Obesity: on the eve of a major conceptual revolution

Obesity has reached epidemic proportions worldwide and will continue to rise at an alarming rate. The drive for a low body weight in many contemporary societies and the association of obesity with severe diseases supports an ever-increasing market for the food and pharmaceutical industries. The biology of obesity has become an exciting scientific field of its own, especially after the cloning and characterization of leptin, the weight-reducing product of the *ob* gene[1]. A boom in the identification of novel peptides and the elucidation of mechanisms involved in weight regulation has marked the 'leptin era'. The Institute Pasteur held a Euroconference entitled *Obesity: Genetics, Pathophysiology and Therapeutics on the Eve of a Major Conceptual Revolution* in Paris, France on 25–27 November 1999. Novel biochemical molecules underlying body weight regulation, recent clinical trials and strategies to advance the understanding of, and drug development for, obesity were the subject of discussion.

Molecular mechanisms of weight regulation

The opening presentation by Bernard Guy-Grand (Hospital Hôtel Dieu, Paris, France) highlighted the inefficiency of current preventive and curative treatments, which consists mainly of behavioral modifications, and pointed out the importance of defining clear molecular targets in the complex task of finding adequate and efficient treatments for obesity. Steve Bloom (Imperial College of Hammersmith Campus, London, UK) gave a comprehensive overview of the brain circuitry regulating food intake. The picture is now much more complex than the classical concept of hypothalamic centers independently regulating satiety and feeding behavior. The key aspect of appetite control is the multitude of interactions between ligands and receptors [including cocaine amphetamine-regulated transcript (CART), melanocortin receptors, orexins, leptin, agouti-related protein and glucagon-like peptide 1 (GLP-1)][2].

Understanding weight regulation involves unraveling the roles played by leptin in appetite control and energy expenditure. Adipose tissue produces leptin that reaches homeostatic hypothalamic centers in the brain and provides information on the state of the energy balance. Thus, investigators have been trying to identify the neural circuits that connect leptin plasma levels with its effect on weight reduction. Peter Kristensen at Novo Nordisk A/S (Bagsvaerd, Denmark) demonstrated that the anorectic effects of the neuropeptide CART were leptin dependent[3]. More interestingly, CART immunoreactivity overlaps with the presence of pro-opiomelanocortin (POMC)-containing neurons in the arcuate nucleus and the administration of CART activates corticotrophin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus.

An innovative approach to investigate leptin-mediated effects was presented by Jeffrey Flier (Harvard Medical School, Boston, MA, USA). The leptin receptor belongs to the cytokine receptor superfamily and its stimulation leads to the activation of intracellular Janus tyrosine kinase (JAK), ultimately leading to gene transcription. Flier and his group tested the role of the newly identified SOCS-3 gene (suppressor of cytokine signaling) in leptin activity[4]. They used chinese hamster ovary cells stably expressing the long form of the leptin receptor and demonstrated that after binding to its receptor, leptin activated the transcription of endogenous SOCS-3 gene. More interestingly, the SOCS-3 gene-product then binds and inactivates JAK, therefore inhibiting proximal leptin signal transduction. These findings are important because they provide the molecular basis of the dysfunction of leptin signal transduction that could be a mechanism involved in leptin resistance.

Recent clinical trials

Steven O'Rahilly (University of Cambridge, UK) reported the use of recombinant human leptin as an effective treatment for a child with early-onset obesity and undetectable serum leptin concentration levels[5]. There are currently six individuals described with a homozygous mutation of the *ob* gene associated with obesity, two first cousins of Pakistani origin and four family members of Turkish origin[6]. O'Rahilly showed the continuous weight-reducing properties of leptin administration during a 12 month period in the older child of Pakistani origin. Mark A. McCamish at Amgen (Thousand Oaks, CA, USA) reviewed their Phase II study results on obese and type II diabetes patients treated with recombinant methionyl human leptin. Both groups showed significant weight loss on a dose of 0.2 mg kg^{-1} day^{-1}. The effects of leptin seem to be solely mediated by reduced calorific intake because there was no detectable decrease in energy expenditure. The level of weight loss varied between the subjects, possibly indicating different types of obesity-responsive individuals.

The dose necessary to reduce weight in obese subjects led to a 20–30-fold increase in serum leptin concentration levels compared with normal values. Interestingly, weight loss in the patient

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with the *ob* gene mutation from O'Rahilly's report had leptin plasma levels close to the normal range during treatment. Unfortunately, the volume of fluid required for administration of the peptide (up to 8 ml per day) in the Amgen study was associated with an inflammatory reaction at the injection site and was one of the reasons they abandoned using the native leptin form. However, further studies are continuing with other forms of leptin.

David J. Heal (Knoll Pharmaceutics R&D, Nottingham, UK) reported on the antiobesity drug, sibutramine, which was recently approved by the Food and Drug Administration (FDA). This compound is a potent reuptake inhibitor of serotonin and noradrenaline, acting on the satiety and appetite centers, respectively, in the hypothalamus. In a recent double-blind, placebo-controlled trial, subjects receiving sibutramine maintained weight loss for a one-year period[7]. Apparently, the compound decreases food intake by enhancing postingestive satiety and stimulates thermogenesis via sympathetic β_3-adrenoceptor activation in brown adipose tissue. The fact that sibutramine acts on both serotoninergic and noradrenergic neurons is advantageous as it theoretically combines the actions of agents such as phentermine and fenfluramine without the major side effects, which include their stimulant effect, abuse liability and association with valvular heart disease. The only reports so far of cardiovascular side effects with sibutramine are a mild elevation in blood pressure and heart rate in some, nonhypertensive subjects.

