion through separate pathways converging on cell depolarization and extracellular Ca^{2+} influx. Responsiveness to hypoxia seems to depend on inhibition of voltage-gated K^+ channels (Weir *et al*. 2005), whereas sensitivity to hypoglycemia depends on activation of a non-selective cationic conductance (Garcia-Fernandez *et al*. 2007).

Besides its sensitivity to acute hypoxia, the CB also exhibits a well-known adaptive hypertrophic response to chronic hypoxemia, whose underlying mechanisms are poorly known. We have investigated whether adult CB growth in chronic hypoxia is due to the activation of a population of resident stem cells. Exposure of mice to a hypoxic (10% O_2 tension) isobaric atmosphere for three weeks induced a marked CB enlargement (~2-3 fold) caused by dilation and multiplication of blood vessels as well as expansion of the parenchyma, with increased number of tyrosine hydroxylase positive glomus cell clusters. Although some glomus cells can enter a mitotic cycle in