# Overview: neurobiology of OB protein (leptin)

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The rapid elucidation of the properties, target tissues and actions of OB protein, which is the product of the *ob* gene, has invigorated and energized obesity research as no other finding in this field has in the last 35 years. The circulating concentrations of OB protein are proportional to adiposity and increase with increasing levels of body fat (Considine *et al.* 1996). The OB protein pathway is the long-sought hormonal signal pathway from adipose tissue to the brain that plays a critical role in the regulation of energy balance (Kennedy, 1953; Campfield *et al.* 1996*a*, 1997*a*). OB protein is a 16 kDa polypeptide hormone that is secreted from adipose tissue, circulates in the blood, bound to a family of binding proteins, enters the brain, binds to its receptor in hypothalamic nuclei and other brain areas and acts on central neural networks. Available evidence suggests that OB protein appears to play a major role in the control of body fat stores through co-ordinated regulation of feeding behaviour, metabolism, neuroendocrine responses, autonomic nervous system and body energy balance in rodents, primates and man (Campfield *et al.* 1996*a*, 1997*a*).

The mechanisms in the brain responsible for determining the level at which body fat content is regulated in man and other animals are not completely understood. A similar lack of knowledge exists for the mechanisms regulating the neuroendocrine rhythms supporting the adaptation to starvation and reproductive function in man and other animals. Elucidation of the OB protein pathway within the brain has begun to provide important insights into these, and possibly other, mechanisms. If OB protein proves to be a useful tool to illuminate the mechanisms controlling body fat content and its corresponding decision rules or algorithms, it may provide the basis for a clearer understanding of the regulation of body fat content and energy balance (Campfield *et al.* 1996*a*, 1997*a*). When these mechanisms are understood at the molecular level, they should provide new targets for therapeutic interventions that will reduce and maintain body fat at reduced levels and, therefore, increase metabolic fitness, reduce risk factors and promote improved health of obese individuals. It is this hope that creates much of the excitement that greets each research advance in the understanding of the OB protein pathway (Campfield *et al.* 1996*a*, 1997*a*).

Since the cloning of the *ob* gene in December 1994 (Zhang *et al.* 1994), research has progressed along four parallel paths: (1) regulation of *ob* gene expression in adipose tissue in mice, rats and man; (2) characterization of the biological actions and definition of the elements of the OB protein pathway in lean and obese mice and rats; (3) studies of the biology of OB protein in lean and obese human subjects; (4) studies of the brain structures and mechanisms through which OB protein acts. The key elements of the OB protein pathway are shown in Figure 1, including a transport system for OB protein to enter the brain, OB protein receptors in hypothalamic nuclei, and neural and neuroendocrine outputs to peripheral tissues (Campfield *et al.* 1996*a*, 1997*a*).

In the present review we will discuss the available knowledge about the OB protein pathway within the brain. This understanding is based on *in vitro* experiments as well as studies conducted in laboratory animals and human subjects. This research has revealed that the OB protein, by acting on diverse brain structures and mechanisms, regulates ingestive behaviour, metabolism, neuroendocrine rhythms and controls body energy balance. These brain structures and mechanisms form the central OB protein pathway. The role of OB protein, the concept of reduced brain sensitivity to OB protein in obesity, and possible therapeutic approaches based on OB protein will also be presented. Finally, the interaction of OB protein with other brain mechanisms is summarized. The role of OB protein in obesity and the future of neurobiology of OB protein will also be discussed.

## Obesity and the context of the neurobiology of OB protein

Obesity is a major health problem throughout the world. Obesity is the most common nutritional disorder in the developed world and is associated with significant chronic metabolic diseases (hypertension, non-insulin-dependent diabetes mellitus, hypercholesterolaemia) as well as stroke, sleep apnoea, joint diseases and certain cancers (Bjorntorp & Brodoff, 1992). It is a complex multifactorial disease characterized by behavioural, endocrine and metabolic alterations, with an increasing prevalence (Bjorntorp & Brodoff, 1992; Bouchard & Perusse, 1993; Thomas, 1995). Obesity is a cause of significant morbidity and is having an increasing negative impact on the health care systems in both developed and developing world (Chadwick & Cardew, 1996). Although treatment (e.g. diet, exercise, drugs) is available and most people can achieve medically-significant weight loss (5–10 % initial body weight), the long-term maintenance of that weight loss is, unfortunately, very rare. Thus, obesity remains a poorly managed medical condition that is a major cause of morbidity and mortality (Bjorntorp & Brodoff, 1992; Campfield, 1995; Thomas, 1995; Campfield *et al.* 1996*a*, 1997*a*).

**Abbreviations:** CRH, corticotrophin-releasing hormone; CSF, cerebrospinal fluid; DIO, diet-induced obesity; DIO-R, DIO resistance; NPY, neuropeptide Y; OB-R, OB protein receptor; OB-$R_L$, long form of OB-R; OB-$R_S$, short form of OB-R; POMC, pro-opiomelanocortin.

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https://doi.org/10.1079/PNS19980062 Published online by Cambridge University Press

Obesity is characterized by the following pathophysiological alterations: (1) high rates of lipid deposition in adipose tissue; (2) reduced insulin sensitivity of muscle and fat; (3) exaggerated insulin responses to meals; (4) hyperinsulinaemia; (5) increased OB protein concentrations; (6) reduced brain and peripheral sensitivity to OB protein (Thomas, 1995; Campfield *et al.* 1996*a*, 1997*a*).

