Workshop

All roads take to the brain
- neural control of energy homeostasis in health and disease

September 21-26, 2014
Centro Stefano Franscini
Ascona
Switzerland
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Sponsors

We thank the following institutions and corporations for their generous support:
# Program Summary

**Sunday, September 21**

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# Monday, September 22

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<td>Sex differences in the physiology of eating <strong>Nori Geary</strong></td>
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<td>Jejunal satiation in a rat model of Roux-en-Y gastric bypass surgery (RYGB) <strong>Lori Asarian</strong></td>
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<td>All roads take to the brain, but estrogens facilitate getting there correctly <strong>Debbie Clegg</strong></td>
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<td><em>Chair: Nori Geary</em></td>
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<td>Brain-derived neurotrophic factor mediates estrogenic reduction of dietary obesity in female rats <strong>Min Liu</strong></td>
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<td>Maternal obesity: influences on offspring development <strong>Kellie Tamashiro</strong></td>
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<td>Developmental changes in the dopamine system link maternal infection to reward deficits <strong>Urs Meyer</strong></td>
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<td><strong>ILLNESS ANOREXIA / CANCER CACHEXIA-ANOREXIA</strong></td>
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<td>The TTGG-b cytokine MIC-1/GDF15, a physiological regulator of energy homeostasis and a mediator of cancer anorexia/cachexia <strong>Sam Breit</strong></td>
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<td>Serotonin and beyond: brain mechanisms of disease-associated anorexia <strong>Alessandro Laviano</strong></td>
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<td>Central targets for the treatment of cancer anorexia <strong>Thomas Riediger</strong></td>
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<td>Mountain roads can lead to hypoxia, and metabolic dysregulation <strong>Biff Palmer</strong></td>
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<td>What is the role of GLP-1 in regulating appetite in humans <em>[Christoph Beglinger]</em></td>
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<td>Role of GLP-1 in bypass surgery <em>[Jens Holst]</em></td>
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<td>Peripheral GLP-1 and satiation <em>[Wolfgang Langhans]</em></td>
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<td>09:30 – 10:00</td>
<td>PPG neurons in the mouse NTS: an important relay for visceral and central satiety signals <em>[Stefan Trapp]</em></td>
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<td><strong>Chair: Jens Holst</strong></td>
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<td>A possible CNS mechanism mediating GLP-1R agonist effects on eating <em>[Shin Lee]</em></td>
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<td>GLP-1 receptor stimulation of the lateral parabrachial nucleus reduces food intake: neuroanatomical, electrophysiological and behavioral evidence <em>[Karolina Skibicka]</em></td>
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<td>GLP-1 receptor signaling in the mesolimbic reward system regulates palatable food intake by modulating glutamate-AMPA/kainate signaling <em>[Matt Hayes]</em></td>
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<td>GLP-1 signaling in the hippocampus: a novel site and mechanism for food intake reduction <em>[Scott Kanoski]</em></td>
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<td>Alterations in energy expenditure (EE) in Roux-en-Y gastric bypass (RYGB) rats <em>[Thomas Lutz]</em></td>
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<td>08:00 – 08:30</td>
<td>Bitter transduction: from tongue to brain <em>Wolfgang Meyerhof</em></td>
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<td>08:30 – 09:00</td>
<td>Behaviorally discerning the functional role of gustatory cortex in a rat model <em>Alan Spector</em></td>
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<td>Selected dietary fatty acids and amino acids have potent effects on upper GI function, energy intake and glycaemic control in humans <em>Christine Feinle-Bisset</em></td>
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<td>Circumventricular organs as sensors and integrators of circulating signals controlling feeding and drinking <em>Al Ferguson</em></td>
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<td><em>Chair: Harvey Grill</em></td>
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<td>10:30 – 11:00</td>
<td>Activation of central vagal afferent endings: a putative mechanism for control of food intake by hindbrain melanocortin-4 receptors <em>Bob Ritter</em></td>
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<td>11:00 – 11:30</td>
<td>Orphan G-Protein-coupled receptors and energy homeostasis <em>Rick Samson &amp; Gina Yosten</em></td>
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<td>Nutrient metabolite receptology – signals from gut to brain and body <em>Thue Schwartz</em></td>
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<td>Low calorie sweeteners and weight management -- new data, new perspectives <em>Danielle Greenberg</em></td>
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<td>The possible role of enterocyte fatty acid oxidation in the control of eating</td>
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<td>Nutrient control of energy homeostasis via gut-brain neural circuits</td>
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<td>The gut brain GLP-1 dependent axis and the control of glucose homeostasis: pharmacological implications</td>
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<td>Apo-AIV as the third incretin</td>
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<td>β-Mercaptoacetate antagonizes fatty acid-induced secretion of insulin and GLP-1 in vivo and in vitro</td>
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<td>Hypothalamic fatty acid sensing and the regulation of energy and glucose homeostasis</td>
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<td>ENERGY AND NUTRIENT HOMEOSTASIS</td>
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<td>Reduced thermogenesis counteracts weight loss: a role for leptin</td>
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<td>Role of Golgi-associated GTPases for lipid storage</td>
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<td>The role of the endocannabinoid system in the hypothalamic control of energy balance and its interaction with the mTOR signaling pathway</td>
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<td>Does energy balance determine protein-induced energy-intake and energy-expenditure?</td>
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<td>Histone deacetylase-5 mediates hypothalamic leptin action</td>
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<td>Hypothalamic-hindbrain interaction in glycemia control</td>
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<td>The NTS is an intake inhibitory processing hub</td>
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<td>Brain insulin and novel treatments of obesity</td>
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<td><strong>HEDONICS / REWARD</strong>&lt;br&gt;&lt;em&gt;Chair: Alan Spector&lt;/em&gt;&lt;br&gt;08:00 – 08:30 Food addiction: fact or fiction &lt;em&gt;Stephen Benoit&lt;/em&gt;&lt;br&gt;08:30 – 09:00 Effects of long term exendin-4 on hypothalamic and reward signaling gene expression &lt;em&gt;Tim Moran&lt;/em&gt;&lt;br&gt;09:00 – 09:30 Satiety reduces food-seeking in a mesolimbic dopamine-dependent conditioned approach task &lt;em&gt;Saleem Nicola&lt;/em&gt;&lt;br&gt;09:30 – 10:00 Galanin and leptin action in nutrient reward &lt;em&gt;Heike Münzberg-Grüning&lt;/em&gt;</td>
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<td><strong>CGRP-FAMILY PEPTIDES AND FOOD INTAKE (Joint Session with CGRP Meeting)</strong>&lt;br&gt;&lt;em&gt;Chair: Tim Moran&lt;/em&gt;&lt;br&gt;10:30 – 11:00 Role of the hindbrain in the eating inhibition by amylin &lt;em&gt;Thomas Lutz&lt;/em&gt;&lt;br&gt;11:00 – 11:30 Mechanism of amylin as a leptin sensitizer &lt;em&gt;Barry Levin&lt;/em&gt;&lt;br&gt;11:30 – 12:00 Role of parabrachial CGRP-expressing neurons in mediating anorexia and aversive events &lt;em&gt;Richard Palmiter&lt;/em&gt;&lt;br&gt;12:00 – 12:30 Amylin acts in the mesolimbic reward system to control food intake &lt;em&gt;Matt Hayes&lt;/em&gt;</td>
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<td>Concluding remarks &lt;em&gt;Thomas Lutz&lt;/em&gt;</td>
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Oral presentations

(in alphabetical order by presenting author)
REDUCED THERMOGENESIS COUNTERACTS WEIGHT LOSS: A ROLE FOR LEPTIN

R Adan*, R Pandit

Dept. Translational Neuroscience, Brain Center Rudolf Magnus, UMCU, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands, *email: r.a.h.adan@umcutrecht.nl

Exposure of rats to a choice diet of chow, sucrose water and saturated fat (fCHFS) rapidly results in obesity and leptin resistance. We use withdrawal from such a diet (by giving rats ad lib access to chow after being 4 weeks on the fCHFS diet) as a weight loss model to study physiological responses to dieting. We find these rats to be more energy efficient by reducing thermogenesis in an attempt to protect a higher level of body adiposity. Although these rats remain leptin resistant, administration of leptin increases thermogenesis. This suggests a dissociation of leptin sensitivity in neural circuits controlling food intake and thermogenesis which contributes to the difficulty to lose weight. We hypothesize the dorsomedial hypothalamus as one of the brain nuclei connecting energy balance to regulation of thermogenesis.
JEJUNAL SATIATION IN A RAT MODEL OF ROUX-EN-Y GASTRIC-BYPASS SURGERY (RYGB)

T Bächler¹,², N Geary³, M Bueter²,⁴, TA Lutz¹,⁴, L Asarian¹,⁴*

¹Inst of Vet Physiol, Univ Zürich (UZH), CH-8057 Zürich; ²Dept Surgery, UZH Hospital, CH-8091 Zürich; ³CH-8603 Schwerzenbach; ⁴Zurich Center for Integrative Human Physiol, UZH, CH-8057 Zürich; *email: lasarian@vetphys.uzh.ch

RYGB is an effective and increasingly common treatment for obesity. RYGB decreases meal size and selection of sweets, fats and high-calorie foods. Changes in the pre-absorptive satiating actions of ingested food due to the surgical re-arrangement of the gastrointestinal tract are thought to contribute importantly to these changes in eating. The contributions of delivery of food to the jejunum per se and of adaptation to such delivery in RYGB, however, remain unclear. To investigate this issue, we implanted chronic intra-jejunal (IJ) catheters that ended ~5 cm distal to the gastro-jejunal anastomosis in female RYGB rats and at an equivalent jejunal location in female sham-operated rats and tested the effects of IJ lipid infusions on eating. Rats were maintained on Ensure liquid diet before and after surgery. For tests, rats were food deprived for 4 h during the light phase, placed in infusion cages, received IJ infusions of 20% Intralipid (IL; 0.44 mL/min; 10 min) or saline, returned to their home cages, and offered a non-caloric saccharine-flavored gelatin (NCG) for 2 h. NCG was used to minimize the satiating effects of ingested nutrients. IL infusion reduced NCG intake in RYGB, but not in sham rats, with the saline vs. IL difference between surgical groups statistically significant. These data show for the first time that the satiating action of food in the jejunum is increased by RYGB. Next, to investigate the contributions of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) to this effect, rats were pretreated with the Ccka receptor antagonist devazepide or the GLP-1 receptor antagonist exendin(9-39) before IL infusions. Both devazepide and exendin(9-39) reversed the satiating effect of IJ IL significantly more in RYGB rats than in sham rats. These data indicate that endogenous CCK and endogenous GLP-1 satiation are increased by RYGB. The GLP-1 data extend reports that RYGB leads to (i) jejunal hypertrophy with an increased number of L-cells and (ii) increased basal and prandial GLP-1 secretion by showing for the first time that the contribution of endogenous GLP-1 to the satiating action of food in the jejunum is increased by RYGB. The CCK data similarly extend reports that RYGB increases prandial CCK secretion in human patients and should increase attention to this peptide in the therapeutic effect of RYGB.

Supported by NIH grant DK 092608.
Diabetes mellitus is a proinflammatory condition that is associated with cognitive impairments. Besides being a risk factor for Alzheimer’s disease and multi-infarct dementia, diabetes is also associated with a type of cognitive decline noted for particularly affecting executive function. One mechanism by which diabetes could lead to cognitive decline is because of the associated disruption in the blood-brain barrier (BBB), which has been documented in both humans and in streptozotocin-induced diabetes in animals. Our work has shown that loss of pericytes underlies the BBB disruption of diabetes. Pericytes are pluripotent cells in physical contact with brain endothelial cells (BECs), with the BECs forming the BBB. Pericytes secrete immune active substances, including cytokines, chemokines, and nitric oxide, both constitutively and in response to stimulation with lipopolysaccharide. Pericytes and endothelial cells are in crosstalk with one another so that pericytes induce barrier functions in BECs, induce transport of insulin across monolayer cultures of BECs, and enhance lipopolysaccharide-stimulated transport of the AIDS virus (HIV-1) across the BBB. Evidence further suggests that BEC secretions affect the cytokine secretion pattern of pericytes. Loss of pericytes in diabetes likely occurs because of oxidative stress arising from the excess mitochondrial respiration that occurs with high glucose levels. This increased respiration can be curtailed by inhibiting mitochondrial carbonic anhydrases (mCAs). The mCAs are responsible for the production by the mitochondria of bicarbonate, a necessary ingredient for mitochondrial metabolism of pyruvate. Inhibition of mCAs with topiramate decreases oxidative stress, preserves pericytes in the face of high glucose, and prevents disruption of the BBB. In conclusion, these studies illustrate the importance of pericytes and their crosstalk with BECs in preserving BBB integrity in diabetes.
WHAT IS THE ROLE OF GLP-1 IN REGULATING APPETITE IN HUMANS?

C Beglinger

University Hospital, Basel, Switzerland, email: beglinger@tmr.ch

Is glucagon-like peptide a physiological regulator of appetite? Intravenous infusion of physiological doses of GLP-1 reduce meal size in the absence of side effects in normal-weight men; pharmacological doses affect appetite both in normal weight persons and in patients with type 2 diabetes mellitus. In a recent study we demonstrated that GLP-1R blockade increased appetite, but not meal size. The receptor blockade also led to unusually high levels of glucagon and PYY(3-36) concentrations; these increases may have interfered with antagonist’s desatiating effect. The results were confirmed by another group. Thus, the hypothesis that GLP-1’s status is a physiological satiation signal is supported by most physiological-dose studies, but not antagonist studies.
The concept of "food addiction" has gained much popular attention in the last several years. Addiction psychiatrists, however, remain divided over whether or not food or ingestive behavior can be classified as "addictive." This talk will briefly review the hallmarks of addiction and addictive-like behavior, comparing intake of food to intake of known drugs of abuse. It will emphasize the significance of learned environmental cues and important effects on the meso-limbic dopamine system. Finally, the talk will pose the question of whether "food addiction" is a useful construct for research and/or clinical interventions and offer a tentative solution for discussing the issue with patients.
Reduced food intake is crucial for at least the initial body weight loss and improvement of glycemic control after gastric bypass surgery in humans. This was demonstrated in several recent studies providing the very low calorie diet typically consumed after bypass surgery to non-surgical control subjects for 1-3 weeks and finding similar improvements in insulin sensitivity and glycemic control. While the limited available data in humans show that food intake remains suppressed for up to 10 years after surgery, food intake in rodents returns to pre-surgical levels after about one month, even though body weight and adiposity remain suppressed for much longer. One implication is that rodents rely more on fecal energy loss and/or increased energy expenditure to maintain the lower body weight after surgery. The other implication is that some mechanism prevents rodents from eating above pre-surgical levels to return to their preferred pre-surgical body weight/adiposity. It is now clear rodents with gastric bypass surgery can easily double their energy intake and accumulate large fat depots if appropriately challenged, refuting the notion that there is a physical limit of energy intake and weight gain. We have thus been asking the question: what is the mechanism keeping food intake down? The most popular hypotheses that the greatly increased postprandial levels of the two anorectic L-cell hormones GLP-1 and/or PYY(3-36) act either directly or via vagal afferents on the brain to suppress appetite have not been confirmed in recent studies with RYGB using GLP-1 receptor deficient mice and pharmacological antagonists, suggesting that other mechanisms contribute. One such mechanism may be aversive conditioning during the early postsurgical period that may lead to plastic changes in brain anorexia circuits in the lateral parabrachial nucleus (LPBN) and lateral subdivision of the central amygdala (CEL). Specifically, we have found exaggerated meal-induced activation of calcitonin-gene related peptide expressing neurons in the LPBN in mice 10 days after RYGB as compared with sham-operated mice. Similarly exaggerated activation in the NTS and CEL suggest that a brainstem to forebrain anorexia circuit may be crucial for appetite suppression after RYGB. We are currently testing this hypothesis using pharmacological and pharmacogenetic approaches for the manipulation of specific components along this anorexia circuit. The output of this anorexia pathway may produce changes in appetite by engaging executive, reward, and homeostatic systems and may be a new target for the development of anti-obesity treatments.
MECHANISMS OF INFLAMMATION AND TUMOR INDUCED ANOREXIA-CACHEXIA

A. Blomqvist

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While anorexia-cachexia is a common and serious sequel of chronic inflammatory diseases and cancer, the central mechanisms underlying this syndrome remain obscure. Here we examined in mice the role of prostaglandin and cytokine signaling for food intake and body weight development following peripheral inflammation and tumor transplantation, and we screened for proteins that are differentially expressed in the hypothalamus in tumor bearing anorexic mice and pair-fed controls. Whereas anorexia after peripheral immune challenge with lipopolysaccharide was abolished by inhibition of cyclooxygenase (Cox)-2, but not by inhibition of Cox-1, anorexia in a tumor model (MCG101) was unaffected by Cox-2 inhibition, but attenuated by both unselective Cox-inhibition and selective Cox-1 inhibition. In neither case, however, induced PGE$_2$ production seemed to be involved, because mice with deletion of the PGE$_2$-synthesizing enzyme mPGES-1 displayed normal anorexia in response both to peripheral inflammation and cancer, and deletion of EP-receptors was also without effect. Deletion of myeloid differentiation primary response gene 88 (MyD88), an adaptor protein critical for Toll-like and IL-1 receptor family signaling which has been shown to be involved in inflammation-induced anorexia, attenuated tumor induced anorexia. Using an irradiation-transplantation strategy, we found that MyD88-knockout mice challenged with peripheral injection of lipopolysaccharide displayed anorexia when transplanted with wild-type bone marrow cells, whereas MyD88-knockout mice transplanted with knock-out bone marrow showed normal food intake. Mice with a targeted deletion of MyD88 in hematopoietic or myeloid cells were largely protected against lipopolysaccharide-induced anorexia and displayed attenuated weight loss, whereas mice with MyD88 deletion in hepatocytes or in neural cells or the cerebrovascular endothelium developed anorexia and weight loss of similar magnitude as wild-type mice. In tumor-bearing mice, deletion of MyD88 in hematopoietic cells attenuated the anorexia and protected against body weight loss. These findings demonstrate that MyD88-dependent signaling within the brain is not required for eliciting inflammation-induced anorexia, and identify instead MyD88 signaling in hematopoietic/myeloid cells as a critical component for acute inflammatory-driven anorexia, as well as for chronic anorexia and weight loss associated with malignant disease. Using an unbiased proteomic screening of the hypothalamus, based on two-dimensional gel electrophoresis and liquid chromatography-tandem mass spectrometry, we found that dynamin 1, which is required for the internalization of e.g. the melanocortin 4 receptor was specifically changed in tumor-bearing mice with anorexia. Immunohistochemical data show that dynamin 1 is specifically expressed in the paraventricular nucleus of the hypothalamus upon peripheral immune stimulation, and that its expression is linked to the presence of melanocortin 4 receptors, suggesting that tumor-induced anorexia involvesdynamin 1 regulated melanocortin signaling.
THE TGF-B CYTOKINE MIC-1/GDF15, A PHYSIOLOGICAL REGULATOR OF ENERGY HOMEOSTASIS AND A MEDIATOR OF CANCER ANOREXIA/CACHEXIA.

