weight sparing effect of detemir insulin, relative to other long-acting insulin formulations, is associated with increased transport into the central nervous system [2]. We hypothesised that the effects of detemir insulin on energy balance would be mediated by an increase in central nervous system insulin signalling. Chronic treatment with detemir insulin resulted in reductions in both food intake and weight gain relative to insulin glargine or normal insulin treatment in C57BL/6J mice. Acute peripheral detemir insulin treatment resulted in reduced food intake, with increased phosphorylated Akt also observed in the arcuate nucleus of the hypothalamus of detemir insulin treated mice, relative to other insulin treatments. When mice were maintained on a high fat diet the acute effects of detemir insulin on both energy balance and phosphorylated Akt were inhibited. Furthermore, when specific neuronal populations of insulin receptors were knocked out, animals were insensitive to the acute effects of detemir insulin on energy balance. These data demonstrate that detemir insulin reduces weight gain by acting on the central nervous system to reduce food intake. The inhibition of this effect in high fat diet treated animals indicates that detemir insulin is subject to resistance of insulin transport into the brain.

References

[1] Begg DP, Mul JD, Liu M, Reedy BM, D'Alessio DA, Seeley RJ, et al. Reversal of diet-induced obesity increases insulin transport into cerebrospinal fluid and restores sensitivity to the anorexic action of central insulin in male rats. Endocrinology 2013;154(3):1047—54.

[2] Begg DP, May AA, Mul JD, Liu M, D'Alessio DA, Seeley RJ, et al. Insulin detemir is transported from blood to cerebrospinal fluid and has prolonged central anorectic action relative to NPH insulin. Diabetes 2015;64(7):2457—66.

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Signal transduction pathways activated by the orexigenic gut derived hormone insulin-like peptide 5 at relaxin family peptide receptor 4

Dana S. Hutchinson*, Sheng Y. Ang, Bronwyn A. Evans, Roger J. Summers, Martina Kocan

Monash University, Parkville, VIC, Australia

Introduction: Insulin-like peptide 5 (INSL5) is a two-chain, three-disulphide bonded peptide belonging to the insulin/relaxin superfamily expressed in the enteroendocrine L-cells of human and mouse colon [1]. It is the cognate ligand for relaxin family peptide receptor 4 (RXFP4) a GPCR mainly expressed in the colorectum and enteric nervous system. Currently little is known of the signal transduction pathways activated by RXFP4.

Aims: This study examined intracellular signalling pathways activated by INSL5 acting at the human RXFP4 receptor stably expressed in CHO cells.

Methods: Cell signalling was investigated using AlphaScreen® assays. Ca^{2+} flux was monitored in a Flexstation® using X-rhod-1AM. RXFP4 recruitment of $G\alpha_{i/o}$ protein isoforms were determined by rescue of ERK1/2 responses by PTX-insensitive $G\alpha_{i/o}$ C351I mutants ($mG\alpha_{i/o}$). Cell proliferation was studied by bromo-deoxyuridine (BrdU) cell proliferation ELISA. RXFP4 interactions with β-arrestins 1/2, G protein-coupled receptor kinase 2 (GRK2), KRas and Rab5a were examined using real-time BRET.

Results: Mouse INSL5 inhibited forskolin-stimulated cAMP accumulation and activated ERK1/2, p38MAPK, Akt-Ser473, Akt-Thr308 and S6 ribosomal protein (S6RP) more potently than human INSL5. No Ca^{2+} mobilisation was observed. PTX-abolished INSL5-stimulated ERK1/2 signal was rescued by $mG\alpha_{oA}$, $mG\alpha_{oB}$, $mG\alpha_{i2}$ and to a lesser extent by $mG\alpha_{i1}$ and $mG\alpha_{i3}$. RXFP4 interacted with GRK2, β-arrestins 1/2 and Rab5a but dissociated from KRas.