Ample evidence exists that obesity is, at its basis, a disease of biological dysregulation. The integration of multiple biological factors (including endocrine and metabolic factors), which are at least partially genetically determined, is thought to result in the steady-state body weight of an individual. When the steady-state weight is perturbed in either direction (increased, decreased), changes in body weight are resisted and corrected by robust physiological mechanisms in laboratory rodents and human subjects (Leibel *et al.* 1995). The physiological mechanisms that resist changes in body fat content (e.g. central neural networks, autonomic neural, metabolic and neuroendocrine) are not completely known (Campfield *et al.* 1996*a*, 1997*a*). It is not understood how these physiological mechanisms are responsible for the unfortunate and very frustrating weight regain that usually follows weight loss. Results of available studies suggest that OB protein acts on the brain and probably plays a role in this 'resetting response' that is responsible for weight regain following weight loss (Campfield *et al.* 1996*a*, 1997*a*).

### *Control of food intake: a behavioural response dependent on OB protein*

Feeding behaviour is the result of the complex central nervous system integration of central and peripheral neural, hormonal and neurochemical signals relating to brain and metabolic states. Meals are initiated, maintained and terminated by specific sets of these central and peripheral signals several times daily, separated by inter-meal intervals without food intake (Campfield & Smith, 1990). These signals include patterns of neural afferent traffic, metabolites (glucose), energy flux (fatty acid oxidation, ATP) and hormones (insulin concentrations in plasma and brain; OB protein concentrations in plasma and neuropeptide concentrations in brain). The brain structures and mechanisms involved in the detection of these signals and the mapping of them into altered feeding behaviour are beginning to emerge from the darkness. One hypothesis for this integrated neural, metabolic and hormonal control of food intake postulates the interaction of five classes of signals: (1) hypothalamic neuropeptides; (2) brain insulin; (3) OB protein; (4) metabolic signals, including transient declines in blood glucose concentrations; (5) ascending and descending neural inputs (Bray & Campfield, 1975; Campfield & Smith, 1990; Le Magnen, 1992; Campfield *et al.* 1996*a*,*b*). These signals interact and provide the central and/or peripheral integration necessary to regulate food intake and match energy intake to energy expenditure to maintain body energy balance and composition.

Many experimental studies in mice, rats and many other species demonstrate that several neuropeptides modulate food intake when injected centrally and, in some cases, peripherally. The neuropeptides that affect food intake include neuropeptide Y (NPY), galanin, cholecystokinin, corticotrophin-releasing hormone (CRH), and enterostatin (Kaiyala *et al.* 1995). Synaptic concentrations of central neuropeptides and classical monoamine neurotransmitters are thought to be modulated by the central representations of peripheral metabolic state, and act on post-synaptic receptors to control energy intake (Le Magnen, 1992; Kaiyala *et al.* 1995). Although some investigators still seek the identity of the 'one' major neuropeptide controlling human feeding behaviour, most of the field has adopted a 'parallel' model in which multiple neuropeptides each play a role in determining human feeding behaviour (Campfield *et al.* 1997*a*).

### *Experimental models of obesity due to altered OB protein pathway: obese* ob/ob *and* db/db *mice*

The obese *ob/ob* mice were discovered on the C57BL/6J background in 1950 by animal caretakers at the Jackson Laboratories (Bar Harbour, ME, USA). This mutation results in profound obesity. The *db/db* mouse, which arose on the C57BL/KsJ background, is similarly obese and is also characterized by hyperglycaemia. When the *ob* gene was transferred to the C57BL/KsJ background, an almost identical phenotype to *db/db* mice was observed. Likewise, when the *db* gene was transferred to the C57BL/6J background, an obese phenotype almost identical to *ob/ob* mice resulted (Coleman & Hummel, 1973; Herberg & Coleman, 1977; Coleman, 1978, 1981).

When cross-circulation (or parabiosis) experiments were performed between lean and obese rats by Hervey and colleagues (Hervey, 1971, 1988; Parameswaran *et al.* 1977) at the University of Leeds (Leeds, UK), the lean partner reduced its food intake and lost weight, while the obese partner continued to gain weight. These studies suggested that the obese partner was producing increased amounts of a factor that was proportional to body fat, and the factor crossed into the circulation of the lean partner where it acted on the brain to reduce food intake and body weight. When cross-circulation (or parabiosis) experiments were performed in *ob/ob* and *db/db* mice by D. Coleman and colleagues (Coleman, 1973, 1978, 1981; Coleman & Hummel, 1973; Herberg & Coleman, 1977) at the Jackson Laboratories, they also observed that the *ob/ob* partner reduced its food intake and lost weight, while the *db/db* partner maintained both its food intake and body weight. These studies led Coleman (1973, 1978) to conclude that *ob/ob* mice fail to secrete a circulating factor from adipose tissue, but their brain can respond to it and reduce food intake, while *db/db* mice secrete the circulating factor from their adipose tissue, but their brain cannot respond to it. The insightful hypotheses of Hervey (1971, 1988) and Coleman (1973, 1978, 1981) have been proven correct by the findings on the OB protein pathway in the past 3 years. Despite numerous attempts to isolate and identify this protein, the primary defect, the site of synthesis and the nature of the *ob* gene product were not known until cloning of the *ob* gene. It remains a great privilege for us to work with these very special animals, *ob/ob* mice, and to be part of the emerging OB protein story.