Strategies for new molecular targets

The discovery of leptin and its use as a therapy are certainly landmarks in the quest for effective pharmacological treatments for obesity. From its positional cloning in 1994, it took less than five years for recombinant leptin to become the first example of a rationally based, hormone replacement therapy for a rare form of human obesity[1]. This certainly provides optimism for obesity research, although the picture for most obese people is more challenging.

If the general assumption that most cases of obesity in humans reflect a polygenic inheritance is true, what are the available strategies to test this hypothesis? Quantitative trait loci (QTL) approach is one of the most reasonable genetic techniques to identify genes associated with obesity. Philippe Froguel (Institute Pasteur, Lille, France) summarized recent results of genome-wide scans using QTLs performed in samples of obese individuals worldwide. Two genome regions strongly associated with leptin and a high body mass index (BMI) were identified in Mexican American families, one on chromosome 2p at the *POMC* gene and the other on chromosome 8 at the β_3-adrenoceptor region[8,9]. Evidence of linkage in a region on chromosome 10p was shown in a study investigating the association of obesity and chromosomal regions in affected French Caucasian sibling-pairs[10]. Another region of linkage was found on chromosome 20q in Caucasian sibling-pairs, a region where a diabetes-susceptibility locus was also demonstrated[11]. These latest findings add to the almost 200 genes and markers that have been associated with human obesity phenotypes[12]. A comprehensive list of genes and markers can be found at http://www.obesity.chair.ulaval.ca/genes.html.

Another approach that complements genome-wide scans is the discovery of new candidate genes from studies investigating the molecular basis of gene expression in tissues relevant for obesity. Martine Laville (Hôpital Edouard Herriot, Lyon, France) showed that it is possible to utilize quantitative reverse transcription reaction followed by competitive polymerase chain reaction (RT-PCR) to study *in vivo* regulation of gene expression in humans[13]. Two molecules important in glucose metabolism, p85α-phosphatidylinositol 3-kinase (PI-3K) and hexokinase II, were expressed differently in muscle and adipose tissue biopsies taken from type II diabetic patients compared with controls or patients with obesity[14]. This finding demonstrates the potential use of RT-PCR, not only to understand mechanisms leading to obesity, but to guide research on drug action at the molecular level.

Conclusions

This conference elegantly covered most recent subjects in the field: new peptides controlling appetite, the biology and pharmacology of leptin, Phase II clinical trials and genome-wide scans. Research on leptin and other forms of monogenetic-induced obesity will continue and could be promising in the search for new therapies in the near future. As for leptin itself, efforts will concentrate on discovering molecules that participate in leptin signal transduction in hypothalamic target cells, elucidating the transport mechanisms of leptin across the blood–brain barrier, and the production of leptin analogs. By contrast, both the common and complex forms of human obesity that are not caused by a single gene mutation will be far more challenging for investigators.

Many more genes must be identified, enabling the creation of models to elucidate the interaction of those genes, their products and metabolic pathways, with environmental and psychosocial factors in the genesis of obesity. Hence, there is a general expectation that more resources will be allocated to the identification of new QTLs, genetic studies for polygenic traits (including association and linkage approaches), and dissection of CNS pathways that regulate food intake and energy expenditure.

Because of its multifactorial basis, the regulation of food intake and body weight will remain a challenging but promising area of investigation. It is

hoped that the elucidation of the relative contributions of psychological, social and genetic factors that cause obesity will result in the identification of several novel therapeutic targets for this common and complex disorder.

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An SOS for HIV

The development of a safe, effective vaccine against HIV will be crucial to halting the AIDS pandemic. Three years after President Clinton declared that it should be possible to produce one within a decade, many obstacles remain. Such a vaccine would almost certainly require several components, including a subunit vaccine based on the envelope glycoprotein to induce neutralizing antibodies[1].

Although most trials of HIV subunit vaccines to date have used a soluble form of the gp120 subunit, it is a poor immunogen because of its poor stability. Now, John Moore, James Binley and their colleagues at Aaron Diamond AIDS Research Center (ADARC; NY, USA), working in collaboration with Progenics Pharmaceuticals (NY, USA), have produced a genetically engineered, stable envelope glycoprotein complex that mimics the virion-associated structure[2], which they hope to use as a potential component of a vaccine against HIV.

The native HIV-1 envelope glycoprotein, the so-called 'viral spike' familiar from graphical reproductions of the virus, is a non-covalently weakly bonded complex of three gp120 subunits with three gp41 subunits. Hence, the gp120 subunit often dissociates from the complex (Fig. 1a). Consequently, it has proven very difficult to produce a stable recombinant form of this glycoprotein complex (Fig. 1b).

Stabilizing the complex

The virus produces the glycoprotein complex from a larger gp160 monomer by proteolytic cleavage. Although some groups have attempted to generate a stable complex by mutating gp160 to remove the cleavage site[3] (Fig. 1c), this monomer does not elicit antibodies capable of neutralizing HIV-1. Researchers believe that the confor-

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