Editor's Choice

Jean-Claude Carel and Ze'ev Hochberg

New genes involved in disease: a growth-promoting oncogene

Germline KRAS mutations cause Noonan syndrome

Schubbert S, Zenker M, Rowe SL, Boll S, Klein C, Bollag G, Van der Burgt I, Musante L, Kalscheuer V, Wehner LE, Nguyen H, West B, Zhang KY, Sistermans E, Rauch A, Niemeyer CM, Shannon K, Kratz CP
Department of Pediatrics, University of California, San Francisco, Calif., USA
Nat Genet 2006;38:331–336

Introduction: Noonan syndrome (MIM 163950) is characterized by short stature, facial dysmorphism and cardiac defects. Heterozygous mutations in PTPN11, which encodes SHP-2, cause ~50% of cases of Noonan syndrome. The SHP-2 phosphatase relays signals from activated receptor complexes to downstream effectors, including Ras.

Results: De novo germline KRAS mutations that introduce V14I, T58I or D153V amino acid substitutions were found in 5 individuals with Noonan syndrome and a P34R alteration in an individual with cardio-facio-cutaneous syndrome (MIM 115150), which has overlapping features with Noonan syndrome. Recombinant V14I and T58I K-Ras proteins show defective intrinsic GTP hydrolysis and impaired responsiveness to GTPase-activating proteins, render primary hematopoietic progenitors hypersensitive to growth factors and deregulate signal transduction in a cell lineage-specific manner.

Conclusion: Germline KRAS mutations are a cause of human disease. The constellation of developmental abnormalities seen in Noonan syndrome spectrum is, in large part, due to hyperactive Ras.

Approximately 40% of individuals with clinically diagnosed Noonan syndrome have gain-of-function mutations in protein-tyrosine phosphatase SHP2. But what about the rest, and why do so many case reports link the syndrome with leukemia and other malignancies? We now learn of the Ras connection. The 3 Ras oncogenes, HRas, KRas and NRas, encode 21-kDa proteins called p21s, and provide a regulated change in activity of an oncogene (acquire malignant properties by single point mutations) in a physiologic growth process. Transforming KRas genes were identified in 8 different carcinomas and sarcomas. HRas gene mutations were found in 12 of 13 individuals with the similar Costello syndrome, with a tendency to develop rhabdomyosarcoma. The new finding of KRas mutations in Noonan syndrome suggests that the activation of the Ras-Mapk pathway is the common underlying mechanism of Noonan syndrome and Costello syndrome, and possibly of cardio facio cutaneous syndrome. For the pediatric endocrinologist, this may open an inquiry into the role of the Ras in child growth and Noonan's short stature (though it is to be kept in mind that adult height is in the normal range in 30%).

Food for thought: and glucose for your brain

The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism

Koo SH, Flechner L, Qi L, Zhang X, Sreaton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P, Takemori H, Montminy M
Peptide Biology Laboratories, Salk Institute for Biological Studies, La Jolla, Calif., USA
Nature 2005;437:1109–1111

Introduction: Glucose homeostasis is regulated systemically by hormones such as insulin and glucagon, and at the cellular level by energy status. Glucagon enhances glucose output from the liver during fasting

by stimulating the transcription of gluconeogenic genes via the cyclic AMP-inducible factor CREB (CRE-binding protein). When cellular ATP levels are low, however, the energy-sensing kinase AMPK inhibits hepatic gluconeogenesis through an unknown mechanism.

Results: Hormonal and energy-sensing pathways converge on the coactivator TORC2 (transducer of regulated CREB activity 2) to modulate glucose output. Sequestered in the cytoplasm under feeding conditions, TORC2 is dephosphorylated and transported to the nucleus where it enhances CREB-dependent transcription in response to fasting stimuli. Conversely, signals that activate AMPK attenuate the gluconeogenic program by promoting TORC2 phosphorylation and blocking its nuclear accumulation.

Implications: Individuals with type 2 diabetes often exhibit fasting hyperglycemia due to elevated gluconeogenesis; compounds that enhance TORC2 phosphorylation may offer therapeutic benefits in this setting.

Kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin

Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC

Department of Systems Biology, Harvard Medical School, Boston, Mass., USA

shaw@salk.edu

Science 2005;310:1642–1646

Background: The Peutz-Jegher syndrome tumor-suppressor gene encodes a protein-threonine kinase, LKB1, which phosphorylates and activates AMPK (adenosine monophosphate (AMP)-activated protein kinase).

Results: The deletion of LKB1 in the liver of adult mice resulted in a nearly complete loss of AMPK activity. Loss of LKB1 function resulted in hyperglycemia with increased gluconeogenic and lipogenic gene expression. In LKB1-deficient livers, TORC2, a transcriptional coactivator of CREB (cAMP response element-binding protein), was dephosphorylated and entered the nucleus, driving the expression of peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), which in turn drives gluconeogenesis. Adenoviral small hairpin RNA (shRNA) for TORC2 reduced PGC-1 α expression and normalized blood glucose levels in mice with deleted liver LKB1, indicating that TORC2 is a critical target of LKB1/AMPK signals in the regulation of gluconeogenesis. Metformin, one of the most widely prescribed type 2 diabetes therapeutics, required LKB1 in the liver to lower blood glucose levels.

Conclusion: Kinase LKB1 is an important mediator of liver glucose homeostasis and metformin's effects.