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MIC-1/GDF15 is present in the circulation of all people and levels can increase markedly when overexpressed in injury, inflammation and cancer. With many malignancies, serum levels can increase 10-100 fold leading to cancer cachexia, which in mice can be reversed by monoclonal anti-MIC-1/GDF15 antibodies. Transgenic overexpression or systemic administration of recombinant protein causes weight loss due to reduction in fat and lean mass, and over time, cachexia. Studies of energy expenditure and pair feeding indicate the weight loss is mediated solely by reduced food intake and is centrally mediated. Intracerebroventricular injection of recombinant protein into the lateral ventricle or stereotaxic injection of adeno-associated virus (AAV) expressing MIC-1/GDF15 into the ARC, recapitulates the anorexia and body weight changes seen with systemic administration.

In mice systemic administration of MIC-1/GDF15 leads to rapid activation of neurons in the Arcuate (ARC) and paraventricular (PVN) nuclei of the hypothalamus and brainstem area postrema (AP) and nucleus of the solitary tract (NTS), which in the ARC is associated with decreased NPY and increased POMC expression. To help understand the relative importance of the hypothalamic and brainstem nuclei in the anorexigenic actions of MIC-1/GDF15, we have selectively lesioned brainstem AP/NTS nuclei. Mice who have been lesioned in this way did not reduce their food intake or loose any body weight being completely resistant to MIC-1/GDF15’s anorexigenic actions.

To determine if MIC-1/GDF15 may also participate in physiological regulation of food intake, we have studied MIC-1/GDF15 KO (MIC-1⁻/⁻) mice. These mice, on average are heavier, have a greater fat mass and eat more than syngeneic control mice, with the phenotype being greater in female than male mice. Further this phenotype could be reversed by infusing sufficient MIC-1/GDF15 protein to raise its serum levels into the normal human range.

These studies suggested that disease-associated anorexia/cachexia occurs because of the subversion of a physiological pathway of energy homeostasis, in which circulating MIC-1/GDF15 acts on hypothalamic and brainstem nuclei involved in regulation of energy homeostasis. The precise nature of the neural pathways mediating these actions is still to be elucidated.
GLP-1 ON THE CONTROL OF THE GUT-BRAIN AXIS: PHARMACOLOGICAL IMPLICATIONS.

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GLP-1 controls glucose homeostasis through mechanisms involving endocrine and neural systems. Hence, the treatment of type 2 diabetes with GLP-1 based therapies such as DPP4 inhibitors and GLP-1 receptor agonists should involve differently the endocrine and neural routes. Data from our laboratory and others show that sitagliptin, a DPP4 inhibitor, triggers the gut brain GLP-1 dependent axis through mechanism involving the vagus nerve firing rate whereas data from others demonstrate the direct role of pharmacological doses of GLP-1 receptor agonist on the beta cell GLP-1 receptor for the glycemic control. Inhibiting intestinal DPP4 was sufficient to improve the glycemic control which demonstrates the importance of the enteric GLP-1 signal on glucose metabolism. Interestingly, a first and second phase neural activation are observed in response to enteric GLP-1 receptor activation. Low dose of liraglutide, a long term GLP-1 receptor agonist, also involve similar neural profiles in addition to its role on the beta cells. During high-fat diet-induced type 2 diabetes in mice the first phase of GLP-1 induced vagus nerve activation was impaired suggesting a state of glucose unresponsiveness. Importantly, it was restored by a chronic liraglutide treatment suggesting an impact of glucose sensitive system.

The recruitment of the gut brain GLP-1 dependent axis controls peripheral glucose fluxes by setting up muscles for glucose utilization. Conversely, the gut glucose signal could be inhibited by the brain GLP-1 receptors which reduce the gut-glucose signal to favor hepatic glucose deposition and glucose homeostasis through a mechanism requiring brain PKCs and peripheral blood flow changes. Recent data from our laboratory further demonstrate in human the importance of the gut brain GLP-1 dependent axis on the control of GLP-1 dependent features such as glucagon secretion. Hence, regulating the gut-brain to periphery axis favors the control of glucose homeostasis.
Estrogens and estrogen receptor alpha (ERα) are critical regulators of food intake, body fat distribution, and energy expenditure. We found knocking out ERα from the ventral medial nucleus (VMN) resulted in increased body weight in the females as a result of reductions in energy expenditure. Alternatively, we found that reductions in ERα in the arcuate nucleus resulted in increased body weight as a result of increased food intake in females. Here we extend these initial findings and focus on a potential role for estrogens/ERα to regulate inflammation in the brain. Consumption of diets high in fat (HFD) increases the prevalence of obesity and results in inflammation in the central nervous system (CNS). Here we demonstrate estrogens directly, or acting through ERα has neuroprotective, and anti-inflammatory effects.

We observed male mice exposed to HFD show increased hypothalamic inflammation and this is associated with reductions in ERα in the males but not the females. Furthermore, we find that following exposure to the HFD males, but not females, have dysmorphic mitochondria and reductions in mitochondrial number, function, and activity. To test the mechanisms by which the HFD might be altering ERα and mitochondrial function, we used an in vitro approach. In vitro, we took a neuronal cell culture that expressed ERα and exposed these cells to palmitic acid (PA) which induced inflammation, while at the same time we observed a reduction in ERα. To determine if it is estrogens or ERα which protects against the PA-induced inflammation, we pretreated the neuronal cells with estrogens and found that estrogen pre-treatment protects against the inflammation. We then manipulated the level of ERα using a RNAi-mediated knockdown of ERα, and found that this potently promoted PA-induced inflammation, whereas over expression of ERα inhibits it. We further demonstrate that neuronal Era expression is regulated by PGC-1α, and PGC-1α is reduced in neurons as well as in astrocytes in vitro and in vivo following PA-treatment or HFD-feeding in males. We find reductions in PGC-1α lead to down-regulation in ERα which is associated with increased inflammation. To determine if reductions in PGC-1α or ERα is permissive for the induction of inflammation, we knocked down PGC-1α while ‘rescuing’ with over-expression of ERα and found that the critical determinant of modulation of inflammation is ERα.

Our results demonstrate for the first time that Era expression is regulated by HFD/PA-driven reductions in PGC-1α leading to suppression of ERα in the hypothalamus which facilitates CNS inflammation and reductions in mitochondrial activity.
Endocannabinoid signaling through the cannabinoid type-1 (CB1) receptor plays a key role in energy balance regulation. Here, we have investigated the exact function of CB1 receptors within the hypothalamus or in distinct hypothalamic neuronal populations. Viral-mediated deletion of the CB1 receptor gene in the adult mouse hypothalamus (Hyp-CB1-KO) leads to approximately 60% decrease in hypothalamic CB1 receptor mRNA levels. Hyp-CB1-KO mice maintained on standard chow show decreased body weight gain over time, which is associated with increased energy expenditure and thermogenesis, likely due to increased sympathetic nervous system (SNS) activity. Additionally, Hyp-CB1-KO mice are insensitive to the anorectic action of the hormone leptin. These findings therefore suggest that hypothalamic CB1 receptor signaling is a key determinant of energy expenditure and reveal its specific role in conveying the effects of leptin on food intake. To then precisely assess the role of CB1 receptor signaling in specific hypothalamic neuronal populations, we generated mice lacking the CB1 receptor gene specifically in steroidogenic factor 1 (SF1) expressing neurons, which are highly expressed in the hypothalamic ventromedial nucleus (VMN), or in Single minded 1 (Sim1)-positive neurons, which account for the majority of neurons of the hypothalamic paraventricular nucleus (PVN). In chow, conditional deletion of CB1 receptor in SF1-expressing neurons decreases adiposity by increasing sympathetic activity and lipolysis, and facilitates anorexigenic and metabolic effects of leptin. Conversely, under high-fat diet, lack of CB1 receptor in SF1-expressing neurons produces leptin resistance, blunts peripheral use of lipid substrates and increases adiposity. Thus, CB1 receptors in VMN neurons regulate metabolic flexibility and actions of leptin and provide a molecular switch adapting the organism to dietary change. On the other hand, studies carried out in Sim1-CB1-KO and their WT littermates show that CB1 receptors on Sim1-positive neurons do not impact energy balance in chow, but hinder energy expenditure via SNS inhibition during high-fat diet consumption. Overall, these findings imply that the function of the CB1 receptor within the hypothalamus is cell-type and diet-dependent. Finally, recent studies carried out in our laboratory demonstrate that a link exists between the endocannabinoid system and the mammalian target of rapamycin complex 1 (mTORC1) pathway in the hypothalamic regulation of energy balance. In particular, our data reveal that mTORC1 inhibition requires increased hypothalamic endocannabinoid levels and CB1 receptor activation to alter hypothalamic synaptic plasticity and to induce hyperphagia.
Differential Effects of Fatty Acids and Amino Acids on Upper Gastrointestinal Function, Energy Intake and Blood Glucose in Healthy Humans

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Receptors located in the small intestinal mucosa play a key role in sensing the arrival of nutrients, and these nutrient-receptor interactions initiate signals that convey this information to the CNS, activating feedback loops that lead to adjustments in the rate of gastric emptying, the release of gut hormones and the regulation of energy intake and blood glucose. Intraluminal lipid has potent effects on these functions, and previous work, by ourselves and others, has established that the digestion of triglycerides, and the subsequent release of fatty acids with a chain length of ≥12 carbon atoms, is critical for these effects, including stimulation of plasma CCK, PYY and GLP-1, suppression of ghrelin, modulation of antropyloroduodenal pressures, and potent suppression of energy intake. Moreover, we have determined that the magnitudes of both the stimulation of pyloric pressures and increases in plasma CCK are independent determinants of subsequent energy intake in response to lipid.

Dietary protein is the most satiating of the macronutrients and also has blood glucose-regulatory effects. These effects may, at least in part, be mediated by changes in gastrointestinal (GI) motor and hormone functions, although the GI effects of protein appear to be less potent than those of lipid. We have recently evaluated in a series of studies the effects of a number of amino acids, incl. L-leucine and L-tryptophan, on gut functions, energy intake and blood glucose. While both amino acids, when infused intraduodenally in healthy, lean men, significantly suppressed subsequent energy intake, well in excess of their energy content, their patterns of effects on GI functions varied substantially, between each other, as well as in comparison with the fatty acid, lauric acid. L-tryptophan, modestly stimulated plasma CCK, GLP-1 and PYY, as well as tonic and phasic pyloric pressures, while L-leucine stimulated only CCK. In the absence of any effect on GLP-1, L-leucine was associated with a very small stimulation of insulin, but not glucagon, and a small reduction in fasting glucose. Despite small effects on insulin and substantial stimulation of glucagon, L-tryptophan did not affect plasma glucose. Moreover, the GI effects of L-tryptophan and L-leucine were very modest when compared with those of lauric acid. In conclusion, it appears that amino acids vary substantially in their ability to modulate GI functions, and that their potency is less, when compared with fatty acids. Despite these differences, L-tryptophan, L-leucine and lauric acid all have substantial energy intake-suppressant effects, which, thus, appear to be mediated through different mechanisms.
CIRCUMVENTRICULAR ORGANS AS SENSORS AND INTEGRATORS OF CIRCULATING SIGNALS CONTROLLING FEEDING AND DRINKING.

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Central nervous system (CNS) structures involved in the regulation of energy balance gather information from the variety of different peripherally derived signaling molecules that we now believe provide an integrated perspective of energy status of the organism. However, the existence of the blood brain barrier means that the CNS is theoretically unable to directly monitor many of these circulating signals such as adiponectin, amylin, cholecystokinin (CCK), glucose, ghrelin, leptin, and peptide YY (PYY) which do not freely diffuse across this barrier. A number of mechanisms have been suggested to play important roles in facilitating the ability of the CNS to monitor this essential sensory information. My presentation will describe briefly potential roles of vagal afferent signaling and peptide transporters in providing access routes for such information transfer, but will focus primarily on the potential roles of specialized CNS structures which lack the blood brain barrier known as the sensory circumventricular organs (CVOs). In particular I will highlight the complex sensory abilities of single CVO neurons in sensing multiple satiety signals and also describe the efferent projections of these neurons to essential autonomic control centers behind the blood brain barrier.
Recent data obtained in our laboratory revealed that some proteins produced by gut bacteria may influence host appetite as molecular mimetics of the host peptide hormones acting on the host molecular pathways controlling hunger and satiety. This action can be direct, depending on the production rate of such bacterial proteins, and/or indirect, where bacterial proteins act as antigen-mimetics, stimulating production of antibodies cross-reactive with peptide hormones. In this talk, I will present the data showing that the identification of a bacterial antigen-mimetic protein of α-melanocyte-stimulating hormone (α-MSH) may help to shed light on the origin of eating disorders (ED). In fact, previous data indicated that immunoglobulins (Ig) or autoantibodies reactive with α-MSH are involved in regulation of feeding and emotion and that their plasma levels correlate with psychological traits in ED patients, however, the origin of such autoantibodies was unknown. Using proteomics, we identified ClpB heat-shock disaggregation chaperone protein of gut bacteria E. coli as a conformational antigen-mimetic of α-MSH. We showed that ClpB-immunized mice produce anti-ClpB IgG cross-reactive with α-MSH, influencing food intake, body weight, anxiety, and melanocortin receptor 4 signaling. Furthermore, chronic intragastric delivery of E. coli in mice decreased food intake and stimulated formation of ClpB- and α-MSH-reactive antibodies, while ClpB-deficient E.coli did not affect food intake or antibody levels. Finally, we showed that plasma levels of anti-ClpB IgG cross-reactive with α-MSH are increased in patients with anorexia nervosa, bulimia and binge-eating disorder and that the Eating Disorder Inventory-2 scores in ED patients correlate with anti-ClpB IgG and IgM similar to our previous findings for α-MSH autoantibodies. In conclusion, these data support that the bacterial ClpB protein, which is present in several commensal and pathogenic microorganisms, can be responsible for the production of autoantibodies cross-reactive with α-MSH, associated with altered feeding and emotion in humans with ED. Thus, our data suggest that ClpB-expressing gut microorganisms may influence host satiety mechanisms by modulating the melanocortin pathway, and might be involved in the etiology of eating disorders.
SEX DIFFERENCES IN THE PHYSIOLOGY of EATING

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Interest in sex differences in physiology and medicine is increasing; for example, the NIH of the USA is phasing in a requirement to balance sex in cell and animal studies (Clayton & Collins, Nature 509: 282, 2014), similar to the humans-subjects policy announced in 1993. Although markedly more women than men suffer from morbid obesity in the USA and other countries (Flegal, JAMA 303: 235, 2010) and sex differences in physiology apparently contribute this, sex-difference work in our field is relatively neglected. Therefore, this presentation will highlight several examples of sex-specific effects in the physiologies of obesity and eating selected from a recent critical review (Asarian & Geary, Am J Physiol 305: R1215, 2013).