A mutation in the *ob* gene results in severe obesity in mice (*ob/ob* mice). In December 1994, in a *tour de force* of positional cloning, the laboratory of Dr J. M. Friedman succeeded in cloning the *ob* gene (Zhang *et al.* 1994). A

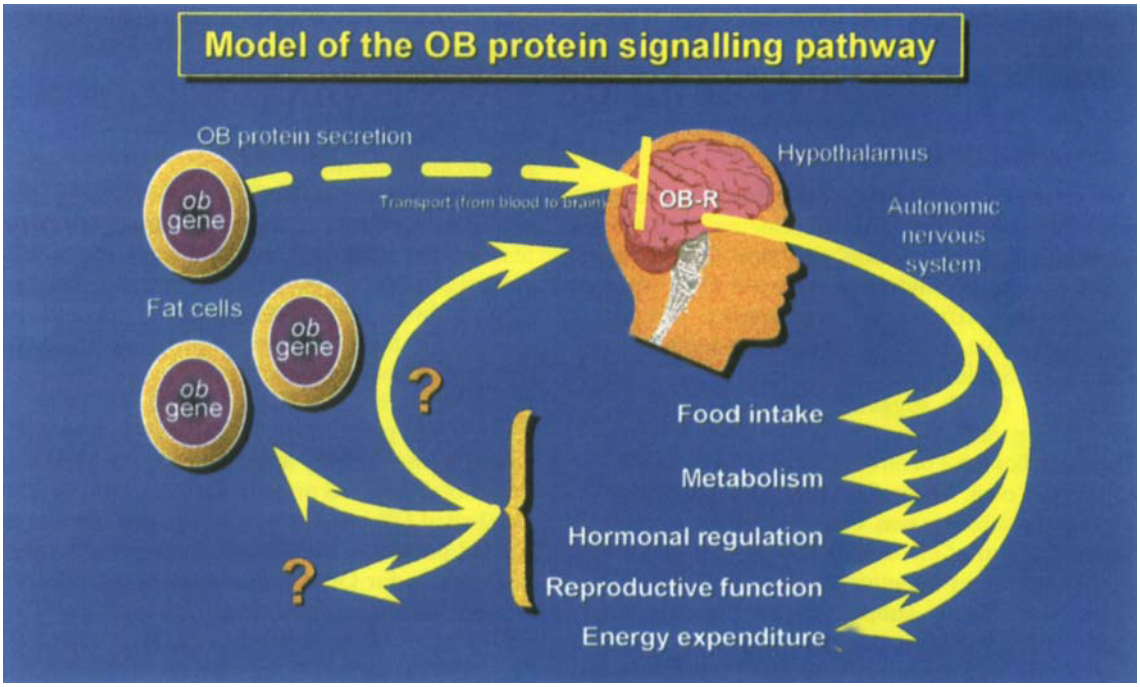


**Fig. 1.** A schematic model of some of the important elements of the OB protein signalling pathway that regulates body energy balance. OB-R, OB protein receptor. For further, details, see p. 429.

mutation in the *db* gene results in severe obesity that is phenotypically similar to that of the *ob/ob* mice. Following the expression cloning of the OB protein receptor (OB-R) in late 1995 by scientists at Millennium Pharmaceuticals Inc. (Cambridge, MA, USA) and Hoffmann–La Roche (Nutley, NJ, USA) (Tartaglia *et al.* 1995), three groups demonstrated that the *db* gene encodes the OB protein receptor in early 1996 (Chen *et al.* 1996; Chua *et al.* 1996; Lee *et al.* 1996).

After screening the coding region of the *ob* gene in several thousand obese subjects, two obese cousins with *ob* gene mutations were identified. These two individuals were normal weight at birth, but both have become severely obese children (Montague *et al.* 1997). Their identification suggests the operation of an OB protein pathway in man.

### *Diet-induced obesity*

A model with potentially more applicability to human obesity is diet-induced obesity (DIO) in mice and rats. When very palatable, high-fat diets (remarkably similar to chocolate chip cookie dough without the chocolate chips; mainly composed of sugar and shortening added to powdered rodent food) are given to normal mice and rats, DIO occurs in approximately 60–75 % of some, but not all, strains of mice and rats. Animals will rapidly gain weight over a 4–6-week period, and have increased circulating concentrations of insulin and OB protein, and become very obese when fed on these diets (West *et al.* 1994). These DIO animals, also, will have decreased sensitivity to the actions of circulating insulin and OB protein. The operational definition for DIO is a body weight > average body weight of animals fed on normal food + two standard deviations. Diets with a high fat content, high energy density, or multiple palatable items that are presented simultaneously (called supermarket or cafeteria diets) can produce DIO in otherwise normal rodents. Strains that 'resist' DIO and its metabolic alterations (e.g. SHR mice) and individual 'resistant' members of a susceptible strain are being studied because they may provide important biological clues to help reduce weight gain, and regain, in susceptible human subjects (West *et al.* 1994). DIO is an experimental model that is very relevant for studying obesity, particularly with regard to the OB protein pathway. This is because it offers us a chance to study a polygenic obese state in animals that may mimic the situation in most obese human subjects; the interaction of genetic predisposition, rather than mutation, with the environment.