If your slide on glucose control has not been loaded enough, you can now supplement it with the TORCs, a family of latent cytoplasmic coactivators that enhance cellular gene expression via interaction with the cAMP-responsive factor CREB leucine zipper (bZIP). CREB in turn stimulates hepatic gluconeogenesis and fatty acid oxidation by inducing expression of PPAR- γ and its coactivators. The authors found that both stimulated hepatic CREB phosphorylation. TORC2 was phosphorylated under ad libitum feeding or insulin-stimulated conditions, and were dephosphorylated in response to glucagon or fasting. Thus, TORC2 promotes glucose output in response to fasting signals, and promotes hyperglycemia. The report elaborates on several other details, but the bottom line is that TORC2 is a critical switch that modulates the gluconeogenic program in response to both hormonal and intracellular signals. On the practical side, a number of adipokines such as adiponectin and resistin have been shown to modulate hepatic gluconeogenesis via an AMPK-dependent mechanism; these results provide a regulatory framework with which to test these regulatory inputs. The other paper addresses the role of LKB1, a protein kinase involved in Peutz-Jegher syndrome (known to pediatric endocrinologists by its risk for gonadal tumors and gynecomastia). This protein kinase targets TORC2. Mice whose livers are deficient in LKB1 are diabetic with a profile reminiscent of type 2 diabetes and increased liver gluconeogenesis. Metformin, widely used for the treatment of type 2 diabetes, activates AMPK. Its actions are due to the blocking of hepatic gluconeogenesis and stimulation of glucose uptake in muscle. Mice with a specific liver LKB1 deficiency are resistant to the blood glucose-lowering effects of metformin, indicating that the liver LKB1/TORC2 pathway is essential to its action (and therefore minimizes the importance of the drug's effects on glucose uptake in the periphery). Finally, these observations also provide a further link between tumorigenesis (in Peutz-Jegher syndrome) and nutrient metabolism.

Regulation of blood glucose by hypothalamic pyruvate metabolism

Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L

Departments of Medicine and Molecular Pharmacology, Diabetes Research Center, Albert Einstein College of Medicine, Bronx, N.Y., USA

Science 2005;309:943–947

Introduction: The brain keenly depends on glucose for energy, and mammals have redundant systems to control glucose production. An increase in circulating glucose inhibits glucose production in the liver, but this negative feedback is impaired in type 2 diabetes.

Results: A primary increase in hypothalamic glucose levels lowered blood glucose through inhibition of glucose production in rats. The effect of glucose requires its conversion to lactate, followed by stimulation of pyruvate metabolism which leads to activation of adenosine triphosphate (ATP)-sensitive potassium channels.

Implications: Interventions designed to enhance the hypothalamic sensing of glucose may improve glucose homeostasis in diabetes.

Hypothalamic glucose sensing plays an important role in preserving nutrient homeostasis. This becomes important in the paradoxical diabetic hyperglycemia, when the rate of liver glucose production increases further and inappropriately. You recall that blood glucose gains access to the CNS by means of a facilitated transport system, and indeed, the extracellular concentration of glucose in the brain is significantly lower ($\sim 2\text{ mM}$) than its concentration in blood ($\sim 5\text{ mM}$). However, it remains controversial whether neurons directly metabolize glucose to pyruvate or use lactate derived from anaerobic glycolysis of glucose within astrocytes; the transfer of glucose-derived lactate from astrocytes to neurons is referred to as the 'astrocyte-neuron lactate shuttle'. This paper reports that an increase in hypothalamic glucose levels lowers blood glucose through inhibition of glucose production in rats, and that the effect of glucose requires its conversion to lactate, followed by stimulation of pyruvate metabolism, which leads to activation of ATP-sensitive potassium channels. Neurons appear to have developed mechanisms for glucose sensing designed to protect them from hypoglycemic injury by triggering the rapid secretion of counterregulatory hormones in response to low extracellular glucose levels. A moderate increment of extracellular glucose levels within a specific region of the hypothalamus is sufficient to lower blood glucose levels through a robust inhibition of liver glucose production. This discovery paves the way for biochemical interventions designed to restore central glucose sensing and glucose homeostasis.

A new hormone: spinning for longevity

Suppression of aging in mice by the hormone Klotho

Kurosuo H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M

Department of Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas, Tex., USA

Science 2005;309:1829–1833

Introduction: A defect in Klotho gene expression in mice accelerates the degeneration of multiple age-sensitive traits.

Results: Overexpression of Klotho in mice extends life span. Klotho protein functions as a circulating hormone that binds to a cell-surface receptor and represses intracellular signals of insulin and insulin-like growth factor 1 (IGF-1), an evolutionarily conserved mechanism for extending life span. Alleviation of aging-like phenotypes in Klotho-deficient mice was observed by perturbing insulin and IGF-1 signaling.

Conclusions: Klotho-mediated inhibition of insulin and IGF-1 signaling contributes to its anti-aging properties. Klotho protein may function as an anti-aging hormone in mammals.

Klotho, daughter of Zeus and Themis, is the Greek goddess of spinning, and linked with Lachesis and Atropos as one of a trio of the Moirai or Fates. Klotho was originally reported as a mutated gene in a mouse strain that accelerates age-dependent loss of function in several age-sensitive traits. It encodes a transmembrane protein that is expressed mostly in distal convoluted tubules of the kidney and the choroid plexus in the brain. Because a defect in the Klotho gene results in age-dependent degeneration, the Klotho protein may function through a circulating humoral factor that regulates the development of age-related disorders or natural aging processes. It was later observed that polymorphisms in the human Klotho gene are associated with stroke, osteoporosis, altered life span and altered risk for coronary artery disease. We now learn that the extracellular domain of Klotho is shed from the cell surface (remember GH-binding protein?) and the extracellular soluble product inhibits intracellular insulin and IGF-1 signaling. It increases resistance to IGF-1 and insulin, and mice with Klotho deficiency have reduced blood glucose and insulin levels and enhanced insulin sensitivity directly in peripheral tissues. The mechanism of this action includes suppressing tyrosine phosphorylation of insulin and IGF-1 receptors, which results in reduced activity of IRS proteins and their association with PI_3 -kinase, thereby inhibiting insulin and IGF-1 signaling. That negative regulation of insulin and IGF-1 extends life span has been previously suggested as an evolutionarily conserved mechanism to suppress aging, and Klotho seems to be a novel hormone to modulate such signaling and thereby mediate insulin metabolism and aging. Additional reports on Klotho (definitely a hot topic) identify its β -glucuronidase activity capable of hydrolyzing an ion channel and involved in calcium homeostasis [1].