1, Melanocortin signaling: Loss-of-function mutations in MC4R are the commonest monogenic cause of obesity and lead to ~2-fold greater BMI gain in women than in men (Stutzmann, Diabetes 57: 2511, 2008). There is a similar effect in Mc4r⁻/⁻ mice (Sutton, Endocrinol 147: 2186, 2006).

2, Development of the control of eating: The four-core genotype model indicates sex differences in eating are programmed by both genes and gonadal hormones: for example, gonadal females (XX mice, XY mice without Sry) ate more than gonadal males (XY, XX with Sry) (Chen, PloS1 Genet 8: e1002709, 2012).

3, Sweet-taste hedonics: Data in orangutans, baboons and humans indicate that male-female sex-differences in sweet-taste hedonics are likely to contribute to sex differences in total food intake. In humans, the hedonics differences (Hayes & Duffy, Physiol Behav 95: 77, 2008) were related to a prominent sex difference in the density of fungiform taste papillae on the anterior tongue (Bartoshuk, Physiol Behav 56: 1165, 2002).

4, Binge-eating: Female rats were also many fold more prone than males to develop binge-like eating when maintained with limited access to palatable food (Klump, Int J Eat Dis 46: 729, 2013).

5, Effects of estrogens: Eating is inhibited in female animals and women by an activational (i.e., non-developmental) effect of estrogens. The estrogenic inhibition eating in women is expressed as a decrease in food intake in the follicular phase of the ovarian cycle, which we estimate to translate into a difference of ~3000 kcal/month, more than enough to affect body weight. In rats and mice, removal of estrogens by ovariectomy (OVX) leads to hyperphagia and marked increases in adiposity, which can be prevented by estrogen treatment. In rats and mice, estrogens decrease in the eating-stimulatory effect of ghrelin and increase the satiating effects of CCK and GLP-1.

6, Estrogens and CCK: The effect of estrogens on CCK satiation has been investigated most. Endogenous CCK satiation is increased during the peri-ovulatory phase of the rat ovarian cycle and is increased by estradiol treatment in OVX rats. RNAi knockdown of estrogen-receptor-1 (Esr1 or ERα) in the caudal medial nucleus tractus solitarii (cmNTS) of rats completely prevented the ability of a near-physiological estradiol treatment to maintain normal levels of meal size, daily food intake and body weight in ovariectomized rats. Additionally, cmNTS Esr1 knockdown prevented estradiol from increasing the satiating potency of endogenous CCK, as assessed with a specific CCK₄ receptor antagonist.

6, Estrogens and leptin: Knockdown of cmNTS leptin receptors also prevented estradiol’s ability to restrain food-intake and body-weight or to amplify endogenous CCK satiation in OVX rats. Further investigation of sex differences and sex-specific effects in eating should lead to improved sex-specific, physiologically-based therapies for disordered human eating and weight regulation.

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Controversy has existed for decades over the effectiveness of low calorie sweeteners (LCS) as a weight management tool. The replacement of calories from sugar with zero calorie or negligible calorie substitutes provides opportunity for individuals to facilitate weight loss by lowering total caloric intake. Additionally, use of LCS may improve adherence to weight loss or maintenance plans by minimizing feelings of deprivation that may occur from dietary change or restrictions. On the other hand it has been suggested that LCS use may promote weight gain. The scientific literature supporting the concept of weight gain with LCS use is derived from several disciplines; human observational, animal, and invitro studies. Observational cohort studies often demonstrate a positive relationship between LCS consumption and body weight. These findings have generally been interpreted as an indication that LCS consumption could lead to weight gain. However, commonly left unstated is that these prevalent associations may, with similar certainty, be the result of “reverse causality”. Specifically, it is equally likely that obesity tends to result in higher LCS use just as dieting is more commonly self-reported as weight increases. In vitro studies demonstrate that LCS are potent stimulators of sweet taste receptors, being 100 or more times sweeter than sucrose. It has been argued that foods and beverages containing LCS are sweeter and thus lead to a positive shift in hedonic response to sweet. This concept is not, however, consistent with how food manufacturers utilize LCS. Typically LCS is incorporated at less than 1/100th the amount of sucrose with resulting products that are not sweeter but similarly sweet to those sweetened with sucrose. Findings in rodents suggest that LCS could confuse the ability to sense the ingestion of energy-containing sweet foods and thus lead to deregulation of eating with overconsumption of sweet foods. However, the ability of such animal models to predict human behavior remains undefined and controversial.

Recent human research supports the use of LCS in weight management. Two different investigative groups find that weight loss program participants assigned to use low calorie sweetened beverages are more successful with weight loss efforts than those assigned to consume water. A new meta-analysis of randomized controlled trials (n=15) demonstrates that LCS use modestly but significantly reduces: body weight, body mass index, fat mass and waist circumference. Prospective cohort studies (n=9), suggest that LCS intake is not significantly associated with body weight or fat mass. There is however an association in these trials with a small increase in BMI. A new study examining use of LCS from the National Weight Loss Registry confirms that successful weight maintainers use LCS to a greater extent than those who have always been at normal weight. Finally, preliminary findings from NHANES nutritional survey data on the pattern of use of LCS in combination with other sources of calories, indicates that animal conditioning studies suggesting dissociation between sweet taste and caloric content may not be applicable to humans.

Taken together these new findings suggest that LCS are likely to be a beneficial tool for weight loss and weight maintenance. Research is needed to determine if LCS use will help prevent weight gain in those at normal weight.
Obesity is a disease. Effective long-term treatments for the hyperphagia that underlies obesity are, however, currently unavailable. This outcome is likely driven by the fact that the food scarcity that marked human evolutionary history gave rise to a neural control system that insures humans eat when food is available by involving contributions from multiple and redundant neurochemical and signaling pathways. Given this redundancy of energy balance control mechanisms it is likely that effective obesity treatments will require the joint actions on several neurochemical systems and/or actions at nodes of the control system that are common to the processing of multiple food intake inhibitory signals. This presentation makes the case that neurons of the medial nucleus tractus solitarius (mNTS) in the dorsal medulla is a hub for the processing of multiple intake inhibitory signals. Highlighted are data linking mNTS neurons to the processing of four sources of intake inhibitory signals - gastrointestinal vagal afferents reporting gut energy availability, leptin receptors reporting stored energy availability, glucagon like receptor 1 signaling, and oxytocin receptor signaling involving hypothalamic-hindbrain projections. Also highlighted are interactions between the processing of these signals by mNTS neurons that result in the amplification of the intake inhibitory effects of one signal type by the processing of signals from other sources.

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FAT SENSING IN THE SMALL INTESTINE AND SIGNALING TO THE BRAIN

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Several mechanisms exist in the small intestine for sensing the metabolites of dietary fat, i.e. fatty acids and 2-monoacylglycerol. Such mechanisms involve sensors, transducers and responses. Dependent on the responses various sensors may be involved, e.g. the GLP-1 release in humans induced by an oral intake of 20 ml olive oil may involve only GPR119 as sensor, while the accompanying CCK release may involve only a fatty acid sensor. On the other hand, self-administration of an intragastric Intralipid emulsion by trained mice, may involve both GPR119, as a 2-monoacylglycerol sensor, plus a fatty acid sensor, which may be GPR40. Data will be presented for the various sensors/responses.
Glucagon-like peptide-1 receptors (GLP-1R) expressed in both the ventral tegmental area (VTA) and nucleus accumbens (NAc) core are pharmacologically and physiologically relevant for the regulation of palatable food intake. In separate sets of experiments we have begun to demonstrate similar behavioral, molecular and neurophysiological mechanisms mediating the intake suppressive effects following GLP-1R activation in either the NAc core or VTA. Within the VTA, intraparenchymal administration of the GLP-1R agonist exendin-4 (Ex-4) reduces palatable high-fat food intake in rats primarily by reducing meal size; these effects are mediated in part via glutamatergic AMPA/kainate, but not NMDA, receptor signaling. Additional behavioral data indicate that GLP-1R expressed specifically within the VTA can partially mediate the intake- and body weight-suppressive effects of systemically administered Ex-4, offering the intriguing possibility that this receptor population may be clinically relevant for food intake control. Intra-VTA Ex-4 rapidly increased tyrosine hydroxylase levels within the VTA, suggesting that GLP-1R activation modulates VTA dopaminergic signaling. Further evidence for this hypothesis was provided by *ex vivo* electrophysiological data showing that Ex-4 increased the frequency of AMPA-mediated currents and reduced paired-pulse ratio in VTA dopamine neurons. *Within the NAc core* we first investigated whether GLP-1R signaling modulates GABAergic medium spiny neurons (MSNs) through presynaptic glutamatergic and/or dopaminergic signaling to control feeding. *Ex vivo* fast-scan cyclic voltammetry showed that Ex-4 does not alter dopamine release in the NAc core. Rather, similar to our findings in the VTA, support for a glutamatergic mechanism was provided by *ex vivo* electrophysiological analyses showing that Ex-4 activates presynaptic GLP-1Rs in the NAc core to increase the activity of MSNs via a glutamatergic, AMPA/kainate receptor-mediated mechanism, indicated by increased mEPSC frequency and decreased paired pulse ratio. Only a small direct excitatory effect on MSNs by Ex-4 was observed, suggesting that the contribution of postsynaptic GLP-1R in the NAc core to MSN activity is minimal. The behavioral relevance of the electrophysiological data was confirmed by the finding that intra-core injection of the AMPA/kainate receptor antagonist CNQX attenuated the ability of NAc core GLP-1R activation by Ex-4 microinjection to suppress food intake and body weight gain; in contrast, intra-core NMDA receptor blockade by AP-5 did not inhibit the energy balance effects of NAc core Ex-4. Collectively, these experiments offer complementary evidence showing that GLP-1R signaling in the mesolimbic reward system modulates glutamatergic signaling to affect palatable food intake.

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AMYLIN SIGNALING IN THE MESOLIMBIC REWARD SYSTEM REGULATES FOOD INTAKE

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Amylin is a pancreatic-derived hormone that acts centrally to reduce food intake. Although its effects have historically been attributed to the area postrema, recently our lab has shown that the ventral tegmental area (VTA), a nucleus in the mesolimbic reward system, is a pharmacologically and physiologically relevant site of action for amylin-mediated control of energy balance and food reward. These discoveries encourage mechanistic investigations of VTA amylin signaling, as well as the broader assessment of the CNS circuitry mediating amylin’s effects on food intake/reward. The lateral dorsal tegmental area (LDTg) of the brainstem is an amylin binding site that is well-positioned to integrate feeding-related signals. The LDTg receives input from feeding-relevant centers of the hindbrain and forebrain, and sends cholinergic/glutamatergic input to the VTA to modulate rewarding behavior. Given these data, we tested the hypothesis that amylin receptor signaling in the LDTg controls food intake in rats. First, qPCR analyses show that the components of the amylin receptor are expressed in the LDTg, with CTR-A and RAMP1 being the most highly expressed. Unilateral LDTg injection of the amylin receptor agonist salmon calcitonin (sCT; 0.01, 0.04, 0.1μg) dose dependently reduced chow intake and body weight at 24h at doses subthreshold for effect when applied to the ventricle. Meal pattern analyses showed that injection of sCT (0.04μg) in the LDTg predominantly reduced food intake by suppressing meal size. Importantly, intra-LDTg injection of sCT (0.01, 0.04, 0.1μg) did not produce pica (ingestion of kaolin clay), suggesting that the suppression in food intake is not due to nausea/malaise. Ongoing immunohistochemical analyses show that CTR-positive neurons are not expressed on cholinergic neurons (CHAT-positive) in the LDTg, but instead indicate co-localization with GABA-positive neurons using a GAD2-tdTomato mouse. Finally, ongoing analyses are using a novel AAV-shRNA construct to knockdown CTR in the LDTg, thereby assessing the role of endogenous LDTg amylin receptor signaling in the chronic control of energy balance. Rats maintained on chow with reduced LDTg CTR expression gain more body weight and consume more energy over the initial 2 weeks post-AAV injection than AAV-control-treated rats. Together with our evolving analyses of amylin signaling in the VTA, these data identify the LDTg, and nuclei of the mesolimbic reward system as a whole, as physiologically relevant sites-of-action mediating the energy balance effects of amylin receptor signaling. DK096139 (MRH).
ROLE OF GLP-1 IN BYPASS SURGERY

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GLP-1 SIGNALING IN THE HIPPOCAMPUS: A NOVEL SITE AND MECHANISM FOR FOOD INTAKE REDUCTION

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Activation of central GLP-1 receptors (GLP-1Rs) reduces feeding and body weight. The neural circuits and mechanisms mediating these effects are only partially understood. Here we investigate the inhibition of food intake and motivated responding for food in rats following GLP-1R activation in the ventral hippocampal formation (HPFv), a region only recently highlighted in food intake control. Increased HPFv GLP-1R activity following local exendin-4 delivery potently reduced food intake (both chow and Western diet) and body weight, whereas HPFv GLP-1R blockade increased food intake. These hypophagic effects were based on reduced meal size, and likely do not involve nausea as HPFv exendin-4 did not induce a conditioned flavor avoidance. HPFv GLP-1R activation also reduced effort-based responding for food under an operant progressive ratio reinforcement schedule, but did not affect food conditioned place preference expression. To investigate possible routes of HPFv GLP-1 signaling, immunohistochemical analysis revealed the absence of GLP-1 axon terminals in the HPFv, suggesting volume transmission as a mechanism of action. Consistent with this, the presence of active GLP-1 was detected in both the cerebral spinal fluid (CSF) and the HPFv. The source of CSF GLP-1 may be NTS GLP-1-producing neurons, as 1) ~30% of NTS GLP-1 neurons colocalized with the retrograde tracer fluorogold following lateral ventricle fluorogold injection, and 2) GLP-1-immunoreactive axon terminals were observed adjacent to the ventricular ependymal layer. Collectively these findings illuminate novel neuronal and behavioral mechanisms mediating food intake reduction by GLP-1.
PERIPHERAL GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AND SATIATION

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Enteroendocrine L-cells release GLP-1 in response to luminal nutrient (primarily carbohydrate and fat) stimulation. Peripheral and central administration of GLP-1 inhibit eating, and peripheral GLP-1 inhibits gastric emptying and enhances glucose-induced insulin release. GLP-1 receptors (GLP-1R) are expressed in the periphery and in several brain areas implicated in eating control, but the sites and mechanisms through which intestinal endogenous or peripherally administered exogenous GLP-1 inhibit eating are uncertain.

I will present findings indicating that intestinal endogenous GLP-1 released during and after a meal inhibits eating primarily via a paracrine effect on vagal afferents originating in the small intestine. Peripherally administered exogenous GLP-1 inhibits eating by recruiting at least two different pathways: (1) after intravenous infusion GLP-1 activates hindbrain GLP-1R, presumably in the area postrema (AP). This mechanism may mimic situations in which the circulating levels of endogenous GLP-1 are markedly elevated, such as after bariatric surgery or pharmacological treatments with GLP-1R agonists or DPP-IV inhibitors. (2) In contrast, after IP administration GLP-1 appears to activate intestinal vagal afferent GLP-1R to inhibit eating, mimicking the effect of intestinal endogenous GLP-1 in response to a normal meal. Both mechanisms are clinically relevant, but it remains to be investigated whether they recruit the same central nervous system circuitries to inhibit eating.
Appetite is frequently affected in patients suffering from acute and chronic diseases, leading to anorexia and consequently insufficient food intake. Consistent experimental data indicate that hypothalamic serotonergic activity is impaired during disease and contributes to anorexia development. Most of the results pointing to a role for serotonin in disease-associated anorexia have been obtained in cancer models, although the serotonergic mechanism appears to operate in other clinical conditions as well.

Initially, the role of increased serotonergic activity in disease-associated anorexia has been inferred by clinical data reporting higher blood levels of the precursor of serotonin, the amino acid tryptophan (TRP), which suggested increased brain serotonin synthesis and activity. Similarly, therapeutic strategies aiming at reducing brain TRP entry yielded reduced anorexia and improved food intake in patients suffering from cancer, liver cirrhosis, and uremia. More recently, new analytic techniques allowed a better understanding of the molecular mechanisms linking deranged hypothalamic serotonin levels and disease-associated anorexia.

