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The role of allosteric regulation of muscle glycogen synthase *in vivo*

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Skeletal muscle is the major site of insulin-stimulated glucose uptake, with the majority of glucose that enters muscle fibres being stored as glycogen. Insulin promotes dephosphorylation and activation of glycogen synthase (GS) by inactivating GSK3. Insulin also promotes glucose uptake and glucose-6-phosphate (G6P) production, which allosterically activates GS. However, the relative contribution of these two regulatory mechanisms *in vivo* is not well understood. We have recently shown that a knock-in mice carrying a mutated form of GSK3 that cannot be inactivated by insulin have normal rate of glycogen synthesis and glycogen content in skeletal muscle, suggesting that allosteric activation by G6P could be the major mechanism by which muscle GS is activated *in vivo* (1, 2).

Using a mammalian expression system, we carried out a mutagenesis analysis using point mutants of arginine residues situated in the putative G6P binding region of GS. We identified one point mutation that completely abrogates G6P-mediated activation of GS without altering GS expression levels or insulin-mediated activation through dephosphorylation. To address the physiological role of the allosteric regulation of GS in the control of glycogen synthesis *in vivo*, a knock-in mouse carrying this mutation has been generated. The GS knock-in mice are viable and present a normal growth. They express the enzyme at a similar level than their wild type counterparts in different muscle types, in liver and heart. Their muscle GS is completely resistant to G6P-mediated activation and insulin administration results in the same level of PKB and GSK3 phosphorylation and GS desphosphoylation as the wild type mice. We are carrying out the full characterisation of these mice and providing direct genetic evidence of the major role that G6P allosteric activation plays in the maintenance of physiological glycogen levels in skeletal muscle.

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

PC10

Hypothalamic CRTC2 is regulated by metabolic state via AMPK-mediated phosphorylation

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AMP activated protein kinase (AMPK) has been identified as a cellular fuel gauge, which is able to sense cellular energy status and control energy homeostasis by regulating ATP generating and consuming pathways. In the hypothalamus AMPK is able to integrate information from nutrient levels and metabolic hormones in order to regulate downstream systems. So far few downstream targets of AMPK in the hypothalamus have been described. We have identified the CREB coactivator CRTC2 (CREB regulated transcription factor 2, formerly TORC2) as a novel target of hypothalamic AMPK.

In the liver CRTC2 was shown to be nuclear and active in the fasted state, becoming phosphorylated in response to feeding. Phosphorylation at a crucial S171 residue induces translocation of CRTC2 from the nucleus to the cytoplasm, where it is sequestered by a 14-3-3 family protein. Hepatic CRTC2 integrates signals from Ca2+ and cAMP signaling pathways, mediated by the kinases AMPK and SIK2, and the phosphatase Calcineurin, which regulate the stability of the CRTC2:14-3-3 complex. Active (nuclear) CRTC2 is able to induce the transcription of CRE regulated genes by coactivating CREB.

In studies using mice, we have found that the cellular localization of hypothalamic CRTC2 is opposite to hepatic CRTC2 under the same conditions. That is, hypothalamic CRTC2 is dephosphorylated, nuclear and active in the fed state, and in the fasted state hypothalamic CRTC2 is cytoplasmic and inactive. Phosphorylation at S171 is sufficient to inactivate hypothalamic CRTC2

We have identified AMPK as the kinase responsible for inactivating CRTC2 in the hypothalamus. By incubating dissected hypothalami or primary neuronal cultures under different glucose concentrations we have shown that high glucose is sufficient to activate CRTC2, while under low glucose conditions CRTC2 is cytoplasmic. Addition of AICAR (an AMPK activator) or Compound C (an AMPK inhibitor) reverses the subcellular localisation of CRTC2.

Active (nuclear) CRTC2 is able to promote the transcription of cAMP response element (CRE) regulated genes. We have identified insulin receptor substrate 2 (IRS2) as one of these hypothalamic CRE genes. Mutation of the crucial S171 residue has been shown to produce a constitutively active mutant of CRTC2. Transgenic mice over-expressing this constitutively active CRTC2(S171A) in the CNS indeed show a large increase in IRS2 mRNA under fed conditions compared to wild type animals.

Using transgenic mice over-expressing this constitutively active form of CRTC2 only in the CNS we will identify other downstream targets of CRTC2 by microarray, and define a physiological role for CRTC2 in the regulation of energy homeostasis.

In summary, our data suggests an important role for CRTC2 in linking hypothalamic metabolic sensing pathways with gene transcription.

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