## Important findings from the human biology of OB protein

### *Serum concentrations*

Radioimmunoassays and ELISA assays for human OB protein were rapidly developed (Considine *et al.* 1996). These studies can be summarized as follows: when obese subjects were compared with lean individuals, it was observed that the serum OB protein concentrations were higher in obese individuals; OB protein concentrations increased with increasing percentage body fat (Maffei *et al.* 1995; Considine *et al.* 1996). Thus, obese human subjects are not deficient in OB protein, but rather they have elevated circulating OB protein concentrations. Women have higher OB protein concentrations than men, even when corrected for the percentage body fat. Subjects with non-insulin-dependent diabetes mellitus have lower OB protein levels than obese subjects but higher levels than lean subjects. When obese subjects lost

https://doi.org/10.1079/PNS19980062 Published online by Cambridge University Press

weight by energy restriction, OB protein concentration decreased and then rose slightly when the lower weight was maintained (Considine *et al.* 1996).

### *Circulating binding proteins*

A group of scientists at Thomas Jefferson University (Philadelphia, PA, USA) provided convincing evidence, using gel filtration to separate 'bound' and 'free' OB protein, for a family of circulating binding proteins for OB protein (Sinha *et al.* 1996). Affinity chromatography revealed that these proteins have molecular masses ranging from 40 kDa to 280 kDa. Studies with an antibody to the extracellular domain of OB-R suggested that approximately 10 % of the binding protein might be the 'soluble form' of OB-R. These studies have been confirmed in many laboratories, including our own. Most of the OB protein in the serum of lean human subjects was bound to other proteins, while the major fraction of circulating OB protein in obese human subjects was in an unbound or free form. When a small number of obese subjects were fasted, the total (free + bound) OB protein concentration in the serum decreased, with the major change occurring in the free fraction, while the bound fraction remained unchanged (Sinha *et al.* 1996). The radioimmunoassays used by the Thomas Jefferson University group (Considine *et al.* 1996), including the commercial radioimmunoassay by Linco (St Charles, MO, USA), and the human OB protein-specific ELISA that we used in the studies summarized previously, all measure the total OB protein concentrations (Sinha *et al.* 1996).

### *Cerebrospinal fluid OB protein concentrations*

Measurements of the concentrations of OB protein in the cerebrospinal fluid (CSF) and the serum, and the calculation of CSF : serum values for OB protein of lean and obese human subjects have been reported, first by Schwartz *et al.* (1996*b*) and later by Caro *et al.* (1996). The CSF concentrations of OB protein were correlated with BMI, but were much lower than the serum concentrations in individuals studied in both reports (CSF : serum values ranged from 0·01 to 0·15 in the Schwartz *et al.* (1996*b*) study and from 0·01 to 0·09 in the Caro *et al.* (1996) study). Although the serum concentrations of OB protein were much higher in obese individuals compared with lean subjects, the CSF levels in obese subjects were low and similar to those of lean subjects in both studies. Comparison of the CSF and serum concentrations of OB protein in the lean and obese subjects reveals that OB protein concentrations were much lower in obese subjects than in lean subjects (mean CSF : serum values were 0·013 (SE 0·001) in obese subjects and 0·068 (SE 0·01) in lean subjects in the Schwartz *et al.* (1996*b*) study and were 0·011 (SE 0·002) in obese subjects and 0·047 (SE 0·001) in the lean subjects in the Caro *et al.* (1996) study). Thus, when obese and lean subjects are compared, the appearance of OB protein within the CSF is much lower than expected and is not proportional to the serum concentration, given the elevated concentrations of total OB protein. This observation has led to the suggestion that the brain uptake of OB protein and/or its appearance in the CSF compartment is defective in obese subjects and it may be a component of the decreased sensitivity to OB protein (Caro *et al.* 1996; Schwartz *et al.* 1996*b*). However, this suggestion may have to be reconsidered as the role of the circulating binding proteins is elucidated and the serum concentrations and CSF concentrations of 'free' OB protein are measured.

Like much else in the OB protein pathway, the functional significance of low CSF concentrations of OB protein must also await additional experiments conducted in human subjects. However, the transport of radiolabelled OB protein into the brain of mice has been reported by Banks *et al.* (1996). In these studies, radiolabelled OB protein was injected intravenously into mice and autoradiography was conducted in brain sections after the mice were killed. They showed the presence of $^{125}I$ in the arcuate nucleus of the hypothalamus shortly after the injection. The rate of uptake of radiolabelled OB protein was decreased by co-injection of unlabelled OB protein, suggesting that the transport system for OB protein in mice may be saturable. A saturable transport system for OB protein in isolated human brain microvessels has been discovered and reported (Golden *et al.* 1997). Many of the features of the brain transport system for OB protein appear to be similar to the transport system already described for brain insulin (Schwartz *et al.* 1991, 1994; Kaiyala *et al.* 1995).