We recently reported the hypothalamic gene expression profile of a cancer cachectic mouse model with increased food intake. In this model, mice bearing C26 tumour have an increased food intake subsequently to the loss of body weight. We hypothesized that in this model, appetite-regulating systems in the hypothalamus, which apparently fail in anorexia, are still able to adapt adequately to changes in energy balance. By applying transcriptomics, many appetite-regulating systems in the hypothalamus were assessed, which provided an overview of changes that occur in the hypothalamus during tumour growth. In this model, food intake increased significantly in cachectic tumour-bearing mice, synchronously to the loss of body weight. Hypothalamic gene expression of orexigenic neuropeptides NPY and AgRP was higher, whereas expression of anorexigenic genes CCK and POMC were lower in tumour-bearing compared to controls. In addition, serotonin and dopamine signalling pathways were found to be significantly altered in tumour-bearing mice. Serotonin levels in brain showed to be lower in tumour-bearing mice compared to control mice, while dopamine levels did not change. Moreover, serotonin levels inversely correlated with food intake. Therefore, transcriptomic analysis of the hypothalamus of cachectic tumour-bearing mice with an increased food intake showed changes in NPY, AgRP and serotonin signalling. Serotonin levels in the brain showed to correlate with changes in food intake.

More recently, we tried to further define the role of hypothalamic serotonin on the pathogenesis of disease-associated anorexia. By using cancer models with opposite impact on food intake, we showed that the NPY system appears to fail to respond to changes in energy balance during tumour growth. Furthermore we showed that this failure might be mediated by changes in serotonin signalling. In particular, two tumour cachectic mouse models with different food intake behaviours were used: a C26-colon adenocarcinoma model with increased food intake and a Lewis lung carcinoma model with decreased food intake. This contrast in food intake behaviour between tumour-bearing mice in response to tumour growth was used to distinguish between processes involved in cachexia and mechanisms important in food intake regulation. Hypothalamus was used for transcriptomics. In C26 tumour-bearing mice, food intake increased, while in mice bearing Lewis lung carcinoma food intake decreased. In both models, hypothalamic gene expression of orexigenic neuropeptides NPY and AgRP was higher compared to controls. Expression of genes involved in serotonin signalling showed to be different between C26 tumour-bearing mice and Lewis lung tumour-bearing mice and were inversely associated with food intake. In vitro, serotonin repressed hypothalamic NPY excretion, while not affecting messenger NPY.

When considered together, these data indicate that serotonin signalling is important in food intake behaviour in cancer cachexia, probably by mediating its inhibitory effect on food intake via affecting the NPY system. Serotonin regulation might therefore be a therapeutic target to prevent the development of cancer-induced eating disorders.
A POTENTIAL CNS MECHANISM MEDIATING GLP-1R AGONIST EFFECTS ON EATING


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GLP-1R agonists are approved for the treatment of type II diabetes, but the CNS mechanisms mediating GLP-1’s effects on eating and glucose homeostasis are still unclear. Our group showed that neuronal activation in the hypothalamic paraventricular nucleus (PVH) may be critical in this context, and that hindbrain dopamine-β-hydroxylase (DBH) neurons are potential mediators of the effects of peripheral GLP-1. In fact, intraperitoneal (IP) injection of the GLP-1 receptor (GLP-1R) agonist exendin-4 (Ex-4) stimulated the HPA axis and increased plasma corticosterone levels, suggesting that GLP-1R activation influences eating and glucose metabolism via HPA axis activation. Here we bilaterally injected anti-DBH saporin into the PVH to selectively eliminate DBH projections to the PVH (DSAP lesion), and measured the effects of this lesion on the HPA axis, eating, and the brain responses to IP Ex-4. IP Ex-4 (1 μg/kg BW) rapidly increased serum corticosterone in controls, and this effect was attenuated in DSAP rats, indicating that effects of IP Ex-4 on corticosterone secretion require intact DBH neuronal projections to the PVH. The acute hyperglycemic effect of IP Ex-4, however, was preserved in DSAP rats. Moreover, the PVH DSAP lesion enhanced the satiating effect of IP Ex-4 (1 μg/kg BW), which was possibly related to the dysregulation of HPA axis activity. Interestingly, the PVH DSAP lesion increased the number of c-Fos expressing cells in the POMC neurons of the hypothalamic arcuate nucleus (Arc), in line with the enhanced satiation induced by IP Ex-4 in these animals. Furthermore, IP Ex-4 down-regulated hindbrain preproglucagon (PPG) mRNA expression in control rats, and this effect was blunted in DSAP rats. The findings raise the possibility that IP Ex-4 controls eating by activating hindbrain catecholamine neurons and the HPA axis, and suppresses hindbrain PPG gene expression as a negative feedback mechanism.
HYPOTHALAMIC FATTY ACID SENSING AND THE REGULATION OF ENERGY AND GLUCOSE HOMEOSTASIS

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Metabolic sensing neurons monitor and regulate peripheral energy and glucose homeostasis by altering their activity in response to changes in ambient concentrations of metabolic substrates and hormones. In the hypothalamus, select neurons monitor levels of long chain fatty acids (LCFA) utilizing CD36, a metabolism-independent receptor that senses altered levels of LCFA transported into the brain from the blood. This CD36-mediated FA sensing is critical for homeostatic regulation. Depletion of ventromedial hypothalamic (VMH) CD36 with AAV CD36 shRNA has little effect on food intake and body weight gain in outbred rats fed a high fat diet. But, although it drives ingested fat into subcutaneous rather than visceral depots, CD36 depletion causes marked insulin resistance. In selectively bred diet-induced obese rats, hypothalamic CD36 depletion causes increased food intake, weight gain, stunted growth, hepatic steatosis and marked insulin resistance. But FA sensing neurons do not act alone. Astrocytes utilize LCFA to produce ketone bodies which alter the ability of hypothalamic neurons to sense LCFA. Intake of a 60% fat diet at dark onset produces a spike of ketone bodies in the VMH which inhibits intake 3-5h later. Blockade of VMH ketogenesis during the first 2h after dark onset high fat intake completely abolishes this delayed feeding reduction. These studies demonstrate a critical role for the interaction between hypothalamic FA sensing neurons and astrocyte ketogenesis in the regulation of energy and glucose homeostasis during intake of a high fat diet.
ROLE OF AMYLIN SIGNALING IN THE ONTOGENY OF THE POSTNATAL DEVELOPMENT, LEPTIN SENSITIVITY AND PROTECTION FROM OBESITY

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Exogenous amylin has previously been shown to increase leptin signaling and sensitivity in the ventromedial hypothalamus (VMH) of rats. We found that amylin acts to increase leptin signaling by stimulating VMH microglia to produce IL-6 which presumably acts through its IL-6Rα/gp130 receptor to synergistically activate and phosphorylate STAT3 (pSTAT3) downstream of the leptin receptor. IL-6 ko mice treated with amylin fail to increase VMH leptin-induced pSTAT3 as amylin-treated wild type mice do. We also found that administering amylin (50µg/kg, 3x/d) from birth (P0)-P16 increased leptin-induced expression of VMH pSTAT3 and corrected the defective arcuate (ARC) to paraventricular (PVN) hypothalamic projections of ARC AgRP and α-MSH of the leptin resistant, selectively bred diet-induced obese (DIO) rat. While the DIO rats lost weight while amylin was administered, this conferred no long-term protection from obesity on high fat diet. Impairing VMH amylin signaling using an AAV expressing shRNA for the Ctr1a component of the amylin receptor complex, caused diet-resistant (DR) rats to become presumptively obese (3x increase in leptin levels) on low fat chow and showed increased body weight gain, carcass adiposity, leptin levels and severe insulin resistance after 5wk on a high fat diet. Collectively, these results lend strong support to the hypothesis that VMH amylin signaling is required for full expression of VMH leptin signaling and sensitivity.
BDNF MEDIATES ESTROGENIC REDUCTION OF DIETARY OBESITY IN FEMALE RATS

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Estrogens, especially estradiol (E2), potently facilitate satiation and energy expenditure, leading to lower body weight in many species, including humans. However, the underlying mechanisms for estrogenic regulation of energy homeostasis are largely unknown. Considerable evidence suggests that E2 exerts its catabolic action indirectly via enhancing the strength of other physiological signals implicated in the control of energy balance. Brain-derived neurotrophic factor (BDNF) is a compelling candidate.

BDNF is widely distributed in adult CNS, with the highest levels in the hippocampus, hypothalamus, amygdala, and brainstem. BDNF binds to tyrosine kinase receptor B (TrkB), promoting development and differentiation of neurons, long-term potentiation, synaptic plasticity, and other actions. More importantly, BDNF is critical in maintaining energy balance by regulating both energy intake and expenditure. Chronic intra-3rd ventricular (i3vt) delivery of BDNF reduces food intake and weight gain. Selective deletion of brain bdnf gene causes a phenotype characterized by hyperphagia, obesity, and increased abdominal white adipose tissue that is significantly more pronounced in females.

Recent studies demonstrated that bdnf gene expression in the VMH varies across the ovarian cycle, being lowest at diestrus, and highest at the estrus phase of the ovarian cycle in intact female rats. OVX significantly decreases VMH bdnf gene expression, and this is reversed by cyclic E2 treatment, suggesting that E2 normally regulates bdnf gene expression in the VMH. i3vt administration of low doses of BDNF reduces food intake to a significantly greater extent in sham-operated estrous rats, as well as OVX rats cyclically treated with E2 than in vehicle-treated OVX rats or male rats, implying that E2 enhances the anorectic effect of BDNF.

Using a cultured rat hypothalamic cell line, we demonstrated that E2 elicited time- and dose-dependent activation of the MAP kinase (MAPK) signaling pathway and the production of BDNF protein, which was significantly attenuated by pretreatment with UO126 (5 \(\mu\)M), an inhibitor of the MAPK signaling pathway. These data imply that the effect of E2 on BDNF protein expression is, at least partially, mediated through non-genomic mechanisms. Interestingly, while UO126 almost completely abolished the activation of MAPK signaling pathway induced by E2, it only attenuated the effect of E2 on BDNF protein production, indicating the involvement of a classical mechanism of E2.

To further understand the mechanisms mediating E2’s action on bdnf gene expression, primary neuronal cells from embryonic rat brainstems were cultured and predesigned rat SRC-1 siRNA (40 nM) and negative control (scrambled) siRNA were transfected into the cells. Treatment with SRC-1 siRNA, but not scrambled siRNA,
ALTERATIONS IN ENERGY EXPENDITURE (EE) IN ROUX-EN-Y GASTRIC BYPASS (RYGB) RATS

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Compared to traditional weight loss strategies, the compensatory decrease in energy expenditure (EE) in response to body weight loss is attenuated after RYGB surgery. Most measures of EE in rodent models of RYGB surgery have been performed at ambient temperatures that are markedly below the thermoneutral zone (TNZ) of rats and mice. Hence, EE in RYGB and sham operated control rats at room temperature is increased due to adaptive thermogenesis, and this may mask potential differences between groups. We therefore speculated that reported alterations in EE of RYGB rats are caused by a shift in the TNZ or by reduced thermal insulation and hence higher thermogenesis. Rats on chow diet underwent RYGB or sham surgery and were ad libitum fed (AL) or weight matched to RYGB (BWM). EE was significantly higher in AL and RYGB compared to BWM rats at all ambient temperatures (22-32°C). In AL and RYGB rats, EE was lowest between 28 and 30°C. AL, but not RYGB rats showed an increase in EE at all other temperatures. The difference in EE between AL and RYGB rats was smaller at thermoneutrality than at room temperature. The TNZ in BWM rats was between 30 and 32°C, and total EE was markedly below that of AL and RYGB rats at all ambient temperatures. In separate experiments, we tested the contribution of elevated glucagon like peptide-1 concentrations to higher EE after RYGB because GLP-1 had been shown to affect EE. However, neither acute increase in GLP-1 signaling by injection of the GLP-1 agonist exendin-4 (5µg/kg) nor acute blockade of GLP-1 receptors with the GLP-1 antagonist exendin-9 (30µg/kg) had an effect on EE in any group of rats. We conclude that the reported alterations in EE of RYGB rats are not due to an artifact introduced by measuring EE below the TNZ; the TNZ of AL and RYGB rats did not differ. Further, an acute increase in GLP-1 signaling does not seem to be responsible for higher EE after RYGB surgery in rats.
Amylin is co-released with insulin from pancreatic beta-cells in response to eating. Amylin has been shown to reduce eating in animals and humans and to reduce body weight when administered chronically. One important site of action for amylin to reduce meal size and total food intake is the area postrema (AP) in the hindbrain. Other sites of amylin action may include the ventral tegmental area and the ventromedial nucleus of the hypothalamus but the interaction between these sites is currently unknown. Within the AP, noradrenergic neurons that project to the lateral parabrachial nucleus mediate amylin’s effect on eating. Amylin sensitive neurons differ from other AP neurons that are sensitive to anorectic (GLP-1) or aversive (LiCl) stimuli because only the former contain the amylin receptor. In fact, we recently identified all necessary components of the amylin receptor (CTR and RAMPs) within single amylin sensitive AP neurons. So far, we found no evidence for reduced amylin secretion or for a malfunction of the amylin signaling system that may explain overeating and ensuing obesity, or for reduced amylin sensitivity in obesity. Rather, amylin seems to sensitize obese rats or humans to the body weight reducing effect of other signals, in particular leptin. Circulating amylin levels are increased after Roux-en-Y gastric bypass (RYGB) but the clinical relevance of this increase is currently unknown. However, because RYGB may lead to abnormally high postprandial glucose excursions including the frequent occurrence of hypoglycemic episodes, the effect of amylin to reduce gastric emptying and glucagon secretion may be of potential clinical benefit; in other words, due to its effect on glucagon secretion, amylin may blunt the sharp rise in postprandial glycemia and hence also reduce the subsequent stimulus for exaggerated insulin release and ensuing hypoglycemia.
THE POSSIBLE ROLE OF ENTEROCYTE FATTY ACID OXIDATION IN THE CONTROL OF EATING

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Fatty acid oxidation (FAO) has been implicated in the control of eating based on studies showing that various FAO inhibitors are able to stimulate eating in laboratory animals and man. The liver was originally proposed as the origin of this eating-stimulatory signal. Several studies, however, failed to establish a direct link between an inhibition of hepatic FAO and a stimulation of eating. This and the fact that the eating-stimulatory effect of the FAO inhibitor mercaptoacetate (MA) critically depends on intact abdominal vagal afferents, prompte hypothesize that FAO in the intestine may generate a signal that affects eating. The small intestine is exposed to all dietary nutrients, needs substantial amounts of energy for nutrient absorption, and its capacity to oxidize fat and produce ketone bodies is well established. Moreover, the vagus and the splanchnic nerves densely innervate the intestine and could therefore link enterocyte FAO to the brain circuitries that control eating. We have shown that pharmacological activation of the peroxisome proliferator-activated receptor alpha (PPAR-α) using the synthetic PPAR-α agonist Wy-14643 or the endogenous PPAR-α ligand oleylethanolamide (OEA) reduced food intake in adult male rats fed high-fat diet mainly through an increase in the latency to eat. These effects were related to an increase in intestinal rather than hepatic FAO. Also, Wy-14643 and OEA increased circulating ketone bodies and the expression of key ketogenic enzymes specifically in the jejunum but not in the liver. These findings support the hypothesis that intestinal FAO and ketogenesis might play a role in the control of eating.
DEVELOPMENTAL CHANGES IN THE DOPAMINE SYSTEM LINK MATERNAL INFECTION TO REWARD DEFICITS