New mechanisms: hormones of affection

Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior

Fries AB, Ziegler TE, Kurian JR, Jacoris S, Pollak SD

Department of Psychology, University of Wisconsin, Madison, Wisc., USA

Proc Natl Acad Sci USA 2005;102:17237–17240

Introduction: The formation of social attachments is a critical component of human relationships. Infants begin to bond to their caregivers from the moment of birth, and these social bonds continue to provide regulatory emotional functions throughout adulthood.

Methods: To examine children who were reared in extremely aberrant social environments, where they were deprived of the kind of caregiving typical for our species.

Results: The vasopressin and oxytocin neuropeptide systems, which are critical in the establishment of social bonds and the regulation of emotional behaviors, are affected by early social experience.

Implications: This might be a potential mechanism whose atypical function may explain the pervasive social and emotional difficulties observed in many children who have experienced aberrant caregiving. The findings are consistent with the view that there is a critical role for early experience in the development of the brain systems underlying basic aspects of human social behavior.

Suckling leads to an increase in oxytocin (OT) levels both within the CNS and peripherally in rats, and increased central and peripheral OT levels have been demonstrated after exposure to an emotional stressor. Human infants require special bonding by caregivers, which are essential for survival and healthy adaptation. It was previously shown that OT and vasopressin (AVP) are integral parts of social bonding, parental care, stress regulation, social communication, and emotional reactivity. Children who had experienced early neglect had lower overall levels of AVP than family-reared children, suggesting that social deprivation may inhibit the development of the AVP system. Functionally, central AVP appears to be critical for recognizing familiar individuals, a key component of forming social bonds. The present study shows the imprinting effect of OT and AVP. OT levels for family-reared children increased after physical contact with their mothers. Children who experienced early neglect did not show this response after physical contact with their mothers. The evolutionary perspective provides a window into the relationship between early postnatal experience and the emergence of a

complex bio-behavioral system. A failure to receive care disrupts the normal development of the OT and AVP systems in young children. Perturbations in this system may interfere with the calming and comforting effects that typically emerge between young children and familiar adults who provide care and protection. Along the same line, here is another perspective on AVP.

The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study

Bielsky IF, Hu SB, Ren X, Terwilliger EF, Young LJ

Department of Psychiatry and The Center for Behavioral Neuroscience, Emory University, Atlanta, Ga., USA

alanwithisa@yahoo.com

Neuron 2005;47:503–513

Introduction: Vasopressin modulates many social and nonsocial behaviors, including emotionality; male mice with a null mutation in the V1a receptor (V1aR) exhibit a profound impairment in social recognition and changes in anxiety-like behavior.

Methods: Site-specific injections of a V1aR-specific antagonist and re-expressing V1aR in the lateral septum of V1aR knockout mice (V1aRKO) using a viral vector.

Results: The lateral septum, but not the medial amygdala, is critical for social recognition. Re-expression of the V1aR resulted in a complete rescue of social recognition, and overexpression in the lateral septum of wild-type (wt) mice resulted in a potentiation of social recognition behavior and a mild increase in anxiety-related behavior.

Conclusions: The V1aR in the lateral septum plays a critical role in the neural processing of social stimuli required for complex social behavior.

The vasopressin receptor family consists of three receptors: the vasopressin-1a receptor (V1aR; expression in liver and to a lesser degree in heart, kidney, brain and skeletal muscle), the vasopressin-1b receptor (V1bR, antidiuretic), and vasopressin-2 receptor (V2R, pressor effects). The extensive distribution of AVP throughout the brain presents a challenge for determining the role of specific AVP receptors in particular behaviors, including flank-marking behavior, paternal behavior, social recognition, aggression, affiliation behavior, and anxiety-like behavior. We now learn that V1aR in the lateral septum, a relay point in the social olfactory circuit, regulates social recognition in delivering necessary information to the hypothalamus. AVP through its V1aR may be the modulator required for social cognition and aspects of anxiety-like behavior.

Concepts revised: another kind of attachment

Drosophila Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins

Hampoez B, Hoeller O, Bowman SK, Dunican D, Knoblich JA

Institute of Molecular Biotechnology, Austrian Academy of Sciences, Vienna, Austria

Nat Cell Biol 2005;7:1099–1105

Introduction: Heterotrimeric G proteins act during signal transduction in response to extracellular ligands. They are also required for spindle orientation and cell polarity during asymmetric cell division.

Results: In *Drosophila*, both functions require the G- α interaction partner Ric-8. *Drosophila* Ric-8 is a cytoplasmic protein that binds both the GDP- and GTP-bound form of the G-protein α -subunit Galphai. In ric-8 mutants, neither Galphai nor its associated β -subunit G β 13F are localized at the plasma membrane, which leads to their degradation in the cytosol. During asymmetric cell division, this leads to various defects: apico-basal polarity is not maintained, mitotic spindles are misoriented and the size of the two daughter cells becomes nearly equal. ric-8 mutants also have defects in gastrulation that resemble mutants in the G α protein concertina or the extracellular ligand folded gastrulation.