## Properties of OB protein and biological activity of OB protein in mice and rats

Some of the major properties of OB protein are as follows: the signal sequence in front of the sequence for OB protein is cleaved and the mature OB protein is secreted into the circulation. OB protein is a 16 000 Da monomeric protein. The C terminal disulfide bond appears to be critical for the activity of OB protein. In addition, OB protein has homology to the class I cytokine family. Finally, the *ob* gene is expressed in adipose tissue, bone marrow and placenta, and OB protein is synthesized and secreted from adipose tissue in proportion to adipocyte size and number (Campfield *et al.* 1996*a*, 1997*a*).

### *Biological activity of OB protein in mice and rats*

To determine if the brain was a target of OB protein, we injected recombinant mouse OB protein intracerebroventricularly (lateral ventricle) to *ob/ob* and lean (±) mice in our first study of the biological activity of OB protein (Campfield *et al.* 1995). These mice were implanted with chronic lateral ventricle cannulas. Single intracerebroventricular injections of 0·001–1 μg/mouse in 1 μl were administered to overnight fasted *ob/ob* and lean (±) mice. Cumulative 7 h and 24 h food intake values were suppressed in a dose-related manner. Post-injection body-weight gains were also reduced in a dose-related manner compared with mice injected with vehicle control (Campfield *et al.* 1995).

However, when the effective dose in lean rats (3·5 μg) of OB protein was placed in the third ventricle in obese Zucker rats, no behavioural effects were observed (Seeley *et al.* 1996). In contrast, Rohner-Jeanrenaud *et al.* (1996) and Lin *et al.* (1996) reported that higher doses of OB protein did suppress food intake of obese Zucker rats. Further studies

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will be needed to determine the impact of the point mutation in the OB-R of the obese Zucker rat on its sensitivity to OB protein.

When a compound, peptide or protein, administered peripherally or centrally results in reduced food intake and the loss of body weight, the possibility that these behavioural effects are due to non-specific action or illness produced by the test substance must be considered. In order to assess this possibility for the biological effects of OB protein, a conditioned taste aversion experiment was conducted (Thiele *et al.* 1997). In these studies, lean Long-Evans rats were offered two drinking bottles containing saccharin solution or water daily for several days. The presentation of saccharin was paired with the intracerebroventricular injection of OB protein, the intraperitoneal injection of LiCl or no treatment. As expected, treatment with a known toxin, LiCl, caused striking rejection of the consumption of saccharin, the taste associated with LiCl treatment, but had no effect on the consumption of water. This decreased preference for a taste paired with a toxic substance is called a conditioned taste aversion. In contrast, intracerebroventricular treatment with OB protein had no effect on the consumption of saccharin or water. These studies demonstrate that OB protein does not support the development of a conditioned taste aversion. These results provide strong support for the idea that the reduction in food intake and body weight observed following the administration of OB protein are specific biological effects of the hormone (Thiele *et al.* 1997).

The results of these experiments, taken together, provide further support for the hypothesis that a circulating protein-based signal, secreted from adipose tissue, acts on central neuronal networks, and suggests that OB protein plays an important role in the regulation of ingestive behaviour and energy balance. The duration of action of OB protein appears longer than that of other neuropeptides which modulate ingestive behaviour (cholecystokinin, NPY, galanin), and is similar to that of centrally-administered insulin. The brain–insulin system is the other well-established system that signals adiposity to the brain (Kaiyala *et al.* 1995). The behavioural and physiological effects following central administration of OB protein into the lateral ventricle suggest that OB protein can act directly on the neural networks in the brain that regulate ingestive behaviour and energy balance.

### *OB protein receptor*

Several significant results were achieved that led to the rapid identification and characterization of OB-R. First, was our demonstration that central administration of OB protein results in reductions in food intake, body weight and alterations in metabolism. These findings were confirmed by others (Stephens *et al.* 1995). Second, scientists at Hoffmann–La Roche, Millennium Pharmaceuticals Inc., and others, identified a central binding site for labelled OB protein in the choroid plexus and pia mater in *ob/ob*, *db/db* and lean mice as well as lean and obese Zucker rats (Devos *et al.* 1996; Lynn *et al.* 1996). Based on this identification, researchers at Millennium Pharmaceuticals Inc. and Hoffmann–La Roche succeeded in the expression cloning of a central receptor, OB-R, from the mouse choroid plexus in December 1995 (Tartaglia *et al.* 1995).

OB-R had considerable homology with the GP130 subunit of the IL-6 receptor. It was concluded that the OB-R has significant homology to a cytokine receptor (Tartaglia *et al.* 1995). OB-R was found to be expressed in the choroid plexus, the hypothalamus and many other brain areas as well as several peripheral tissues (Ghilardi *et al.* 1996; Mercer *et al.* 1996). However, OB-R is a low-abundance message and protein, making unambiguous detection of the receptor message and protein difficult. As a result of alternate splicing, OB-R exists in multiple forms. The two major forms are a short form (OB-$R_S$, with a truncated intracellular domain) and a long form (OB-$R_L$, with the complete intracellular domain). OB-$R_L$ is thought to be the form that signals and mediates the biological effects of OB protein (Tartaglia *et al.* 1995). *In situ* hybridization studies have demonstrated that the mRNA for OB-$R_L$ is localized primarily to the hypothalamus (arcuate, lateral, ventromedial, dorsomedial nuclei; Mercer *et al.* 1996; Schwartz *et al.* 1996*c*). Soon it was demonstrated that the *db* gene encodes the OB-R (Chen *et al.* 1996; Chua *et al.* 1996; Lee *et al.* 1996). Point mutations have been identified in the OB-R in obese *db/db* mice (no OB-$R_L$), obese Zucker (extracellular domain) and corpulent rats (no mRNA for OB-R). The molecular biology and characterization of OB-R has been recently reviewed by our colleague, Dr L. Tartaglia (Tartaglia, 1997; White & Tartaglia, 1997).