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Maternal infection during pregnancy is an early-life environmental risk factor for long-term brain and behavioral dysfunctions in the offspring. Many of the prenatal infection-induced functional brain abnormalities appear to be closely associated with imbalances in the central dopamine system, which in turn plays a pivotal role in reward processing. Against these backgrounds, we explored whether prenatal infection might link developmental changes in the dopamine system to the emergence of deficient reward processing. We used a mouse model of prenatal viral-like immune activation that is based on maternal gestational treatment with poly(I:C), a synthetic analog of virus-specific double-stranded RNA. Longitudinal neuroanatomical investigations revealed that prenatal immune challenge increased striatal levels of dopamine receptor 2 (D2R) in pubescence, but reduced D2R levels in adulthood. Adult offspring born to immune-challenged offspring further displayed decreased D1R and dopamine transporter contents in striatal areas. Compared to adult control offspring, immune-exposed offspring also showed impaired motivation to run for food and reduced conditioned place-preference for sucrose, suggesting decreased sensitivity to food reward in approach-based and associative test conditions. On the other hand, they demonstrated greater consumption of palatable food in an ad-libitum free-access home-cage test and displayed higher propensity to diet-induced obesity. Strikingly, these effects were almost exclusively restricted to female offspring, suggesting that the female sex is more vulnerable than the male sex in terms of prenatal infection-induced abnormalities in central reward processing. Our data suggest that prenatal viral-like infection (sex-dependently) changes the trajectories of dopaminergic development and precipitates long-term deficits in reward processing.
Taste is a major factor of food selection. While sweet and umami taste check for the presence of calories, salty taste evaluates the presence of electrolytes. All three tastes mediate attraction and drive intake of food containing proteins, amino acids, carbohydrates and salts. In marked contrast, bitter and sour tastes detect potential toxicants and acids, respectively, and mediate repulsion, limiting the intake of contaminated or spoiled food that could harm. Each quality is represented by a cognate population of chemoreceptor cells in the mouth dedicated to recognize tastants of only that quality. The receptor molecules expressed not only determine the molecular receptive ranges of these populations but define subpopulations as well. Whereas mammals appear to possess only one or very, very few receptors for sweet and umami, they possess ~30 bitter taste receptors. This results in comparatively homogenous populations of receptor cells for sweet and umami tastes. In contrast, the numerous bitter receptors are expressed in different subsets by their cognate cells generating diverse subpopulations of bitter-sensing cells with distinct yet overlapping molecular receptive ranges. Genetic ablation of one of these subpopulations therefore does not extinguish but only partly diminishes bitter responsiveness. The functional diversity of the peripheral bitter sensing cells is propagated by afferent nerve fibers to 1st order gustatory neurons in the brain stem. In this area as well as in other areas processing taste information neurons are found that express bitter taste receptors. Blockade of synaptic transmission by genetic manipulation in these neurons results in partly impaired bitter responses. However, the extent to which the bitter sensing system is affected is smaller than that seen in the animals with the ablated peripheral bitter cell subpopulation. The data propose that bitter recognition is mediated by functionally distinct yet overlapping lines in the periphery that partially converge in the central nervous system raising the question if and to which extend discrimination of bitter compounds is possible and how this organization affect ingestion of food differing in the content of bitter substances.
The extrinsic gastrointestinal nervous system plays a key role in the sensing of nutrients and its translation in terms of control of food intake by the central nervous system. Regarding major macronutrients as glucose and protein, they are sensed by the gastrointestinal neural system and the transmission of the signals to the brain promotes satiety phenomena. Glucose is sensed in the portal vein by neurons expressing the glucose receptor SGLT3 and activates the main regions of the brain involved in the control of food intake (1). Protein indirectly acts on food intake via intestinal gluconeogenesis and the sensing of released glucose by the portal glucose sensor. Peptides are first sensed by mu-opioid receptors in the neural system of the portal vein to promote intestinal gluconeogenesis via a nervous circuit (2). Similarly, soluble fibers and their products (short-chain fatty acids) mediate their anti-obesity and anti-diabetic benefits via a reflex arc with the brain inducing intestinal gluconeogenesis. Propionate is sensed by the periportal neural system via its agonistic binding to the free fatty acid receptor FFAR3, which induces intestinal gluconeogenesis gene expression. Moreover, propionate serves as an intestinal glucose precursor (3). This new knowledge provides novel mechanisms of control of body weight, which might be useful to envision future approaches of prevention or treatment of obesity and diabetes.

2. Duraffourd et al, Cell, 2012
3. De Vadder et al, Cell, 2014
EFFECTS OF PROLONGED EXENDIN-4 ADMINISTRATION ON HYPOTHALAMIC AND REWARD SIGNALING GENE EXPRESSION

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Repeated administration of the long acting glucagon-like peptide 1 (GLP-1) analog Exendin-4 (EX-4) has been shown to reduce food intake and body weight and do so without a rebound increase in food intake following treatment termination. In the current studies we examined the effects of prolonged EX-4 treatment in rats consuming chow and high fat diets on food intake, body weight and gene expression in the arcuate nucleus, VTA and nucleus accumbens. After 6 weeks maintenance on a standard chow (SC) or a high fat (HF) diet, male Sprague Dawley rats were treated with EX-4 (3.2μg/kg, ip, b.i.d) or vehicle for 9 consecutive days. Pair fed groups were included to control for effects of reduced food intake and body weight. Treatment with EX-4 significantly decreased food intake and BW over the 9-day period in both the SC and HF groups. HF feeding decreased POMC without changing NPY/AgRP gene expression in the ARC. Treatment with EX-4 increased POMC and decreased NPY expression independent of the reduction of food intake and BW. Mesolimbic TH and D1R gene expression were decreased significantly in chronic HF fed rats, and these changes were reversed in both EX-4 and pair-fed conditions. In an acute experiment, we assessed the role of Akt signaling in the anorexia produced by Ex-4. Ex-4 administration increased the phosphorylation of Akt in the arcuate and this effect was blocked by 3rd ventricular wortmanin administration. Wortmanin administration also prevented the ability of Ex-4 to reduce food intake and body weight and elevate ARC POMC expression. Together these results suggest a role for increased POMC and decreased NPY expression in the ARC in the effects of EX-4 on food intake and BW. Our findings also suggest that EX-4 induced recovery of mesolimbic TH and D1R expression in HF fed rats may be secondary to HF intake reduction and/or weight loss.
GALANIN AND LEPTIN ACTION IN NUTRIENT REWARD

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The anorexigenic hormone leptin is known to decrease food intake at least in part by decreasing the rewarding effects of food via direct or indirect effects on the mesolimbic dopaminergic system. However, the exact mechanisms involved are largely unknown. We identified a subset of leptin receptor (LepRb) neurons in the perifornical area/lateral hypothalamus (PFA/LH) that express the inhibitory neuropeptide galanin (Gal-LepRb neurons), which is induced by leptin and Gal-LepRb neurons heavily innervate local orexin neurons. Deletion of LepRb in galanin neurons (Gal-LepRb KO mice) significantly decreased galanin and galanin receptor 1(Gal-R1) expression in the PFA/LH, and enhanced activation of orexin neurons. While galanin deficiency is associated with decreased fat consumption, orexin acts on midbrain dopaminergic neurons to modulate reward behavior, e.g. increased sucrose consumption. Interestingly, in a two-bottle choice test (isocaloric 25%sucrose & 10% intralipid solutions) Gal-LepRb KO mice showed a robust sucrose preference over lipids compared to wild-type mice exhibiting no preference. Conversely, neuronal activation of LH galanin neurons using pharmacogenetic DREADD technology induced a fat preference over sucrose. Our data suggests that leptin inhibits orexin neurons by activating inhibitory acting galanin neurons and via galanin action on GalR1 to modulate the preference and rewarding value of palatable foods.
Satiety is typically measured by observing the decline in consumption over time in subjects provided with freely available food. However, such studies do not assess the influence of satiety on food seeking, which is controlled by neural circuits different from those controlling consumption. For instance, the mesolimbic dopamine projection from the ventral tegmental area to the nucleus accumbens (NAc) is required for certain cued food approach behaviors, but not for consumption of freely available food. Presumably, satiety reduces the willingness to seek food, perhaps via the well-known reduction in NAc dopamine levels that accompany satiety. However, despite this potentially important mechanism of intake regulation, the influence of satiety on food-seeking has not been extensively studied.

To begin to study this mechanism in more detail, we developed a rodent behavioral model in which food-seeking is (1) under stimulus control; (2) dependent on mesolimbic dopamine; and (3) strongly influenced by satiety. Rats were given food and water ad libitum in the home cage, and placed every day in an operant chamber for 2 to 3 hr cued access to dilute sucrose solution. An auditory conditioned stimulus (CS) signaled the availability of sucrose in a receptacle equipped with a photobeam to detect entry. Animals responded to 25 to 75% of CS presentations by entering the receptacle. Responding was reduced by injection of dopamine D1 or D2 receptor antagonists into the NAc core, and increased by injection of D1 or D2 agonists. Moreover, in rats performing a similar task, roughly half of recorded NAc neurons were excited by CS presentation; these excitations preceded initiation of approach movement and predicted its latency (McGinty et al, Neuron 78:910, 2013), and injection of dopamine antagonists into the NAc reduced the magnitude of these excitations. Thus, CS-induced food approach behavior is caused by facilitation of cue-evoked NAc neuronal excitation by dopamine.

Intriguingly, animals began each session by responding to most CS presentations, and then gradually reduced their responding. To test whether this within-session decline in responding was due to satiety, we replaced sucrose with an isohedonic concentration of saccharine; no within-session decline was observed, suggesting that the decline in sucrose CS responding was due to nutrient sensing. To further test this hypothesis, we implanted gastric drains and compared responding when the drains were open (preventing sucrose from reaching the duodenum) to when they were closed. The within-session decline in responding was attenuated in the drain-open condition. Moreover, pre-loading the stomach with sucrose reduced the normally high level of responding at the beginning of the session. These results demonstrate that the within-session decline in CS responding is due to satiety that results from intestinal or post-absorptive nutrient sensing. Ongoing studies investigate the neural mechanisms of this effect.
Oxygen-sensing mechanisms have evolved to maintain cell and tissue homeostasis since the ability to sense and respond to changes in oxygen is essential for survival. The primary site of oxygen sensing occurs at the level of the carotid body which in response to hypoxia signals increased ventilation without the need for new protein synthesis. Chronic hypoxia activates cellular sensing mechanisms which lead to protein synthesis designed to alter cellular metabolism so cells can adapt to the low oxygen environment without suffering toxicity. The master regulator of the cellular response is hypoxia-inducible factor (HIF). Activation of this system under condition of hypobaric hypoxia leads to weight loss accompanied by increased basal metabolic rate and suppression of appetite. These effects are dose dependent, gender and genetic specific, and results in adverse effects if the exposure is extreme. Hypoxic adipose tissue may represent a unified cellular mechanism for variety of metabolic disorders, and insulin resistance in patients with metabolic syndrome.
CGRP-EXPRESSING NEURONS IN THE PARABRACHIAL NUCLEUS MEDIATE AVERSIVE EVENTS

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Both visceral and somatosensory neuronal information is relayed to higher brain regions by CGRP-expressing neurons that reside in the external lateral region of the parabrachial nucleus (ePBN). Activation of these CGRP-expressing neurons either by light activation of channelrhodopsin (ChR2) or CNO activation of hM3Dq DREADD receptors inhibits feeding and chronic activation of these neurons would lead to starvation. Additional experiments demonstrate that the relevant output of these CGRP-expressing neurons that mediates anorexia is a projection to the lateral capsule region of the central nucleus of the amygdala. This circuit, which originates with vagal activation, also mediates the unconditioned stimulus associated with conditioned taste aversion. The CGRP-expressing neurons also mediate the unconditioned stimulus (foot shock) mediated by the somatosensory system in fear conditioning experiments. Inactivation of CGRP expressing neurons in ePBN with tetanus toxin attenuates foot-shock responses and blocks freezing behavior that is normally associated with the conditioning context. How aversive gastrointestinal and somatosensory stimuli are distinguished by activation of this common CGRP-mediated pathway remains to be resolved.
Leptin-melanocortin signaling plays a key role in food intake and energy balance control, and is often impaired in obese individuals. Exact molecular underpinnings for this impairment are incompletely understood, and may involve the interaction between genetic factors and environmental stimuli. Twin cohort studies indeed revealed differential propensities for weight gain despite an identical nutritional and genetic status, and postulated that epigenetic modifications play a crucial role in this process. We here aimed to identify such novel epigenetic regulators of leptin-MC signaling. Expression analyses of hypothalami from leptin-treated mice identified a clear association between leptin-MC signaling and hypothalamic histone deacetylase 5 (HDAC5) gene expression. Global HDAC5 KO mice displayed increased food intake and propensity for diet-induced obesity when chronically exposed to high fat diet. Pharmacological and genetic inhibition of HDAC5 activity in the mediobasal hypothalamus increased food intake and modulated pathways implicated in leptin-MC signaling. Notably, HDAC5 knockdown lead to increased Stat3 acetylation, but decreased Stat3 phosphorylation as well as binding to the POMC promoter. Accordingly, we observed massively impaired leptin sensitivity in HDAC5 KO mice compared to WT littermates. Finally, hypothalamic overexpression of HDAC5 decreased food intake, attenuated HFD-induced leptin resistance, and ameliorated diet-induced obesity in mice. Overall, our data suggests that hypothalamic HDAC5 activity acts as epigenetic regulator of leptin MC signaling to ultimately adapt food intake and body weight to changes in our environment.
BENEFICIAL EFFECT OF PREBIOTIC MILK OLIGOSACCHARIDES ON GUT MICROBIOTA, INTESTINAL BARRIER FUNCTION AND ADIPOSITY IN DIET-INDUCED OBESITY IN MICE

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It is now well established that high fat (HF) diet-induced obesity is associated with significant changes in the gut microbiota and in intestinal permeability. In the current study, we used addition of prebiotic bovine milk oligosaccharides or inulin to determine the protective effects on barrier function in different regions of the intestine. C57BL/6 mice were fed a WE (41% fat), WD + prebiotic (inulin or BMO, 6%), or control diet (C, 16% fat) for 1, 3 or 6 weeks. Barrier function was assessed along the length of the intestine (ex vivo) with small and large molecular probes to measure paracellular and transcellular permeability, respectively. Addition of either prebiotic significantly reduced weight gain induced by a high fat diet. Paracellular and transcellular flux in the ileum were significantly higher in WD fed mice after three weeks; this was completely restored by prebiotic treatment. Transcellular flux in the colon was significantly higher in WD fed mice after six weeks, and was prevented by prebiotic treatment. These results show that prebiotics restore gut barrier function and reverse HF diet-induced obesity.
CANCER ANOREXIA

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Cancer anorexia (CA) contributes to the anorexia-cachexia syndrome, which deteriorates treatment success, survivability and quality of life in cancer patients. Earlier studies demonstrated the involvement of brainstem mechanisms in CA, but the possible effect of the area postrema (AP) has not yet been dissociated from effects of afferent vagal signals. We explored whether AP lesion (APX) or subdiaphragmatic vagal deafferentation (SDA) blocks CA in a rat Morris-7777 hepatoma tumor model. Using a two-diet choice paradigm, we also tested the possible involvement of a tumor-dependent learned diet aversion. In addition to hindbrain mechanisms, the arcuate nucleus (ARC) represents a target for pharmacological approaches against CA. Therefore, we characterized a novel ghrelin agonist (HM01) and its ability to attenuate CA and body weight loss.

In SHAM-APX animals, mean daily food intake (FI) decreased significantly by 38 ± 5% between wk 2 and wk 4 after tumor inoculation. CA was paralleled by a net body weight (BW) loss in wk 4. APX rats did not significantly reduce FI and did not show a net reduction in BW. Tumor-induced anorexia and body weight loss was completely unaffected in SDA rats. Interestingly, in tumor-bearing rats diet preference for chocolate or vanilla flavored diet converted into diet avoidance after the animals had received only their preferred diet for 8 days during the phase of tumor anorexia.

The ghrelin-like action of HM01 was confirmed in electrophysiological recordings. HM01 (10^-7-10^-6 M) increased the neuronal firing rate in 76% of the recorded Arc neurons. Notably, there was a co-sensitivity of 100% (n=17) between the response profiles of HM01 and ghrelin (10^-8 M). HM01 delivered chronically via osmotic minipumps (50 μg/h) increased FI in healthy rats by 24% during the entire experimental period (12 days), leading to increased BW. Moreover, HM01 attenuated the anorectic response induced by tumor-growth leading to 30% higher FI compared to saline-treated tumor-bearing animals. In contrast to tumor-bearing controls, HM01-treated rats did not lose BW during the treatment (-10.4 ±1.7 g vs. 1.1±2 g, p<0.001).

Our findings substantiate the importance of the area postrema in the mediation of tumor-induced anorexia and body weight loss. AP dependent neuromechanisms mediating pathological anorexia/aversion might represent targets for the treatment of cancer anorexia. HM01 mimics the neuronal effect of ghrelin in Arc and positively affects energy balance in both healthy and tumor-bearing rats. Ghrelin agonists like HM01 appear to be therapeutically useful for the treatment of CA and possibly other forms of disease-related anorexia.

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ACTIVATION OF CENTRAL VAGAL AFFERENT ENDINGS: A PUTATIVE MECHANISM FOR CONTROL OF FOOD INTAKE BY HINDBRAIN MELANOCORTIN 4 RECEPTORS.