Conclusions: Both receptor-dependent and receptor-independent G-protein functions are executed at the plasma membrane and require the Ric-8 protein.

You certainly recall that G proteins are composed of non-identical α , β and γ subunits. In most cases, when a $G\alpha$ subunit binds GDP, it forms a complex with a $G\beta$ and a $G\gamma$ subunit, and it functionally dissociates from the $G\beta\gamma$ - $\beta\gamma$ complex when it binds GTP. The pathway is activated by two types of $G\alpha$ -binding proteins, GDP dissociation inhibitors (GDI) and RIC-8, a non-receptor guanine nucleotide-exchange factor (GEF) for $G\alpha$. Three reports now show that RIC-8 is essential for plasma-membrane localization of G-protein subunits and provide further insights into the RIC-8-mediated regulation of heterotrimeric G-protein signaling. In the absence of RIC-8, the G-protein subunits, $G\alpha$ i and $G\beta$ -13F, become cytoplasmic. Hampoelz et al. also show that RIC-8 does not function as a GEF for $G\alpha$, and that RIC-8 binds both the GDP and GTP forms of $G\alpha$ – a result that contradicts previous data.

Review, GH and anti-aging: no alternative to medicine

Provision or distribution of growth hormone for 'anti-aging': clinical and legal issues

Perls TT, Reisman NR, Olshansky SJ

Geriatrics Section, Department of Medicine, Boston Medical Center and Boston University School of Medicine, Boston, Mass., USA

thperls@bu.edu

JAMA 2005;294:2086–2090

Thirty percent of GH prescriptions in the United States are for indications not approved by the Food and Drug Administration (FDA), including anti-aging and abuse by athletes. In addition, homeopathic GH and IGF-1, with concentrations of 10^{-30} M (the odds for the presence of a single molecule is 1 in 10 million) are also sold for a cost of some USD 80 a month (good business for tap water)! Other pills and sprays allegedly containing GH are also on sale. Richard Dawkins nicely put it: *Alternative medicine is defined as that set of practices that cannot be tested, refuse to be tested or consistently fail tests.* This definition is in agreement with that of the FDA for health fraud as *the promotion, for profit, of a medical remedy known to be false or unproven.* Growth hormone has been documented to improve some measures of body composition, including a slight increased muscle mass, reduced total body fat, improved skin elasticity, and reduced rate of bone demineralization, but without positive effects on strength, functional capacity, or metabolism. Furthermore, the positive effects may be short-lived: in a study of 148 patients with adult GHD, the 5% increase in lean body mass disappeared after 24 months of treatment, and 38% of study participants dropped out because of lack of subjective improvement. On the other hand, carpal tunnel syndrome developed in 38% of women and 24% of men taking anti-aging GH, edema in 39 and 30%, and arthralgia in 46 and 41%, respectively [2]. No studies have assessed the long-term efficacy or safety of GH as an anti-aging intervention. Transgenic mice that produce supraphysiological levels of GH have markedly reduced life spans and experience premature onset of age-related cognitive changes, and GH-resistant and GH-deficient mutant mice experience substantially increased life spans.

Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution

Nishio J, Gaglia JL, Turvey SE, Campbell C, Benoist C, Mathis D

Section on Immunology and Immunogenetics, Joslin Diabetes Center, and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, and Harvard Stem Cell Institute, Boston, Mass., USA

Science 2006;311:1775–1778

Background: A cure for type 1 diabetes will probably require the provision or elicitation of new pancreatic islet β cells as well as the re-establishment of immunological tolerance. A 2003 study reported achievement of both advances in the NOD mouse model by coupling injection of Freund's complete adjuvant with infusion of allogeneic spleen cells. It was concluded that the adjuvant eliminated anti-islet autoimmunity and the donor splenocytes differentiated into insulin-producing (presumably β) cells, culminating in islet regeneration.

Results: The authors provide data indicating that the recovered islets were all of host origin, reflecting that the diabetic NOD mice actually retain substantial β -cell mass, which can be rejuvenated/regenerated to reverse disease upon adjuvant-dependent dampening of autoimmunity.

A cure for type 1 diabetes basically requires two interventions: to restore the β -cell mass and to prevent the recurrence of autoimmunity. Current approaches envision either intervening prior to islet destruction (preventive therapies) or after the onset of diabetes via restoration of islet mass by transplantation of allogeneic or stem cell-derived islets, or maybe through islet neogenesis. It is not known to what extent islet neogenesis can occur because the ongoing islet-destructive process keeps destroying the newly produced islets. However, we can just think of another endocrine gland, the adrenal, where we often see intratesticular adrenal rest neogenesis in congenital adrenal hyperplasia, but never in autoimmune adrenal deficiency. In 2003, the group led by Denise Faustman [3] presented data where they could cure diabetes in NOD mice using a combination of complete Freund's adjuvant and allogeneic splenocytes. The use of Freund's adjuvant had been known for a long time to prevent diabetes in NOD mice but, unfortunately, clinical trials in humans were unsuccessful. What was more surprising in Faustman's experiments [3] was that the newly formed islets appeared to be derived from the infused splenocytes, suggesting that hematopoietic cells contained stem cells capable of transdifferentiating into islet endocrine cells. In this recent issue of *Science*, three groups independently refute this experiment [4, 5]. They all show that Freund's adjuvant can cure the disease in some diabetic NOD mice (albeit to a lesser extent than in the earlier report). They completely refute that spleen cells can transdifferentiate into islets. Rather they show that the cure of diabetes is due to islet regeneration from the endogenous pancreas. This is bad news for those believing that lead can turn into gold. However, it is good news for those who think that intervening on the immune process might be sufficient to let the islet mass recover. However, we have to remember that the NOD mouse is a very permissive model, and that most interventions shown to be effective in this model are not so when tested in humans.