## Role of OB protein in obesity and the concept of reduced sensitivity to OB protein in obesity

Most human obesity is probably not due to a deficiency of OB protein, but instead due to a central and/or peripheral resistance or decreased sensitivity to OB protein. Support for the concept of reduced sensitivity to OB protein in obesity is based on the available results in animal models and human subjects, summarized previously (Campfield *et al.* 1995; Considine *et al.* 1996). This is very similar to the case of the insulin-resistant obese patients with non-insulin-dependent diabetes mellitus. However, positive clinical experience with insulin therapy in these patients and our positive results with OB protein treatment of DIO mice (Campfield *et al.* 1995) strongly suggest that therapeutic augmentation of circulating OB protein levels may result in reductions of food intake, body fat mass and body weight in many obese patients (Campfield *et al.* 1996*a*, 1997*a*).

Strong support for the concept of reduced sensitivity to OB protein in obesity is provided by the observation of elevated OB protein concentrations in the blood of obese individuals and the experimental result that higher doses are required to affect feeding behaviour, metabolism and body fat in DIO mice (Campfield *et al.* 1995; Considine *et al.* 1996). At one extreme of the continuum of OB protein responsiveness is the obese *db/db* mouse, with elevated OB protein levels, which is totally unresponsive to OB protein, while at the other end is the very responsive OB protein-deficient, obese *ob/ob* mouse, as shown in Fig. 2. Although these two mutant mice with defects in the OB protein pathway anchor the continuum of OB responsiveness shown in Fig. 2, they have, in our opinion, little relevance to human obesity. Although DIO mice are less responsive than lean mice, they still retain a significant responsiveness to OB

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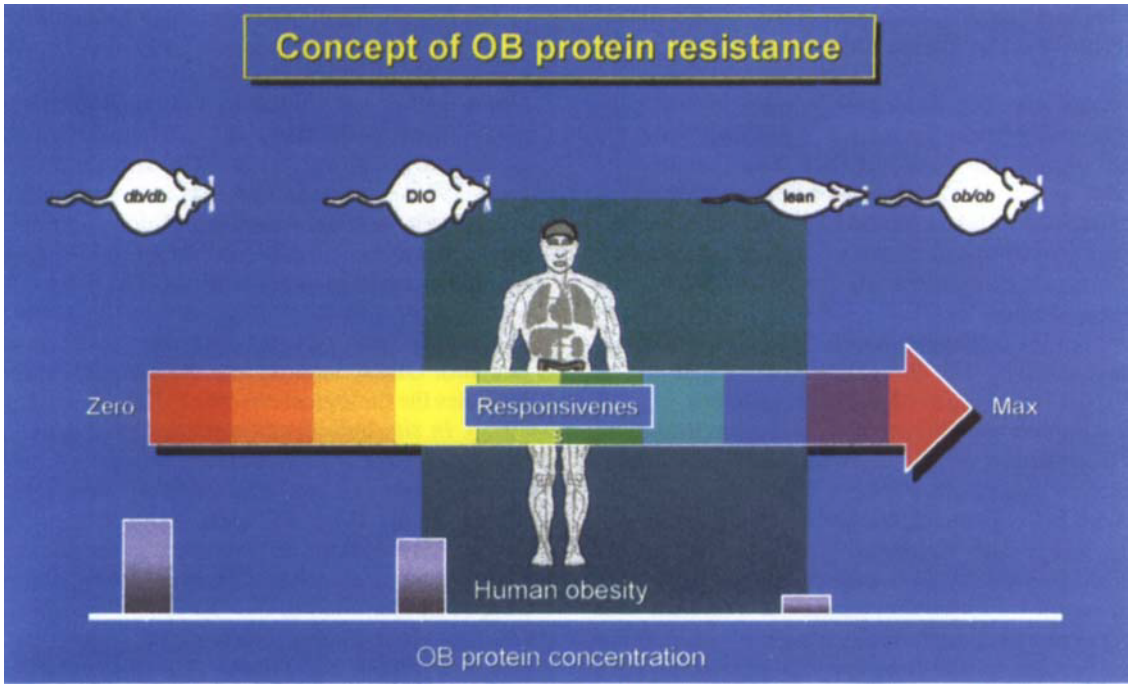


**Fig. 2.** The concept of OB protein resistance. Mice with different sensitivity to OB protein define the range of responsiveness to OB protein. At one extreme are the OB protein-deficient, obese *ob/ob* mice that are maximally responsive, while at the other end are the obese *db/db* mice, with elevated OB protein levels, which are totally unresponsive to OB protein. In the middle, lean and obese diet-induced obese (DIO) mice are shown. Although obese DIO mice are less responsive that lean mice, they still retain a significant responsiveness to OB protein. The OB protein responsiveness of obese human subjects is assumed to range from normal to decreased, but still responsive. Relative plasma OB protein concentrations are shown for unresponsive obese *db/db* mice (high), DIO mice with reduced responsiveness (elevated), lean mice (normal) and maximally responsive obese *ob/ob* mice (zero). (Reprinted from Campfield *et al.* 1996a.)

protein (Campfield *et al.* 1995). The OB protein responsiveness of most obese humans will probably range from normal to decreased, but still responsive, as indicated in Fig. 2. Whether the reduced sensitivity to OB protein is due to one or more intrinsic or regulatory defects in the post-receptor signalling pathway or/and decreased brain transport and uptake of OB protein remains to be determined by further research (Banks *et al.* 1996; Golden *et al.* 1997).