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Vagally transmitted information from the GI tract comprises the sensory basis of satiation, which controls meal size. Melanocortin 4 receptor (MC4R) agonists, released from POMC neuron terminals, also are important central mediators for control of food intake. However, MC4R agonists also control food intake by reducing meal size, suggesting that MC4R activation might enhance or even mimic gastrointestinal satiation signals. MC4R are expressed by vagal afferent neurons, and MC4R activation enhances spontaneous glutamate release from vagal afferent endings in the nucleus of the solitary tract (NTS). Hence, MC4R potentially could modulate or mimic satiation by enhancing central vagal afferent synaptic function. In support of this hypothesis we might expect 1) that POMC neuron endings would make close contacts with vagal afferent endings in the NTS; 2) that activation of MC4R would trigger cellular changes in vagal afferents consistent with enhanced presynaptic function; 3) that inhibition of MC4R cellular effects would attenuate MC4R-induced reduction of food intake; and 3) that activation of central vagal afferents would be necessary for reduction of food intake by MC4R activation. In multilabel immunohistochemical preparations we observed numerous close contacts between alpha MSH immunoreactive endings and vagal afferent endings in the NTS. In fact MSH immunoreactive endings sometimes appeared to wrap vagal afferent fibers. Fourth ventricle injection of the MC3/4R agonist, MTII, increased synapsin 1 phosphorylation in vagal afferent endings at serine 9 (PKA site), an effect that increases synaptic strength in other neurons. MTII-induced increase in synapsin phosphorylation was prevented by an MC4R antagonist and was abolished by protein kinase A (PKA) inhibition. In rats that underwent unilateral nodose ganglion removal, resulting in degeneration of central vagal afferent endings predominantly in the ipsilateral NTS, MTII increased synapsin phosphorylation contralateral to nodose removal, but phosphorylation ipsilateral to nodose removal was attenuated. Fourth ventricle injection of MTII reduced food intake and, as with synapsin phosphorylation, the reduction of intake was prevented by PKA inhibition. Likewise, unilateral nodose ganglion removal attenuated reduction of food intake when MTII was injected into the NTS ipsilateral to nodose removal, but not when MTII was injected into the contralateral NTS. Collectively our results support the hypothesis that reduction of food intake by hindbrain MC4R agonist requires activation of central vagal afferent endings. Moreover, our observations are consistent with a mechanism by which MC4R activation triggers PKA-catalyzed synapsin 1 phosphorylation in vagal afferent endings, thereby increasing vagal afferent synaptic function and reducing food intake. Supported by NIH grants DK-52849 and NS-20561.
MERCAPTOACETATE DIRECTLY ANTAGONIZES FATTY ACID-INDUCED SECRETION OF INSULIN AND GLP-1 AND STIMULATES FEEDING VIA THE MEMBRANE FATTY ACID RECEPTOR GPR40

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β-Mercaptoacetate (MA) stimulates food intake in rats, an action widely attributed to its ability to block fatty acid (FA) oxidation. However, we reported recently (Am J Physiol, 2014) that MA blocks FA signaling in cultured nodose neurons by directly antagonizing the G-protein coupled receptor 40 (GPR40). GPR40 is expressed not only by nodose neurons, but also mediates the stimulatory effects of FAs on β-cell insulin secretion and enteroendocrine cell secretion of CCK and GLP-1. Therefore, our finding that MA blocks GPR40-induced Ca2 influx in nodose neurons suggests that MA may also antagonize these same receptors in other tissue sites. We tested this hypothesis using calcium-imaging techniques to examine the interaction of MA with GPR40 in cultured STC-1 cells, which are derived from intestinal enteroendocrine cells. In support of our hypothesis, we found that MA blocks the effect of linoleic acid (LA, a long chain FA) and GW9508 (a GPR40 agonist) on calcium influx in STC-1 cells. We also found that MA reduced LA-induced secretion of GLP-1 from STC-1 cells and, in vivo, reduced GLP-1 secretion triggered by olive oil gavage or by spontaneous intake of a fatty meal. To further test MA’s interaction with GPR40, we examined the effects of MA on FA-induced potentiation of insulin secretion. In support of our hypothesis, we found that MA potently blocks insulin secretion while greatly prolonging the elevation of plasma glucose in glucose-injected rats and also blocks insulin secretion from cultured INS-1E (insulinoma) cells, the latter suggesting that MA’s effects in vivo result from a direct action of MA on β-cells. These in vivo and in vitro results reveal a novel and direct action of MA on GPR40-mediated effects of FAs on insulin and GLP-1 secretion. The interaction of MA with GPR40 receptors controlling FA-induced secretion of GLP-1, a hormone that influences food intake in part or entirely by actions on the vagus nerve, is consistent with previous results showing that MA’s feeding effects are vagally-dependent. Indeed, using automated meal monitoring and home-cage testing, we found that MA does not stimulate food intake in GPR40 knock out mice but significantly increases intake in wild type littermates. Results strongly suggest that MA’s blockade of GPR40 (presumably at multiple sites) significantly influences or mediates MA-induced stimulation of food intake. Supported by NIH grants R01DK081546, R01DK097437 and R01040498.
ORPHAN G PROTEIN-COUPLED RECEPTORS AND ENERGY HOMEOSTASIS: THE CASE FOR GPR107

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A severe complication of type 1 diabetes (T1DM) is hypoglycemia, which can lead to coma or death. Hypoglycemia in T1DM is due to impairment of counter-regulation that is in part a consequence of “glucose blindness” of the pancreatic alpha cells. In the hypoglycemic T1DM state, alpha cells cannot secrete sufficient amounts of glucagon in a timely manner to overcome the hypoglycemia. We identified a novel regulator of pancreatic cell function, neuronostatin, which enhances glucagon mRNA expression and protein release. Neuronostatin is produced in delta cells of the pancreas and levels in plasma increase in fasting suggesting a role for the peptide in glucagon production and release during hypoglycemic conditions. Using a unique Deductive Ligand-Receptor Matching Strategy, we elucidated the putative receptor for neuronostatin to be the previously orphaned G protein coupled receptor (GPCR), GPR107. GPR107 is expressed most highly in the pancreas and particularly by alpha cells, and appears to be essential for mediating the actions of neuronostatin in those cells. We identified the cellular mechanism of action of neuronostatin in alpha cells to be via activation, under low glucose conditions, of PKA, via a non-cAMP-dependent pathway. Recently, using fluorescence immunohistochemistry we have demonstrated that neuronostatin colocalizes with GPR107 on alpha cell membranes in human islets. We hypothesize that impaired GPR107 signaling or neuronostatin release from the pancreatic delta cells contribute to deficits in counter-regulation leading to insulin-induced hypoglycemia in type 1 diabetes.
ROLE OF GOLGI-ASSOCIATED GTPASES FOR LIPID STORAGE

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Lipid homeostasis is maintained through the coordination of lipid metabolism in various tissues, including adipose tissue and the liver. The disruption of lipid homeostasis often results in the development of metabolic disorders such as obesity, diabetes mellitus, liver steatosis, and cardiovascular diseases. Lipids are oxidized, stored as lipid droplets and/or secreted as lipoproteins (e.g. chylomicrons, very-low-density lipoproteins; VLDL). Generation of both lipid droplets and lipoproteins is initiated in the endoplasmic reticulum (ER), whereas final maturation likely occurs within the Golgi. ADP-ribosylation factor-related protein 1 (ARFRP1), a small GTPase associates with trans-Golgi membranes and is required for the recruitment of ARF-like 1 (ARL1) to this compartment. ARL1 interacts with the scaffolding protein Golgin-245 to which Rab-GTPases (e.g. Rab2, Rab6) bind. The whole cascade plays an essential role for lipid droplet and lipoprotein formation: In intestine-specific Arfrp1 knockout (Arfrp1\textsuperscript{vd+/−}) mice and in Caco-2 cells it modulates the lipidation of chylomicrons and the assembly of ApoA1. Suppression of each protein resulted in the formation of smaller chylomicrons (Jaeschke et al., 2012). A similar phenotype was discovered in liver-specific knockout (Arfrp1\textsuperscript{lv+/−}) mice which exhibit an impaired VLDL lipidation and an inappropriate sorting of ApoCIII to the VLDL particle, which leads to the hepatic release of smaller VLDL particles carrying less triglycerides (Hesse et al., 2013, 2014). Arfrp1\textsuperscript{ad-ER−/−} mice exhibit lower fat mass with smaller adipocytes due to a reduced growth of white and brown adipose tissue depots shortly after suppression of Arfrp1 expression in adipose tissue by tamoxifen treatment. Similarly, 3T3-L1 adipocytes were markedly smaller when Arfrp1 expression was suppressed by siRNA. Thus, we believe that the action of ARFRP1, ARL1 and Rab2 at the trans-Golgi plays an important role in lipid droplet and lipoprotein formation presumably by regulating the sorting of coating proteins during the maturation lipid containing particles.


It has been known for many decades that food intake stimulates the secretion of gut hormones which through endocrine and neural mechanisms control body metabolism (1). However it is first recently that it has become increasingly evident that the nutrient metabolites, which are responsible for this regulation of gut-brain/gut-body signaling, act not only as ‘fuels’ which are being further metabolized inside the cells, but also as signaling agents themselves acting through a series of specific 7TM G protein-coupled receptors.

Three stories will illustrate the complexity of the physiology of this nutrient metabolite receptor system and possibilities to exploit this pharmacologically even beyond its physiology:

1. **The strong signaling effect of dietary triglyceride (TG) is obtained through conjoined sensing of its metabolites: long chain fatty acids (LCFA) acting on GPR40 and stimulating Gq and mono-acyl glycerol (2-MAG) acting though GPR119 and stimulating Gs – functioning in symphony with TRG5 sensing the bile acids and signaling through Gs – whereas the LCFA receptor GPR120 does not appear to play a major role.** This notion is based on ex vivo studies with selective agonists for each of the receptors and in vivo studies of GIP, GLP-1 and PYY responses in KO and double KO animal models for each of the receptors.

2. **Physiological stimulation of GPR40 by LCFAs or selective, orthosteric GPR40 agonists - such as TAK-875 – only leads to Gq signaling and rather poor if any incretin hormone secretion.** However, certain ago-allosteric agonists (3) – such as AM5262 - can induce GPR40 to signal not only through Gq but also through Gs and such agonists are hereby able to stimulate, for example GLP-1 and GIP secretion robustly - conceivably by mimicking the joint Gq plus Gs signaling obtained by physiological coaction of GPR40 with GPR119 and TGR5.

3. **A series of different metabolite receptors are stimulating GLP-1 secretion but are in fact inhibiting ghrelin secretion (5) – that is in accordance with the opposite secretory pattern for ghrelin versus GLP-1 on metabolic targets such as: a) insulin secretion; b) glucagon secretion; c) appetite; d) gastric emptying; f) adiposity/WAT; and g) energy expenditure/ BAT function.** This opens for the possibility of pharmacological opposite control of GLP-1 and ghrelin secretion - and thereby action - through the same receptor target.

GLP-1 RECEPTOR STIMULATION OF THE LATERAL PARABRACHIAL NUCLEUS REDUCES FOOD INTAKE: NEUROANATOMICAL, ELECTROPHYSIOLOGICAL AND BEHAVIORAL EVIDENCE

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The parabrachial nucleus (PBN) is a key nucleus for the regulation of feeding behavior. Inhibitory inputs from the hypothalamus to the PBN play a crucial role in the normal maintenance of feeding behavior, as their loss leads to starvation. Viscerosensory stimuli result in neuronal activation of the PBN, however the origin and neurochemical identity of the excitatory neuronal input to the PBN remain largely unexplored. Here we hypothesize that hindbrain glucagon-like peptide 1 (GLP-1) neurons provide excitatory inputs to the PBN, activation of which may lead to a reduction in feeding behavior. Our data, obtained from mice expressing the yellow fluorescent protein in GLP-1-producing neurons, revealed that hindbrain GLP-1-producing neurons project to the lateral PBN (lPBN). Stimulation of lPBN GLP-1 receptors (GLP-1R) reduced the intake of chow and palatable food and decreased body weight in rats. It also activated lPBN neurons, reflected by an increase in the number of c-Fos-positive cells in this region. Further support for an excitatory role of GLP-1 in the PBN is provided by electrophysiological studies showing a remarkable increase in firing of lPBN neurons after exendin-4 application. We show that within the PBN, GLP-1R activation increased gene expression of two energy balance regulating peptides, calcitonin gene related peptide (CGRP) and interleukin-6. Moreover, nearly seventy percent of the lPBN GLP-1 fibers innervated lPBN CGRP neurons. Direct intra-lPBN CGRP application resulted in anorexia. Collectively, our molecular, anatomical, electrophysiological, pharmacological and behavioral data provide evidence for a functional role of the GLP-1R for feeding control in the PBN.
The gustatory cortex (GC) in the rat has traditionally been defined as an area of taste-responsive neurons in dysgranular/agranular insular cortex flanking the middle cerebral artery. To better understand the roles of GC in taste function, we have been examining the consequences of ibotenic acid lesions in this area on various taste-guided behaviors. To date, we have found that extensive bilateral lesions in GC, as traditionally defined, do not modify concentration-dependent licking to sucrose or quinine, nor ostensibly impair the oromotor taste reactivity profiles elicited by intra-oral delivery of these same stimuli. Perhaps even more unexpectedly, in contrast to the prevailing view that GC is necessary for conditioned taste aversion (CTA) retention, we found that rats with extensive centrally-positioned bilateral lesions in GC exhibited normal post-surgical expression of a pre-surgical LiCl-induced CTA. A follow-up study systematically varied the lesion size and placement to further assess the functional significance of GC with respect to CTA expression. This approach yielded a subset of LiCl-injected rats with lesions that displayed severe deficits in CTA expression, as well as a subset of rats with extensive bilateral GC damage that displayed normal CTA expression as measured in one and two-bottle intake tests. Thus, we developed a lesion analysis system that allowed us to quantify not only the degree of bilateral damage to GC, but also commonalities of lesion size and topography between rats that share a significant deficit in performance relative to unimpaired rats. Using this system, we found that rats with impaired CTA expression commonly had damage positioned in the posterior portion of GC and especially within posterior regions of insular cortex but outside of GC in an area thought to be involved in visceral signal processing. Recently we made very large lesions of GC that encroached upon surrounding areas of insular cortex including the posterior regions implicated in CTA expression. As expected, these rats failed to express a presurgical CTA to sucrose when tested postsurgically. In this same study, rats were subsequently operantly trained and tested for NaCl and KCl taste sensitivity in a psychophysical signal detection task. Psychometric sensitivity functions for NaCl and KCl were significantly shifted to the right in rats with extensive bilateral GC lesions, though these rats were able to competently learn to discriminate between these salts albeit at a retarded rate. Interestingly, lesion-induced deficits on the NaCl and KCl detection tests correlated modestly with each other and with impairments in salt discrimination acquisition, but did not correlate with performance on the CTA retention test. Our collective findings to date paint a picture of the role of GC in taste that appears functionally and topographically complex. We are planning to extend our analysis by using more challenging taste-guided behavioral tasks, including tests of both signal detection in mixtures and short-term taste memory, as well as by incorporating optogenetic techniques to silence discrete regions of insular cortex during the stream of ongoing behavior.

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Exposure to a high-fat (HF) diet increases one’s risk for obesity, diabetes, and the metabolic syndrome, which have been associated with an increased risk of cognitive deficits. Few studies, however, have assessed the effects of maternal diet on the risk of cognitive deficits in offspring. We used a rat model to determine how maternal diet during pregnancy and lactation impacts the behavior, cognition, and hippocampal gene expression of offspring. Pregnant Sprague-Dawley rats were given ad libitum access to standard chow (n = 12) or HF diet (n = 12). On postnatal day (P)21, brains were collected from 2 males per litter for gene expression analysis. All other pups were weaned onto chow. Starting on P90, one male per litter was used for behavioral testing. On P150, brains were collected from one male per litter for gene expression analysis. At P21, expression of insulin receptor (Insr), leptin receptor (Lepr), and GLUT1 (Slc2a1) was decreased in the hippocampus of HF offspring. The decreased expression of Insr and Lepr persisted at P150. As adults, HF offspring weighed more, had increased food intake and increased preference for a HF diet. Further, HF offspring were hypoactive, less responsive to amphetamine, and had impaired object recognition compared to offspring of chow fed dams. We found similar results in female offspring. Together, our results suggest that maternal HF diet during pregnancy and lactation has significant lasting effects on the brain, behavior, and cognition of offspring.
PPG NEURONS IN THE MOUSE NTS: AN IMPORTANT RELAY FOR VISCERAL AND CENTRAL SATIETY SIGNALS?