Noninvasive diagnosis of focal hyperinsulinism of infancy with [¹⁸F]-DOPA positron emission tomography

Otonkoski T, Nanto-Salonen K, Seppanen M, Veijola R, Huopio H, Hussain K, Tapanainen P, Eskola O, Parkkola R, Ekstrom K, Guioy Y, Rahier J, Laakso M, Rintala R, Nuutila P, Minn H
Hospital for Children and Adolescents, and Program of Developmental and Reproductive Biology, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland
timo.otonkoski@helsinki.fi
Diabetes 2006;55:13–18

Background: Congenital hyperinsulinism of infancy (CHI) is characterized by severe hypoglycemia due to dysregulated insulin secretion, associated with either focal or diffuse pathology of the endocrine pancreas. The focal condition is caused by a paternally inherited mutation in one of the genes encoding the subunits of the β -cell ATP-sensitive potassium channel (SUR1/ABCC8 or Kir6.2/KCNJ11) and somatic loss of maternal 11p15 alleles within the affected area. Until now, preoperative diagnostics have relied on technically demanding and invasive catheterization techniques.

Methods: The authors evaluated the utility of fluorine-18 L-3,4-dihydroxyphenylalanine ([¹⁸F]-DOPA) positron emission tomography (PET) to identify focal pancreatic lesions in 14 CHI patients, 11 of which carried mutations in the ABCC8 gene (age 1–42 months). To reduce bias in PET image interpretation, quantitative means for evaluation of pancreatic [¹⁸F]-DOPA uptake were established.

Results: Five patients had a visually apparent focal accumulation of [¹⁸F]-DOPA and standardized uptake value (SUV) >50% higher (mean 1.8-fold) than the maximum SUV of the unaffected part of the pancreas. When these patients were operated on, a focus of 4–5 × 5–8 mm matching with the PET scan was found, and all were normoglycemic after resection of the focus. The remaining 9 patients had diffuse accumulation of [¹⁸F]-DOPA in the pancreas (SUV ratio <1.5). Diffuse histology was verified in 4 of these, and pancreatic catheterization was consistent with diffuse pathology in 4 cases.

Conclusion: [¹⁸F]-DOPA PET is a promising noninvasive method for the identification and localization of the focal form of CHI.

Noninvasive imaging of pancreatic inflammation and its reversal in type 1 diabetes

Turvey SE, Swart E, Denis MC, Mahmood U, Benoist C, Weissleder R, Mathis D
Section on Immunology and Immunogenetics, Joslin Diabetes Center, and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Mass., USA
J Clin Invest 2005;115:2454–2461

Background: A major stumbling block for research on and treatment of type 1 diabetes is the inability to directly, but noninvasively, visualize the lymphocytic/inflammatory lesions in the pancreatic islets. One potential approach to surmounting this impediment is to exploit MRI of magnetic nanoparticles (MNP) to visualize changes in the microvasculature that invariably accompany inflammation.

Results: MNP-MRI did indeed detect vascular leakage in association with insulinitis in murine models of type 1 diabetes, permitting noninvasive visualization of the inflammatory lesions in vivo in real time. The authors demonstrate, in proof-of-principle experiments, that this strategy allows to predict, within 3 days of completing treatment with an anti-CD3 monoclonal antibody, which NOD mice with recent-onset diabetes are responding to therapy and may eventually be cured. Importantly, an essentially identical MNP-MRI strategy has previously been used with great success to image lymph node metastases in prostate cancer patients.

Conclusion: This success strongly argues for rapid translation of these preclinical observations to prediction and/or stratification of type 1 diabetes and treatment of individuals with the disease; this would provide a crucially needed early predictor of response to therapy.

Pancreatic islet cells constitute approximately 1% of pancreatic mass and yet contribute to health and disease through production of various insular hormones. Unfortunately, the only access we have to pathology occurring in islets is through hormonal measurements and their consequences on

circulating substrate levels. Although endoscopic ultrasound is widely used for the diagnosis of insular tumors, we had no current methods to image the islets. These two reports give new hopes that we will be able to follow the functional pancreatic islet mass and use this information to guide our clinical decisions. First in neonatal hyperinsulinism, the group of Jean-Marie Saudubray has deciphered the existence of focal and diffuse forms, the former curable by a partial pancreatectomy. However, complex preoperative approaches, based on portal vein sampling or selective arteriographic delivery of stimuli, had to be used to differentiate the two forms preoperatively. Here, it is shown that PET imaging using fluoro-DOPA can accurately detect the focal forms and allow a preoperative diagnosis. A similar report was also published recently by the group from Necker Hospital [6]. The selective uptake of this tracer by functional islets predicts that the method may be useful in other clinical situations such as type 1 diabetes. The report on the use of magnetic nanoparticles to image pancreatic inflammation relies on the particularity of the islet to drive a large proportion of the pancreatic blood flow and on the nanoparticles to leak into the perivascular space in case of local inflammation (insulinitis). It is shown that this technique can efficiently detect insulinitis with clinical correlates in several mouse models. There is still a lot to do before we can use these techniques to evaluate disease progression in individuals at risk, but we might soon get out of the dark ages of isletology.