Movement of mice along the continuum of OB protein sensitivity as a function of adiposity, as shown in Fig. 2, has been demonstrated in our recent studies of DIO mice. When lean AKR/J mice are fed on a high-fat energy-dense diet, they become obese, with elevated OB protein and insulin concentrations. Our demonstration that DIO AKR/J mice required higher intraperitoneal doses of OB protein to reduce food intake contributed to the concept of OB protein resistance. Recently, we have shown that DIO mice have decreased central sensitivity to OB protein (Campfield *et al.* 1997*b*). In these studies we determined the sensitivity to intracerebroventricular injection of mouse OB protein in DIO mice after changes in diet. Female mice were fed on a high-fat diet for 13 weeks and then switched to chow for 2 weeks. We measured 6 h food intake following intracerebroventricular administration of OB protein to DIO mice as well as lean mice, and non-obese mice on the same high-fat diet (DIO-R). After 13 weeks on the high-fat diet, serum insulin and OB protein levels were markedly increased in DIO mice. When 1 μg OB protein was injected intracerebroventricularly, food intake suppression was similar in lean and DIO-R mice (43 %), but was markedly reduced in DIO mice (23 %). After 2 weeks on chow, DIO mice had reduced body weight to the range of body weight of DIO-R mice, and now intracerebroventricular OB protein reduced food intake similarly in lean and DIO-R and formerly DIO mice (53 %), indicating an increase in brain sensitivity to OB protein in formerly DIO mice. These results demonstrate that expansion of the adipose tissue mass, as a result of high-fat diet feeding, is associated with decreased brain sensitivity to OB protein injected intracerebroventricularly. In contrast, decreased brain sensitivity to OB protein was not observed in mice remaining lean while on high-fat diet. Thus, these studies with DIO mice indicate that the brain sensitivity to OB protein of the neural network in the brain controlling energy balance is decreased by weight gain and can be reversed by weight loss (Campfield *et al.* 1997*b*).

The biological effects of daily peripheral administration of recombinant human OB protein or an analogue of human OB protein in lean and obese human subjects are currently being determined in clinical trials being conducted by Amgen (Thousand Oaks, CA, USA) and Eli Lilly (Indianapolis, IN, USA) respectively. In a press release, Amgen stated that weight loss was observed after daily subcutaneous injections of OB protein in obese subjects. The publication of the results of these trials is eagerly awaited by the obesity research community and obese individuals.

Each advance in our understanding of the OB protein brings us closer to the development of an effective and safe drug that will increase the sensitivity of the OB pathway in

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brain of obese individuals to their own more-than-adequate levels of OB protein (Campfield *et al.* 1996*a*, 1997*a*). Such an increase in the sensitivity of the OB protein pathway to their OB protein should assist them to reduce and then chronically maintain a lower amount of body fat that will result in improved health (Thomas, 1995; Campfield, 1996).

## Interaction of OB protein with other brain hormones and neuropeptides

Research on the brain mechanisms involved in OB protein action has been stimulated by the long-lasting reductions in food intake and body weight of obese *ob/ob* mice observed following intracerebroventricular OB protein administration (Campfield *et al.* 1995; Stephens *et al.* 1995), together with the observation that the circulating OB protein concentration was proportional to body fat in human subjects (Considine *et al.* 1996). Central administration of OB protein reduced food intake and body weight, altered metabolism and inhibited NPY-induced feeding of obese *ob/ob* mice and lean mice and rats (Campfield *et al.* 1995; Stephens *et al.* 1995; Hwa *et al.* 1996; Smith *et al.* 1996).

Some of the recent advances in the neurobiology of OB protein are listed in Table 1. The neurotransmitters and neuropeptides that directly mediate the actions of OB protein have not yet been identified, but experimental evidence is consistent with OB acting through several brain mechanisms (NPY, CRH, pro-opiomelanocortin (POMC)) to co-ordinate the regulation of energy balance. Which, if any, neurotransmitters and neuropeptides are responsible for the decreased sensitivity to OB protein in obesity are not known. However, the neuronal network controlling energy balance and the descending sympathetic nervous system following peripheral and central administration of OB protein is beginning to emerge.

Patterns of activation of brain areas in response to central or peripheral administration of OB protein have been measured using *c-fos* immunoreactivity. Numerous studies have implicated hypothalamic nuclei (arcuate, ventromedial hypothalamus, dorsomedial hypothalamus, paraventricular nucleus) and other brain areas thought to be involved in the control of energy balance (van Dijk *et al.* 1996; Thiele *et al.* 1997; Yokosuka *et al.* 1997).