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Glucagon-like peptide-1 (GLP-1) affects central autonomic neurons, including those controlling the cardiovascular system, thermogenesis, and energy balance. Additionally, GLP-1 influences the mesolimbic reward system to modulate the intake of palatable food. GLP-1 is not only produced in the gut, but also by hindbrain preproglucagon (PPG) neurons, located mainly in the nucleus tractus solitarii (NTS) and medullary reticular formation. Transgenic mice expressing glucagon promoter-driven yellow fluorescent protein or Cre-recombinase revealed that these brainstem PPG neurons not only project to the mesolimbic reward centres and central autonomic control regions, but also strongly innervate spinal autonomic neurons. Therefore, brainstem PPG neurons could directly modulate sympathetic outflow through their spinal inputs to sympathetic preganglionic neurones or interneurons. These observations add another level of complexity to the modulation of autonomic outflow by central PPG neurons. Electrical recordings from identified PPG neurons in vitro have revealed that their activity is modulated by ambient glucose levels in the NTS and that they receive synaptic inputs from vagal afferents entering via the solitary tract. Vagal afferents convey satiation to the brain as a result of signals such as gastric distention following meal ingestion or activation of GLP-1 receptors in the periphery. The satiety peptide cholecystokinin increased the electrical activity of PPG neurons in the NTS, whereas the orexigenic peptide ghrelin failed to alter their electrical properties, further suggesting that satiation is a main driver of PPG neuronal activation. Moreover, the adipocyte-derived hormone leptin increased the electrical activity of PPG neurons. Finally, viral targeting of PPG neurons to express Channelrhodopsin (ChR2) revealed that PPG neurons are amenable to optogenetic activation and that this tool can be used to drive their activity in vivo. These findings indicate that PPG neurons are in a prime position to respond to both immediate and long term indicators of energy and feeding status and to regulate aspects of both energy balance and general autonomic homeostasis.

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Apolipoprotein A-IV is a protein made by the intestinal epithelial cells of the small intestine. Its production by the small intestine is stimulated by fat absorption. It plays an important role as a satiety factor. Recently, we have demonstrated that the apo A-IV knockout mice are glucose intolerant and this is caused by a failure to secrete insulin when stimulated by an elevation in blood glucose. Intraperitoneal administration of apo A-IV restored the insulin secretion by the pancreatic beta cells in the apo A-IV knockout animals in a dose-dependent manner. Using isolated pancreatic islets, we demonstrated that apo A-IV stimulated the release of insulin in the presence of 20 mM glucose concentration but not 3 mM glucose concentration. Apo A-IV is also effective in stimulating insulin secretion in the wild type animals as well as in diet induced obese animals. My talk discusses the various interesting aspects of this potentially important incretin function of apo A-IV. I will also present our most recent data showing that apo A-IV is not working through GLP-1 or its receptor.
Normal variations of blood glucose in healthy mammals are directed back into a relatively narrow range (5-7 mM) by a series of endocrine mechanisms that primarily involve insulin, glucagon, epinephrine, and glucocorticoid. These control mechanisms are compromised in people with diabetes mellitus, either because of failed β-cell function (type 1; T1D), or from insulin resistance and increasingly poor β-cell function (type 2; T2D). With T1D—and now more commonly in T2D—inulin therapy is the treatment of choice. While effective at reducing hyperglycemia, the difficulty of delivering appropriate rates and doses of insulin in real time means that patients can be exposed to bouts of hypoglycemia, which if frequent or if they go unrecognized, lead to serious complications. Brain mechanisms play a primary role in controlling epinephrine, glucocorticoid and glucagon secretion during hypoglycemia. Therefore identifying the various brain regions that receive and process sensory information about glycemia, and how they use this information to formulate appropriate counterregulatory responses, are key to understanding glycemic control in health and disease. We will discuss our recent results that broadly address the structure and function of the brain networks involved with glycemic control, including how the hindbrain and hypothalamus interact, and the role of the ventromedial nucleus of the hypothalamus. We will also provide evidence that distinct but somewhat overlapping brain networks are responsible for the counterregulatory responses that follow hypoglycemia with either a rapid-onset rate (the more common form in experimental models) and a slow-onset rate (probably the clinically more prevalent form).

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DOES ENERGY BALANCE DETERMINE PROTEIN-INDUCED ENERGY-INTAKE AND ENERGY-EXPENDITURE?

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Protein-induced satiety and energy-expenditure have been proposed as main mechanisms behind the beneficial effects of high-protein diets for body weight management. In two randomised crossover design trials in healthy subjects [n 70/67 (M/F); age: 19-70 years; BMI: 18.2-38.7 kg/m²] with diets containing 5En% from wheat protein (5En%-protein diet), and 5En% from wheat protein added with 10En% (15En%-protein diets) and 25En% (30En%-protein diets) from beef, whey with α-lactalbumin or soy protein was consumed ad libitum for 12 consecutive days. Energy intake was significantly lower in the 30En%-protein (8.73 ± 1.93 MJ/d) compared with the 5En%-protein (9.48 ± 1.67 MJ/d) and 15En%-protein conditions (9.30 ± 1.62 MJ/d, P = 0.001), stemming largely from lower energy intake during meals (P = 0.001). Nitrogen balance was maintained on the 5En%-protein diet, and was positive on the 15En%- and 30En%-protein diets. Protein intake was sufficient to meet dietary IAA requirements in all conditions, irrespective of the protein source. In a separate study, post-prandial kinetics of arginine, asparagine, isoleucine, leucine, lysine, and phenylalanine appeared to underscore soy, and whey-protein-induced fullness especially at 10En% of protein, while the post-prandial kinetics of GLP-1, ghrelin, and insulin also underscored the appetite reduction at 25En% of these proteins. Over 12 weeks on a 30 En% vs. 5 En% protein diet in energy balance, satiety, energy-expenditure (TEE, DIT, SMR), protein- and carbohydrate balance changed in opposite directions (p<0.05). However, when immediately after the diet, continuous infusions with L-[ring-^1H3]phenylalanine, L-[1-^13C]-leucine and L-[ring-^1H2]tyrosine were applied (n=15), and whole-body protein synthesis and breakdown rate and net protein balance in a post-absorptive state were determined, whole-body protein balance was more negative in the HighProteinLowCarbohydrate-group compared with the HCLP-group (-0.069±0.01 vs -0.046±0.01 μmol phenylalanine/kg; P<0.001). Whole-body protein breakdown (0.72±0.07 vs 0.63±0.06 μmol phenylalanine/kg; P=0.001), synthesis (0.65±0.07 vs 0.58±0.06 μmol phenylalanine/kg; P<0.01) and phenylalanine hydroxylatation rates (0.069±0.01 vs 0.045±0.01 μmol phenylalanine/kg; P<0.001) were significantly higher in the HPLC-group vs HCLP-group. In conclusion, in energy-balance a high-protein-diet decreases energy-intake through satiation and satiety, underscored by the kinetics of arginine, asparagine, isoleucine, leucine, lysine, phenylalanine, GLP-1, ghrelin, and insulin, and increases energy expenditure (TEE, DIT, SMR) and protein-balance, while nitrogen balance was not negative and dietary IAA intake was sufficient for twelve days with diets low in protein or limited in protein variety. However, the isotope-study showed a more negative whole-body protein balance due to a higher whole-body protein-breakdown despite a higher protein synthesis.

Discussion: These phenomena observed in energy-balance are applied for protein- diets with 0.8-1.2 g/kg/d protein in negative energy-balance. However, when in energy-balance protein intake exceeds to 1.66 g/kg/d, subjects get into positive energy-balance, and gain fat-free body mass.

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BRAIN INSULIN AND NOVEL TREATMENTS OF OBESITY

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It has long been known that insulin has catabolic actions in the brain, reducing food intake acutely and reducing body weight chronically when administered into the cerebral ventricles or directly into the mediobasal hypothalamus. Reduced central insulin action caused either by pharmacologic or genetic intervention leads to hyperphagia and weight gain, implying that endogenous insulin, like endogenous leptin, normally suppresses body weight. Endogenous insulin is secreted by pancreatic B-cells and is thought to enter the brain by first binding to insulin receptors on brain capillary endothelial cells comprising the blood-brain barrier and then being transported through those cells into brain interstitial fluid. Enhancing this transport process might therefore provide a therapeutic strategy to combat obesity.

Insulin detemir (DET; Novo Nordisk) has an attached fatty acid that allows it to reduce glycemia comparably to other long-acting insulin formulations, but causes less weight-gain and often weight loss. We hypothesized that DET reduces weight-gain, relative to other insulins, due to increased transport into the brain and/or due to increased catabolic action within the brain. When administered acutely into the 3rd-cerebral ventricle, both DET and regular insulin reduced food intake and body weight after 24 h, and this persisted after 48 h only following DET. Appearance of DET and regular insulin in the cerebrospinal fluid (CSF) was compared over several hours and following the administration of different doses systemically. Both exhibited comparable, saturable transport when CSF was sampled from the cisternum magnum, and CSF insulin remained elevated significantly longer following IP DET than following regular insulin, consistent with a longer functional half-life if DET in the brain. In direct comparison with a second long-acting insulin formulation, insulin glargine (GLAR), DET led to longer increases in CSF insulin despite a shorter plasma half-life. Additionally, peripheral DET administration reduced weight gain compared with saline or GLAR in mice. These data support the hypothesis that DET’s catabolic action occurs through an enhanced and prolonged centrally-mediated reduction of food intake.

Insulin and cholecystokinin (CCK) interact synergistically to reduce food intake. To determine if CCK influences insulin transport into the brain, we first identified CCK receptors on brain capillary endothelial cells. We next administered insulin systemically in the presence or absence of ip CCK administration. CCK significantly increased appearance of insulin in the CSF. All of these data suggest that procedures that increase the passage of endogenous insulin into the brain, and/or prolong its action once within the brain, are viable therapeutic strategies for obesity.
Posters

(in alphabetical order by presenting author)
Peripheral fatty acid oxidation (FAO) has long been implicated in the control of eating. Recent evidence suggests that intestinal rather than hepatic FAO is important in this context. Several findings indicate a role of abdominal vagal afferents in sensing enterocyte FAO, but how intracellular FAO may affect vagal afferent activity is unknown. Beta-hydroxybutyrate (BHB), the end product of ketogenesis, could link enterocyte FAO to vagal afferent activity and, hence, affect eating. Cellular uptake or release of BHB is mediated via monocarboxylate transporters (MCTs). We therefore investigated whether MCT2, the major neuronal MCT isoform, is expressed in vagal afferent neurons (VAN). Because previous findings indicate that high fat diet (HFD) adaptation stimulates intestinal FAO and ketogenesis, we also looked whether HFD (45% energy from fat) affected the expression of MCT2. Using immunohistochemical techniques in VAN cell cultures and tissue sections as well as western blots we demonstrate the presence of MCT2 in rat nodose ganglia. Five weeks of HFD did not increase the MCT2 expression in rat VAN compared to standard chow (Western blot: density ratio of MCT2 relative to GAPDH, chow: 0.42 ± 0.07, HFD: 0.43 ± 0.07) We also found expression of the MCT2 in VAN of chow-fed mice. Our data show that VAN express MCT2, but its functional relevance in enterocyte FAO sensing and food intake control remains to be further elucidated.
POSSIBLE ROLE OF MITOCHONDRIAL BIOENERGETICS IN THE RELEASE OF GLP-1 FROM AN ENTEROENDOCRINE CELLS

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Dietary fat potently stimulates glucagon-like peptide-1 (GLP-1) release from enteroendocrine L-cells. Fatty acids act as ligands for several G-protein-coupled receptors (GPCR) that are implicated in mediating GLP-1 release. Recently we found that inhibition of diacylglycerol acyltransferase-1 (DGAT-1) inhibited eating, stimulated intestinal fatty acid oxidation and enhanced meal-induced GLP-1 release in rats fed a high-fat diet (HFD). Also, it has been shown that different membrane bound fatty acid transporters play a role in the fatty acid-induced GLP-1 release. It is, however, unknown whether the GLP-1 release is mediated entirely by cell surface GPCRs or whether some consequence of intracellular fat handling contributes. Here we metabolically characterized the murine GLUTag L-cell line model by measuring the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) of the cells using an Extracellular Flux Analyzer. Further, GLP-1 release was assessed in response to addition of different substrates. We found that oleic acid (250 and 500 µM) potently stimulated the release of GLP-1 from GLUTag cells, whereas BSA-bound oleic acid, glucose or butyric acid did not. Oleic acid induced an H+ leak and dose-dependently reduced the amount of oxygen used for the mitochondrial ATP production through the ATP synthase. Thirty minutes incubation with oligomycin, an inhibitor of mitochondrial ATP production, also increased GLP-1 release. The CPT-1 inhibitor etomoxir reduced the OCR of the cells independent of the presence of oleic acid, but had no effect on the release of GLP-1. These data suggest that the OXPHOS pathway, rather than fatty acid oxidation, plays a role in the release of GLP-1 from GLUTag cells.
ROLE OF UCP-1 AND BROWN ADIPOSE TISSUE IN INFLAMMATION-INDUCED HYPERTHERMIA

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Brown adipose tissue (BAT) thermogenesis is considered to be the main mechanism behind the temperature elevation in inflammation-induced fever in rodents. Peripheral inflammatory signals, such as the cytokines IL-1β and IL-6, activate prostaglandin E₂ (PGE₂) production in endothelial cells of the blood brain barrier. The PGE₂ binds to EP₃ receptors in the preoptic area of the hypothalamus which projects to the dorsomedial hypothalamus and rostral raphe pallidus nucleus, which in turn control sympathetic innervation of the BAT. Blockage of presympathetic neurons in the rostral raphe pallidus has been shown to block PGE₂ induced thermogenesis. BAT has also been shown to express cytokine receptors and to respond directly to lipopolysaccharide (LPS). In the BAT, uncoupling protein-1 (UCP-1) has an important function in the heat generation through uncoupling of the oxidative phosphorylation in the mitochondria, with the result that the electrochemical gradient is used to produce more heat and less ATP than during normal cell respiration. UCP-1 knockout mice exposed to cold have to use shivering thermogenesis to maintain their body temperature, whereas wild type mice use their brown fat to produce non-shivering thermogenesis, suggesting that UCP-1 is necessary for the latter phenomenon. The aim of present study was to examine the involvement of UCP-1 and BAT activation in inflammation-induced fever, using UCP-1 knockout mice. LPS was injected through an indwelling intravenous catheter to animals supplied with an abdominal temperature transmitter, permitting the administration of the immune challenge without handling the animals and the recording of the body temperature through telemetry (thus avoiding handling stress induced hyperthermia). BAT activation was also evaluated at the transcriptional level by using quantitative real-time PCR. The results show that the temperature response to LPS is similar in WT and UCP-1 knockout mice, suggesting the presence of UCP-1 independent thermogenesis during the febrile response to peripheral inflammation.
CHARACTERIZATION OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1) RELEASE AND DISTRIBUTION IN BLOOD AND LYMPH USING THE UNRESTRAINED RAT MODEL

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Interstitial fluid surrounds the cells in multicellular animals. It supplies nutrients to the cells and provides a means of waste removal. Lymph constitutes the best read out of interstitial fluid composition. Some lipophilic drugs are also drained from the blood and transported by the lymphatic system. Last but not least, intestinal lymph carries the chylomicrons resulting from fat absorption and gastrointestinal peptides that are released in response to eating. Lymph composition is similar to blood plasma composition, but may differ slightly due to differences in the fluid and substrate exchange processes between tissue and lymph or blood as well as differences in various enzyme levels and their activity. Therefore, we assessed intestinal lymph composition in relation to mixed nutrient meals in rats and after intraperitoneal (IP) administration of the gut peptide glucagon-like peptide-1 (GLP-1).

Rats were equipped each with mesenteric lymph duct, hepatic portal vein (HPV) and IP catheters, allowing for parallel sampling of intestinal lymph and HPV blood as well as for GLP-1 administration. After recovery from surgery, baseline samples of lymph and blood were taken followed by consumption of a 9 kcal test meal (1.7 g 60% high fat diet [HFD] or 2.3 g 10% low fat diet [LFD]). Immediately after the test meal, rats were IP injected with GLP-1 (1 or 10 nmol/kg) or corresponding volumes of vehicle control. Thereafter, intestinal lymph and HPV blood were sampled in parallel at 20, 40, 60, 120, and 180 min after meal onset.

After the LFD meal, HPV GLP-1 levels appeared to increase more (54%) than after the HFD meal, suggesting that carbohydrates stimulated GLP-1 release more than fats. The HFD meal did, however, produce a higher (p < 0.05) increase in intestinal lymphatic GLP-1 than the LFD meal, resulting in a 400% increase compared to baseline (p < 0.05). After the LFD meal, endogenous GLP-1 peaked around 20 min in blood (p < 0.05 vs. baseline) and then returned rapidly to baseline levels, whereas in lymph it reached the maximum later (at 40 min) and stayed elevated (p < 0.05) until 120 min after meal onset, when the prandial increase in HPV blood GLP had almost disappeared (p > 0.05 vs. baseline). IP injection of 10 nmol/kg GLP-1 increased (p < 0.05) the peptide concentration in HPV blood and intestinal lymph at 20 and 40 min compared to vehicle, but the GLP-1 appeared to disappear faster from HPV blood than from intestinal lymph. These findings indicate that IP injection of a GLP-1 dose that reliably inhibits eating (10 nmol/kg) produces increases in intestinal lymph and HPV blood that can still be considered within the physiological range. The findings suggest that the fat content of the meal influences the dynamics of the GLP-1 release and distribution.
Glucagon-like peptide-1 (GLP-1) is an incretin and satiation hormone produced in the intestine in response to eating. Previous studies from our laboratory demonstrated that intact vagal afferent neurons (VAN) are required for the satiating effect of intraperitoneally (IP) administered GLP-1. Therefore, we tested whether activation of VAN GLP-1R relay the satiation signal to the brain. We knocked down GLP-1R expression in the VAN by using lentiviral-mediated RNA interference in male Sprague-Dawley rats. Fifty-five % GLP-1R knockdown (KD) in rats fed normal chow increased meal size and duration compared to controls and accelerated gastric emptying (GE) of a test meal. Post-meal glucose levels tended to be higher and energy expenditure (EE) was transiently increased in GLP-1R KD rats. Moreover, GLP-1R KD blunted the satiating and GE inhibitory effects of GLP-1 after IP, but not ICV (4th ventricle) administration. Surprisingly, GLP-1R KD rats fed a high-fat diet (HFD) showed decreased body weight gain, improved glycemic control and an increased energy expenditure compared to control animals. Preliminary data suggest that this phenotype is associated with an increased brown adipose tissue (BAT) UCP1 expression and increased sympathetic input to interscapular BAT.