Discussion: INSL5 negatively regulates cAMP production and activates multiple signalling pathways important for diverse cellular functions including growth, differentiation and proliferation (ERK1/2, p38MAPK, Akt) and protein synthesis (S6RP). Following INSL5 activation, RXFP4 recruits a variety of $G\alpha_{i/o}$ and is regulated by β-arrestin 1/2 and GRK2 leading to receptor internalisation. Information on

signalling pathways activated by INSL5 at RXFP4 is essential for understanding the biological roles of this novel gut hormone.

Reference

[1] Grosse, et al. Proc Natl Acad Sci U S a 2014;111:33–8.

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Insights into the trajectory of neuronal projections to brown adipose tissue derived from the use of novel ''brainbow'' neurotropic viruses

Brian Oldfield*, Nicole Weidmann, Aneta Stefanidis

Monash University, Clayton, VIC, Australia

The medullary raphe nuclei are regarded as the final common neural relay connecting higher brain centres to the spinal cord and sympathetic outflow to brown adipose tissue (BAT), an observation which is based on functional (electrpophysiological) data. In order to define the trajectory of populations of neurons in the hypothalamus, synaptically linked to the medullary raphe nuclei and ultimately BAT, a novel approach was utilised which involves the injection of a modified form of pseudorabies virus (PRV) into the BAT. This changes the colour of its fluorescent reporter when it comes into contact with Cre recombinase. We hypothesise that contrary to the dogma stated at the outset there will be two populations of neurons projecting to BAT, one which passes through the midline raphe and the other that involves alternate premotor pathways in the brainstem.

An (AAV)-Cre recombinase construct was injected stereotaxically into the raphe nuclei of male Sprague Dawley rats weighing between 230 and 250 g. This injection was followed 2 weeks later by injection of PRV Brainbow virus (PRV-263) into the interscapular BAT. After 4 days survival, rats were killed and their brains prepared for histological analyses.

PRV-263 which is replication competent was transported retrogradely from the BAT through chains of synaptically-connected neurons in the spinal cord, brainstem and hypothalamus including the midline raphe. After transport through neurons expressing Cre recombinase in the raphe there was recombination of the viral genome at either paired lox2272 or loxP sites, resulting in the loss of the red reporter and expression of either cyan (mCerulean) or yellow (eYFP). Importantly there were distinct groups of neurons in the rostroventrolateral medulla, lateral hypothalamus and paraventricular nucleus that retained their red fluorescent reporter consistent with a trajectory other than through the raphe nuclei.

These data define the nature of descending neural projections to BAT.

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Oleoylethanolamine and endocannabinoid responses to intraduodenal lipid infusion in humans: Relationships with BMI and energy intake

Tanya J. Little[1,*], Nada Cvijanovic[1], Donovan Argueta[2], Jaspreet Kaur[2], Christine Feinle-Bisset[1], Richard Young[1], Chris Rayner[1], Nicholas DiPatrizio[2]

[1] *University of Adelaide, Adelaide, SA, Australia*
[2] *Biomedical Sciences, University of California Riverside, Riverside, USA*

Introduction/aims: Fat stimulates intestinal secretion of oleoylethanolamide (OEA) to reduce food intake, while fasting induces intestinal endocannabinoids with an orexigenic effect. Animal studies suggest that high-fat diet-induced obesity impairs intestinal control of endocannabinoid and OEA production, contributing to reduced satiety and weight gain. The aims of this study were to: (i) evaluate effects of intraduodenal (ID) lipid infusion on plasma levels of anandamide (AEA), 2-arachidonylglycerol (2-AG) and OEA in humans, and to examine relationships with BMI and *ad libitum* energy and fat intakes, and (ii) to evaluate effects of ID lipid on duodenal concentrations of 2-AG, AEA and OEA.

Methods: 19 lean, 16 overweight and 17 obese participants underwent ID Intralipid® infusion (2 kcal/min) for 120 min during which blood samples were collected every 30 min. *Ad libitum* energy intake was assessed at a subsequent buffet meal. Endoscopic duodenal biopsies were collected from 4 lean participants, at baseline, and following 30 min ID Intralipid® infusion (2 kcal/min). Plasma and duodenal 2-AG, AEA and OEA concentrations were assessed by HPLC/tandem mass spectrometry