New mechanism: the role of neurogenesis and neuronal plasticity in the control of energy balance

Neurogenesis in the hypothalamus of adult mice: potential role in energy balance

Kokoeva MV, Yin H, Flier JS

Division of Endocrinology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass., USA

Science 2005;310:679–683

Background: Ciliary neurotrophic factor (CNTF) induces weight loss in obese rodents and humans, and for reasons that are not understood, its effects persist after the cessation of treatment.

Results: Centrally administered CNTF induces cell proliferation in feeding centers of the murine hypothalamus. Many of the newborn cells express neuronal markers and show functional phenotypes relevant for energy-balance control, including a capacity for leptin-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3). Coadministration of the mitotic blocker cytosine- β -*d*-arabinofuranoside (Ara-C) eliminates the proliferation of neural cells and abrogates the long-term, but not the short-term, effect of CNTF on body weight.

Conclusion: These findings link the sustained effect of CNTF on energy balance to hypothalamic neurogenesis and suggest that regulated hypothalamic neurogenesis in adult mice may play a previously unappreciated role in physiology and disease.

NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates

Luquet S, Perez FA, Hnasko TS, Palmiter RD

Howard Hughes Medical Institute and Department of Biochemistry, University of Washington, Seattle, Wash., USA

Science 2005;310:683–685

Background: Hypothalamic neurons that express neuropeptide Y (NPY) and agouti-related protein (AgRP) are thought to be critical regulators of feeding behavior and body weight.

Methods: To determine whether NPY/AgRP neurons are essential in mice, the authors targeted the human diphtheria toxin receptor to the *Agrp* locus, which allows temporally controlled ablation of NPY/AgRP neurons to occur after an injection of diphtheria toxin.

Results: Neonatal ablation of NPY/AgRP neurons had minimal effects on feeding, whereas their ablation in adults caused rapid starvation.

Conclusion: These results suggest that network-based compensatory mechanisms can develop after the ablation of NPY/AgRP neurons in neonates but do not readily occur when these neurons become essential in adults.

Identifying the key neuronal population and neurotransmitters in the control of energy homeostasis is a prerequisite for understanding the physiological basis of weight regulation and fighting the obesity epidemic. This is generally tested in experimental animals by selective ablation of hypothalamic nuclei, use of agonists or antagonists or more recently gene invalidation technologies. These two elegant papers highlight the need to take age into account when interpreting these experiments, something pediatric endocrinologists already knew. The Boston group used the effect of the neurocytokine ciliary neurotrophic factor (CNTF) to protect the mice against diet-induced obesity. They show that generation of new neurons is needed for CNTF to exert its long-lasting effects and that neurogenesis is an obligatory mechanism in this effect. Interestingly, leptin-deficient mice remain responsive to the short-term weight-reducing effects of CNTF but lack the long-term effect and do not display hypothalamic neurogenesis. Hypothalamic rewiring and generation of new neurons therefore appear to mediate not only the weight-reducing effect of CNTF but its capacity to rehabilitate the hypothalamus, an effect we are striving for when caring for obese children. Axokine, a recombinant variant of CNTF, has been evaluated with some success in obese patients and more studies are needed [7]. In the second paper, the NPY/AgRP neurons that play an important role in feeding behavior were made sensitive to the toxic effects of diphtheria toxin using transgenic technology. This allows to destroy this neuronal population by subcutaneous injection of the toxin. When the toxin is injected in adult mice, the NPY/AgRP neurons are depleted and the animals lose weight. In contrast, injection of the toxin in the neonatal period has no effect on weight. However, the NPY/AgRP neurons are destroyed and this destruction persists through adulthood. The interpretation of the findings is that rewiring can occur in the neonates to compensate for the loss but that this cannot happen in adults. From a more practical point of view, it highlights the plasticity of the neonatal brain and suggests that early interventions could have a long-lasting effect on feeding behavior.

New mechanism: β cells sensing defects beyond MODY-2: Glut-2 deficiency

Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes

Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Takeuchi M, Marth JD

Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, Calif., USA

Cell 2005;123:1307–1321

Background: Pancreatic β -cell surface expression of glucose transporter 2 (Glut-2) is essential for glucose-stimulated insulin secretion, thereby controlling blood glucose homeostasis in response to dietary intake.

Results: The authors show that the murine GlcNAcT-IVa glycosyltransferase is required for Glut-2 residency on the β -cell surface by constructing a cell-type- and glycoprotein-specific N-glycan ligand for pancreatic lectin receptors. Loss of GlcNAcT-IVa, or the addition of glycan-ligand mimetics, attenuates Glut-2 cell-surface half-life, provoking endocytosis with redistribution into endosomes and lysosomes. The ensuing impairment of glucose-stimulated insulin secretion leads to metabolic dysfunction diagnostic of type 2 diabetes. Remarkably, the induction of diabetes by chronic ingestion of a high-fat diet is associated with reduced GlcNAcT-IV expression and attenuated Glut-2 glycosylation coincident with Glut-2 endocytosis.

Conclusion: β -Cell glucose-transporter glycosylation mediates a link between diet and insulin production that typically suppresses the pathogenesis of type 2 diabetes.

Post-translational protein modification plays a major functional role, as demonstrated by the multiple organs and systems involvement in congenital disorders of glycosylation [8]. The role of protein

glycosylation in glucose homeostasis is less known, and this paper analyzed the role of GlcNAcT-IV glycosyltransferase (GnT-4a), an enzyme involved in the making of glycan structures. Mice deficient in this enzyme are also diabetic (there is obviously more than one way to skin a cat) with fasting and post-prandial hyperglycemia and decreased insulin secretion. The defect was pinpointed to decreased cell surface expression of Glut-2, the islet- and liver-specific glucose transporter which accumulates intracellularly in endosomes and lysosomes. In kinetic experiments, Glut-2 half-life at the cell surface was decreased 4-fold. Glycosylated Glut-2 interacts with lectins at the cell surface, reducing the rate of endocytosis. Glycosylation of Glut-2 therefore slows down the turnover of the molecule at the cell surface.