Our data demonstrate that intact VAN GLP-1R signaling is required for the full expression of the satiating and GE inhibiting effects of endogenous GLP-1. In addition, VAN GLP-1R signaling appears to contribute to some of the hallmarks of HFD-induced obesity.
The role of gastrointestinal (GI) hormones in the pathophysiology of obesity is unclear, although they are involved in the regulation of satiation and glucose metabolism. The objectives of this study were i) to examine glucagon-like peptide 1 (GLP-1), amylin, ghrelin and glucagon responses to a test meal in obese adolescents and ii) to test which GI peptides are most associated with insulin resistance. A total of 16 obese (BMI ≥ 97th percentile for age and gender) and 14 control (BMI between 25th and 75th percentiles) adolescents were included. Subjects were instructed to eat a test meal (490 kcal). Plasma samples were collected for hormone and glucose analysis. Obese adolescents were insulin resistant as expressed by the HOMA index and had significantly increased fasting glucagon and amylin levels compared to the control group (P = 0.003 and 0.044, respectively). In response to the test meal, the increase in GLP-1 levels was reduced in obese adolescents (P < 0.001). In contrast, amylin secretion was significantly increased in the obese population compared to the control group (P < 0.005). Bivariate analysis for HOMA index and post-prandial GLP-1 levels showed a negative correlation (R^2=0.343, P < 0.001). Amylin post-prandial levels and amylin basal concentration demonstrated both a significant positive correlation (R^2 AUC=0.42, R^2 basal=0.46, both P < 0.001). Fasting glucagon showed a positive correlation to HOMA (R^2 0.46, P < 0.001). We conclude that hyperglucagonemia, hyperamylinemia and reduced post-prandial GLP-1 secretion are important pathophysiological steps in the development of metabolic syndrome in adolescents.
Amylin is a peptide hormone produced by pancreatic β-cells that acts in the CNS to reduce food intake. Previous research demonstrates that one mechanism by which the amylin system regulates energy balance involves a cooperative interaction with the adipose tissue-derived hormone leptin; however, the neuroanatomical site(s) mediating this interaction are unresolved. As the ventral tegmental area (VTA) of the mesolimbic reward system is a physiologically relevant site of action for the anorectic effects of both amylin and leptin, we tested the hypothesis that the VTA mediates the cooperative interaction between these hormones to control food intake. Previous research shows that intra-VTA injection of the amylin receptor agonist salmon calcitonin produces hypophagia; here, we show that intra-VTA administration of the native amylin ligand reduces chow intake in rats at doses that are subthreshold for effect when delivered 3rd i.c.v (0.1μg, 0.2μg). Next, we found that intra-VTA co-administration of amylin (0.4μg) and leptin (0.3μg), doses that are each moderately suprathreshold for feeding effect when administered separately in the VTA, produced an enhanced suppression of food intake over 24h that was significantly greater than the effect of either peptide alone. We also tested doses of amylin (0.04μg) and leptin (0.1μg) that alone are subthreshold for feeding effects when administered intra-VTA, and found that the combination of these doses produced a significant reduction in chow intake. Meal pattern analyses showed that the hypophagia produced by the combination of intra-VTA amylin and leptin is driven by a reduction in meal size, rather than meal number. Importantly, the interaction between VTA amylin and leptin receptor signaling appears to be endogenously relevant as VTA amylin receptor blockade by the selective antagonist AC187 (0.3μg) attenuated the intake- and body weight-suppressive effects of intra-VTA leptin (0.6μg). Collectively, these data support the hypothesis that the VTA is a pharmacologically and physiologically relevant site of interaction between amylin and leptin in control of energy balance. Ongoing experiments are evaluating potential intracellular and neuroanatomical mechanisms by which amylin and leptin cooperatively interact in the VTA to promote negative energy balance.

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Failure of weight-reducing dietary regimens is a common complaint of many obese individuals. The current study aims to identify the neurobiological and physiological alterations triggered in response to withdrawal from an obesogenic diet. Here, male Wistar-rats were exposed to either a Chow diet (Control) for 8-weeks or a HFHS diet (lard, 30% sucrose solution) for 4-weeks and Chow for the next 4-weeks (withdrawal). Our data demonstrate that a 4-week withdrawal from a HFHS diet was insufficient to normalize the obese phenotype encountered upon exposure to it. This was evidenced by persistent leptin insensitivity and pronounced adipose tissue levels in the backdrop of decreased caloric consumption. Interestingly, the withdrawal phase was accompanied by lower core body- and brown adipose tissue temperature independent of locomotor activity. Levels of mitochondrial uncoupling protein 1, an indicator of brown adipose tissue thermogenesis were also attenuated. These data thus indicate that during food scarcity, modulation of thermoregulatory pathways is one of the essential mechanisms by which animals defend their elevated adiposity. The neuronal pathways involved in this modulation are currently under investigation.
INCREASED SIRT3 EXPRESSION IN THE ENTEROCYTES LEADS TO INCREASED FOOD INTAKE AND INCREASED ENERGY EXPENDITURE IN MICE ON A CHOW DIET

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Previous studies with a pharmacological modulation of peripheral fatty acid oxidation (FAO) suggest that a stimulation of intestinal FAO inhibits eating in rodents. To further examine this idea we used transgenic mice with a floxed stop codon preceding the Sirtuin-3 gene (SIRT3 is known to transcriptionally activate the FAO enzymes in mitochondria). We crossed these mice with mice expressing the Cre-recombinase (Cre) under the Villin promoter, leading to an over-expression of SIRT3 specifically in the enterocytes. SIRT3 is a NAD⁺-dependent protein deacetylase, which is a global mitochondrial deacetylase important for activating several enzymes involved in FAO. Adult male mice with increased SIRT3 expression specifically in the intestine showed increases in cumulative food intake on chow diet (p < 0.05; 30% increase in total food intake over 48 hours), and energy expenditure (p < 0.05, 46% increase in average energy expenditure per hour), without a significant difference in activity, when compared to control mice with the floxed SIRT3 expression cassette, but no Cre. Body weight gain and total body fat were also unaffected. When placed on a high fat (60% energy fat) diet for 10 weeks, the mice with an overexpression of SIRT3 in the enterocytes increased body weight similarly to control animals, but showed a significant increase in their sensitivity to insulin compared to the control mice (p < 0.05). These findings suggest that a permanent increase in enterocyte FAO increases food intake and energy expenditure in mice on chow diet, but appears to protect from some of the negative effects of high fat diet exposure. Further studies should address the exact mechanisms of these effects, in particular whether the SIRT3 overexpression really enhances enterocyte FAO and whether there is an effect on food intake and energy expenditure when these mice are switched to a high fat diet.
mTORC1 MODULATES FOOD INTAKE BY ACTING ONTO POMC NEURONS: EVIDENCE USING DREADD TECHNOLOGY

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The mammalian target of rapamycin complex 1 (mTORC1) pathway is an intracellular fuel sensing pathway whose hypothalamic activity is known to affect food intake and body weight. Previous data from our laboratory have shown that the central administration of the mTORC1 inhibitor rapamycin rapidly hampers the activity of POMC neurons in the hypothalamic arcuate nucleus (ARC) and decreases hypothalamic α-MSH content during refeeding, which in turn causes hyperphagia. In the current study, we used DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology to provide specific and reversible regulation of POMC neuronal activity in mice, and we demonstrate that induced activation of POMC neurons in the ARC blocks the ability of rapamycin to increase food intake. The human M3-muscarinic receptor was expressed in POMC neurons by stereotaxic infusion of Cre-recombinase-dependent, adeno-associated viral vectors into the ARC of POMC-Cre mice. After injection of the human M3-muscarinic receptor ligand clozapine-N-oxide (1 mg/kg, i.p.) the acute activation of ARC POMC neurons rapidly inhibited the orexigenic effect of i.c.v. rapamycin on fasting-induced food intake at 1 and 2 hours after refeeding. Moreover, our immunofluorescence studies have revealed that DREADD-induced activity of POMC neurons is associated with increased phosphorylation of mTORC1-downstream target S6 in this neuronal population. Altogether, these data demonstrate that the mTORC1 pathway specifically controls activity of refeeding-induced POMC neurons in the ARC and thereby food intake.
INTESTINAL GLUCONEOGENESIS CONTROLS EMOTIONAL BEHAVIOR BY TARGETING HYPOTHALAMUS


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Epidemiological studies strongly support the existence of a bidirectional relationship between both diseases. Depression is associated with a 60% increased risk of type 2 diabetes (T2D) while 10% to 30% of diabetic individuals suffer from major depressive disorders (MDD). Despite these clinical arguments, the neurobiological mechanisms and anatomical substrates linking both diseases remain to be elucidated.

We recently demonstrated that endogenous glucose production by the intestine (IGN: intestinal gluconeogenesis) in a post-absorptive state has beneficial effects on glucose and energy homeostasis. IGN activates glucose sensors present in the portal vein and this “portal glucose signal” is transmitted by vagus nerve projections to brain regions such as the hypothalamus, to decrease food intake and enhance insulin sensitivity. The hypothalamus is a key integrative center of numerous peripheral stimuli, playing a role in both metabolic and emotional processes. In addition, vagus nerve stimulation is effective in reducing the severity of symptoms in patients with MDD but also in relevant animal models of depression. This mechanistic view makes the vagus nerve a key partner in the gut-brain neural circuitry to link peripheral (IGN) and central (emotion) functions. Together, these data raise the possibility that IGN, through its strategic position in the induction of the “portal glucose signal”, represents a putative common regulator linking T2D and MDD.

Based on this hypothesis, we postulated that the absence of IGN might alter both glycemic parameters and emotional behavior. We have generated mice deficient for IGN by targeting the glucose-6-phosphatase catalytic subunit (G6PC) specifically in the intestine (I- G6pc–/ mice). We demonstrated in a parallel study that I-G6pc–/ mice developed a pre-diabetic state. In the open-field test, I-G6pc–/ mice’s immobility is significantly increased by 74% compared to wild-type (WT) animals. The exposure to a novel object intensified this behavior and reduced the number of visits to the center area of the open-field. I-G6pc–/ mice thus displayed a decrease in exploratory behavior compared to WT mice, suggesting the development of anxiety-like phenotype. In the forced swim test, I-G6pc–/ mice stopped swimming earlier and spent more time immobile than WT mice, suggesting the development of depression-like phenotype. Dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis has been found to be linked to depression. I-G6pc–/ mice presented high plasma corticosterone levels and a reduced negative feedback of HPA axis by glucocorticoids.

We propose that IGN, through the regulation of the portal glucose sensing and vagal transmission, might be a key regulator of hypothalamic functions, including control of metabolism and mood.
EFFECT OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR ANTAGONISM ON APPETITE AND FOOD INTAKE IN HEALTHY MEN

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OBJECTIVE: The GLP-1 receptor antagonist exendin(9-39)NH2 (ex9-39) was used to further explore the role of GLP-1 as an endogenous satiation signal.

DESIGN: Two double-blind, 4-way crossover studies were performed, each of which included 10 healthy men. In study A, subjects received an intravenous infusion of ex9-39 or saline (control) plus an oral glucose preload and an intraduodenal infusion of saline or glucose for 60 min. In study B, intravenous infusions were identical, but an oral mixed-liquid meal preload and a 60-min intraduodenal infusion of saline or oleic acid were administered. Thirty minutes after the oral preloads, subjects ate and drank ad libitum, and amounts and calories ingested and the time to meal completion were quantified. In addition, appetite and plasma GLP-1, peptide YY (PYY), insulin, glucagon, and blood glucose concentrations were measured.

RESULTS: In both studies, GLP-1, PYY, and glucagon were substantially higher with intravenous ex9-39 than with intravenous saline (P ≤ 0.001). Insulin was lower with intravenous ex9-39 during intraduodenal glucose (P ≤ 0.05). The decrease in prospective food consumption and desire to eat during ad libitum eating after glucose ingestion was slightly attenuated (P ≤ 0.05 and P ≤ 0.01, respectively) with ex9-39. However, with intravenous ex9-39, food and fluid intakes and eating duration were not changed in either study.

CONCLUSIONS: GLP-1 receptor antagonism slightly modulates appetite during ad libitum eating, but food and fluid intakes and meal duration remain unchanged, suggesting that endogenous GLP-1 is a weak satiation signal. Concomitant substantial increases in plasma PYY and glucagon may, however, counteract a desatiating effect of ex9-39. The effect of ex9-39 on PYY secretion supports an auto-inhibitory feedback mechanism that controls L cell secretion; the effect on insulin and glucagon confirms the role of GLP-1 in glycemic control through its action on pancreatic α and β cells.
THE ANORECTIC ACTIONS OF THE TGF-B CYTOKINE MIC-1/GDF15 REQUIRE AN INTACT BRAINSTEM AREA POSTREMA AND NUCLEUS OF THE SOLITARY TRACT


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Macrophage inhibitory cytokine-1 (MIC-1/GDF15) modulates food intake and body weight under physiological and pathological conditions by acting on the hypothalamus and brainstem. When overexpressed in disease, such as in advanced cancer, elevated serum MIC-1/GDF15 levels lead to an anorexia/cachexia syndrome. To gain a better understanding of its actions in the brainstem we studied MIC-1/GDF15 induced neuronal activation identified by induction of Fos protein.

In mice 60 minutes after a single intraperitoneal injection of MIC-1/GDF15 activated fos protein expressing neurons could be identified in the brainstem area postrema (AP) and the medial (m) portion of the nucleus of the solitary tract (NTS). These activated neurons did not stain with tyrosine hydroxylase (TH). To determine the importance of these brainstem nuclei in the anorexigenic effect of MIC-1/GDF15, we selectively ablated the AP alone or the AP plus the NTS. The latter combined lesion completely reversed the anorexigenic effects of MIC-1/GDF15.

This study identified neurons in the AP and NTS, as being critical for the regulation of food intake and body weight by MIC-1/GDF15.
SERUM LEVELS OF THE TGF-B SUPERFAMILY CYTOKINE MIC-1/GDF15 VARY IN A CIRCADIAN PATTERN, ARE NOT INFLUENCED SIGNIFICANTLY BY FOOD INTAKE AND CORRELATE WITH BMI IN HUMAN MONOZYGOTIC TWIN PAIRS.

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The TGF-b superfamily cytokine MIC-1/GDF15 circulates in the blood of all humans, with a wide normal range of 150-1150pg/ml. These levels may rise markedly in cancer and other diseases leading to anorexia/cachexia, which is mediated by its actions on feeding centres in the hypothalamus and brainstem. More recent studies in mice also suggest it may play a physiological role in control of energy homeostasis.

To determine whether MIC-1/GDF15 also plays a role in physiological regulation of energy homeostasis in man, we examined data and serum from genetically identical, non-obese monozygous twins from the Swedish twin cohort. By studying these samples, we could eliminate the effect of heritable and disease-related confounding factors, and consequently increase the sensitivity of our analysis. In monozygotic twins, between pair differences in serum MIC-1/GDF15 levels were highly correlated with between pair differences in BMI, suggesting a role for MIC-1/GDF15 in the regulation of energy balance in humans.

To understand what physiological factors may regulate serum levels of MIC-1/GDF15 in man, we have examined its serum levels under various conditions. In healthy fasting subjects, MIC-1/GDF15 serum levels varied in a circadian pattern, but did not rise significantly with food intake. However, the gut-derived satiety hormones, CCK and PYY, but not GLP-1, when infused intravenously, induced small increases in serum levels of MIC-1/GDF15.

Taken together, our findings suggest that MIC-1/GDF15 may be a physiological regulator of energy homeostasis in man and that these actions are not explained by satiety factor-like effects, but are more likely to be due to actions on long-term regulation of energy homeostasis.