Sensing extracellular glucose is the first step of insulin secretion, mediated by the glucose transporter Glut-2. Decreased Glut-2 expression on β -cells has been observed in several animal models of diabetes. The second step is phosphorylation by glucokinase, whose deficiency leads to MODY type 2. This paper shows that altering the kinetics of Glut-2 at the cellular surface has profound consequences on glucose sensing. Of note, the human ortholog of the gene encoding GnT-4a is coded within a region linked to type 2 diabetes susceptibility. More generally, we have to remember that there is more to a protein than its amino acid sequence and that a normal DNA sequence does not mean no involvement in a pathogenic process.

New mechanism: intracellular transfer of hormones: no longer a passive step

Role of endocytosis in cellular uptake of sex steroids

Hammes A, Andreassen TK, Spoelgen R, Raila J, Hubner N, Schulz H, Metzger J, Schweigert FJ, Lippa PB, Nykjaer A, Willnow TE
Max Delbrueck Center for Molecular Medicine, Berlin, Germany
Cell 2005;122:751–762

Background: Androgens and estrogens are transported bound to the sex hormone-binding globulin (SHBG). SHBG is believed to keep sex steroids inactive and to control the amount of free hormones that enter cells by passive diffusion.

Results: Contrary to the free hormone hypothesis, it is shown that megalin, an endocytic receptor in reproductive tissues, acts as a pathway for cellular uptake of biologically active androgens and estrogens bound to SHBG. In line with this function, lack of receptor expression in megalin knockout mice results in impaired descent of the testes into the scrotum in males and blockade of the vagina opening in females. Both processes are critically dependent on sex-steroid signaling, and similar defects are seen in animals treated with androgen- or estrogen-receptor antagonists.

Conclusion: The findings uncover the existence of endocytic pathways for protein bound androgens and estrogens and their crucial role in development of the reproductive organs.

We have all been taught that fat-soluble small hormonal molecules that bind to nuclear hormone receptors pass the plasma membrane freely to enter the cytoplasm. In 1999 it was shown that mice deficient in megalin, a member of the low-density lipoprotein receptor-related protein family, had features of vitamin D deficiency. This apparent deficiency was due to their inability to internalize the vitamin D-binding protein complex and resulted in a rickets-like phenotype. Last year, we learnt that passive intracellular entry is no longer true for thyroid hormones with the discovery of the thyroid hormone transport MC T8 and severe mental retardation when this protein is deficient. Here, the gonadal phenotype of megalin-deficient mice was assessed and led to the conclusion that endocytic mechanisms are involved in sex steroids uptake. Most megalin-deficient mice die perinatally from a defect in forebrain development. In the few survivors, features of sex steroid hormone resistance (high testosterone values with undermasculinization) were noticed, including vaginal opening abnormalities in females (estrogen-dependent) and of testicular descent in males (androgen-dependent). In addition, it is shown that megalin can internalize complexed SHBG-testosterone. However, it should be noted that megalin-deficient mice are not phenocopies of androgen or estrogen receptor-deficient

mice. There are certainly megalin-independent pathways for sex steroid entry into the target cells. From a basic standpoint, the results suggest a megalin-dependent SHBG-sex steroid complex internalization in an endocytic vesicle. How the sex steroid hormone finally finds its way to the cytoplasm to encounter its cognate receptor still needs to be deciphered, and several hypotheses can be made [9]. From a clinical standpoint, it suggests that the concept of measuring 'free' testosterone in the serum as an index of biologically relevant androgen might have to be re-examined.

Concepts revised. Unexpected news from an old friend: growth hormone produced in the brain

Growth hormone is produced within the hippocampus where it responds to age, sex, and stress

Donahue CP, Kosik KS, Shors TJ

Department of Neurology and Center for Neurologic Disease, Harvard Medical School, Boston, Mass., USA

Proc Natl Acad Sci USA 2006;103:6031–6036

Background: Recent studies have demonstrated that growth hormone (GH) is produced endogenously within the hippocampal formation, a brain structure associated with learning and aspects of emotional experience.

Results: This endogenously produced GH is modulated by age, sex differences and the presence of estrogen. GH mRNA levels were higher in females than males, especially during proestrus, a stage of estrus when estrogen levels are elevated. Moreover, GH expression was increased in ovariectomized females that were treated with estradiol. This increase in GH mRNA in response to estrogen was followed by the appearance of GH protein and was negatively correlated with the expression levels of insulin-like growth factor-I mRNA, suggesting a feedback relationship between insulin-like growth factor-I and GH in the brain. GH mRNA levels were also elevated in primary neuronal cultures exposed to 17 β -estradiol in vitro, further confirming the direct influence of estrogen on GH expression. Finally, exposure to an acute stressful event increased the expression and production of GH in both males and females. However, the stress-induced increase of GH in females depended on the stage of the estrous cycle in which they were exposed to the stressful event.

Conclusion: Together, these data further demonstrate that GH is endogenously produced in the adult hippocampal formation, where it is regulated by age, estrogen, and exposure to environmental stimuli. These results suggest that GH may be involved in functions ascribed to the hippocampus, such as learning and the response to stressful experience.

We have come a long way with GH from a purely growth-promoting hormone to one involved in a variety of developmental and metabolic processes. Here it is shown that the GH gene is transcribed in the hippocampus, where it appears to play an important role in learning and response to stress. The authors used transcriptional profiling to identify genes associated with learning in rat hippocampus, and found that acquisition of a learning task increased the expression of the GH gene. Here, they show that the GH and IGF-I genes and the GH protein are expressed in the hippocampus and are positively regulated by estrogens (hence, the known wisdom of females). Moreover, stress increased GH mRNA expression in the hippocampus of male rats and female rats during diestrus, when estrogen levels are low, but not during proestrus and estrus, when estrogen and GH levels are high. Since the hippocampus is involved in learning, the question is whether hippocampal GH might be involved. Although the data only allows speculations, GH might be involved in neuronal growth and development and more generally in neuronal plasticity and learning. We have no clue about the developmental genes involved in hippocampal GH expression and we should certainly pay more attention to subtle learning defects in patients with GH gene defects.

Serum retinol-binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes

Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB

Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass., USA

Nature 2005;436:356–362

Background: In obesity and type 2 diabetes, expression of the GLUT4 glucose transporter is decreased selectively in adipocytes. Adipose-specific Glut4 (also known as Slc2a4) knockout (adipose-Glut4(–/–)) mice show insulin resistance secondarily in muscle and liver.

Results: Using DNA arrays, it is shown that expression of retinol-binding protein-4 (RBP4) is elevated in adipose tissue of adipose-Glut4(–/–) mice. Serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes. RBP4 levels are normalized by rosiglitazone, an insulin-sensitizing drug. Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely, genetic deletion of Rbp4 enhances insulin sensitivity. Fenretinide, a synthetic retinoid that increases urinary excretion of RBP4, normalizes serum RBP4 levels and improves insulin resistance and glucose intolerance in mice with obesity induced by a high-fat diet. Increasing serum RBP4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and impairs insulin signaling in muscle.

Conclusion: RBP4 is an adipocyte-derived ‘signal’ that may contribute to the pathogenesis of type 2 diabetes. Lowering RBP4 could be a new strategy for treating type 2 diabetes.

One more piece in the liver/muscle/adipocyte connection involved in glucose metabolism and type 2 diabetes that culminates in the identification of a new adipokine. The authors started with the observation that variations in the expression of GLUT4 levels in adipocytes are associated with variations of peripheral insulin sensitivity. Increased adipocyte GLUT4 levels were associated with increased whole-body insulin sensitivity. This observation suggested that a circulating factor released from adipocytes is capable of affecting peripheral insulin sensitivity. Expression microarrays identified RBP4 among 5 genes inversely regulated by GLUT4 variations. The paper contains a very complete set of animal data linking increased RBP4 levels to muscle and liver insulin resistance. In accordance with the animal data, serum RBP4 levels were increased ≈ 2 -fold in a limited number of obese vs. control individuals. Pharmacological interventions modulating insulin sensitivity affected RBP4 levels.

The next question is how does it work? RBP4 is known as a retinol (vitamin A) transporter but it is not clear whether this function is related to its effect on insulin action. Retinol is the precursor for the synthesis of ligands of the RAR and RXR nuclear hormone receptors/transcription factors who play a role in the regulation of fatty acid metabolism. In addition, RBP4 could also have a direct effect, although no high affinity receptor is known. From an evolutionary perspective [10], RBP4 could be the link between fat store deprivation during famine and insulin resistance, aimed at reducing glucose uptake in the periphery to spare glucose for the brain. In obesity, adipocyte GLUT4 levels are decreased due to adipocyte insulin resistance. This signal might be mistaken for starvation by the adipocyte and lead to increased RBP4, leading to peripheral insulin resistance. Is the 2005 adipokine going to be rapidly shelved with several others or is this the long sought link between fat and insulin sensitivity as discussed for instance in the famous editorial: ‘What if Minkowski had been ageusic?’ [11]. Although it is still unclear, RBP4 has the potential to become a prime player in the diabetes obesity arena.

References

1. Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG: The β -glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* 2005;310:490–493.
2. Blackman MR, Sorkin JD, Munzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, et al: Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. *JAMA* 2002;288:2282–2292.
3. Kodama S, Kuhreiter W, Fujimura S, Dale EA, Faustman DL: Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 2003;302:1223–1227.

4. Chong AS, Shen J, Tao J, Yin D, Kuznetsov A, Hara M, et al: Reversal of diabetes in non-obese diabetic mice without spleen cell-derived β -cell regeneration. *Science* 2006;311:1774–1775.
5. Nishio J, Gaglia JL, Turvey SE, Campbell C, Benoist C, Mathis D: Islet recovery and reversal of murine type 1 diabetes in the absence of any infused spleen cell contribution. *Science* 2006;311:1775–1778.
6. De Lonlay P, Simon-Carre A, Ribeiro MJ, Boddaert N, Giurgea I, Laborde K, et al: Congenital hyperinsulinism: pancreatic [^{18}F]fluoro-*L*-dihydroxyphenylalanine (DOPA) positron emission tomography and immunohistochemistry study of DOPA decarboxylase and insulin secretion. *J Clin Endocrinol Metab* 2006;91:933–940.
7. Ettinger MP, Littlejohn TW, Schwartz SL, Weiss SR, McIlwain HH, Heymsfield SB, et al: Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *JAMA* 2003;289:1826–1832.
8. Jaeken J, Carchon H: Congenital disorders of glycosylation: a booming chapter of pediatrics. *Curr Opin Pediatr* 2004;16:434–439.
9. Adams JS: ‘Bound’ to work: the free hormone hypothesis revisited. *Cell* 2005;122:647–649.
10. Muoio DM, Newgard CB: Metabolism: A is for adipokine. *Nature* 2005;436:337–338.
11. McGarry JD: What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* 1992;258:766–770.