Early in the story of OB protein it was proposed that NPY, a potent stimulator of feeding (Ezzell, 1995; Rohner-Jeanrenaud *et al.* 1996; Rohner-Jeanrenaud & Jeanrenaud, 1996), was the major mediator of the actions of OB protein (Stanley *et al.* 1986; Ezzell, 1995; Stephens *et al.* 1995). This proposal was based on the inhibitory effects of OB protein on NPY gene expression (Stephens *et al.* 1995; Schwartz *et al.* 1996*a*) and secretion (Stephens *et al.* 1995) observed in early studies of the biological activity of OB protein. We examined the interaction of brain administration of OB protein and NPY on the feeding behaviour of *ob/ob* mice to test this hypothesis. We found that OB protein inhibited the expected feeding following NPY administration. The magnitude and time course of food intake were determined by the presence of OB protein. Although the stimulatory actions of NPY on feeding were dominated by OB protein, the increased food intake following combined OB protein and NPY administration indicated that NPY was still acting at its post-synaptic receptors.

**Table 1.** Recent progress in neurobiology of OB protein

| |
|---|
| Studies of neural activation by central and peripheral OB protein using *c-fos*-like activity in brains of mice and rats |
| Co-localization studies of OB-R- and NPY- or POMC-containing hypothalamic neurones |
| Clear evidence that OB protein modulates *npy*, *crh*, and *pomc* gene expression in hypothalamic neurones |
| Recording of specific peripheral autonomic neural activity following intravenous OB protein administration in rats |
| Demonstration that OB protein modulates synaptic transmission in hypothalamic neurones (intact and slices) |
| Demonstration that central sensitivity of OB protein is a function of glucocorticoid status |
| Interactions between OB protein pathway and melanocortin signal pathways under active investigation |
| Identification of receptor-mediated transport of OB protein by brain microvessels |
| Further evidence that brain uptake and/or transport of OB protein is reduced in obesity |
| Demonstration that central and peripheral sensitivity to OB protein is reduced in DIO rats and mice |
| Localization of OB-$R_L$ in hypothalamic neurones; but majority in intracellular compartment |
| Further support that OB protein modulates 'hypothalamic–pituitary–target organ' axis |
| Clear evidence that OB protein plays a role in the control of reproduction |
| Identification of two obese cousins with *ob* gene mutations suggests operation of OB protein pathway in man |
| Further appreciation of the pulsatile and circadian rhythms of serum OB protein concentrations |

NPY, *npy*, neuropeptide Y; POMC, *pomc*, pro-opiomelanocortin; *crh*, corticotropin-releasing hormone; DIO, diet-induced obese; OB-$R_L$, long form of OB protein receptor.

These results suggest that OB protein determines the sensitivity of the feeding response to exogenous NPY. OB protein, when placed into the lateral ventricle, may alter the binding of exogenous NPY to its receptors that are critical for feeding and/or may inhibit downstream signal transduction (Smith *et al.* 1996). This indicates that OB protein can functionally antagonize the actions of exogenous NPY and suggests that the receptor-mediated actions of NPY on feeding are under the control of OB protein. Thus, it appears that NPY interacts, particularly in the early post-injection period, with OB protein or OB protein-dependent signalling processes that contribute to the regulation of body energy balance. However, our results are also consistent with other mediators, besides NPY, of OB protein action. This possibility has been strengthened by the report that normal-weight mice lacking NPY respond to peripheral administration of OB protein (Erickson *et al.* 1996*a*). Similar results have been reported recently (Stricker-Krongrad *et al.* 1996). When both the *ob* and *npy* loci were disrupted in mice, the double-knockout mice were still obese but the degree of obesity was attenuated by about 25–40 %. These authors conclude that OB protein-mediated antagonism of the hypothalamic NPY system is required for normal regulation of body energy balance (Erickson *et al.* 1996*b*).

When OB protein was administered into the third ventricle of the brain of lean rats, NPY gene expression in the arcuate nucleus was decreased, while CRH gene expression in the paraventricular nucleus was increased (Schwartz *et al.*

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1996*c*). Since CRH inhibits feeding of rodents when administered intracerebroventricularly (Schwartz *et al.* 1994; Kaiyala *et al.* 1995), these results suggest that part of the activity of OB protein to inhibit feeding may be mediated through decreased NPY and increased CRH protein levels in hypothalamic nuclei critical for the regulation of food intake and body energy balance. Recent studies also indicate that central administration of OB protein increases the expression of the POMC gene in the arcuate nucleus (Schwartz *et al.* 1997).

Co-*localization* studies using selective antibodies have demonstrated that OB protein acts through OB-R on neurones that contain NPY, adrenocorticotrophic hormone, CRH, POMC, somatostatin, galanin, tyrosine hydrolase (*EC* 1.14.16.2), and melanocyte-concentrating hormone (Hakansson *et al.* 1998). These findings support an integrative role for OB protein and/or the OB protein pathway.

The long duration of food intake suppression induced by OB protein in obese *ob/ob*, lean and DIO mice (Campfield *et al.* 1995) was reminiscent of the effects of central administration of insulin. Studies to test this hypothesis are currently underway in many laboratories, including ours. The striking finding that adrenalectomy can restore sensitivity of obese Zucker (*fa/fa*) rats to centrally-administered insulin (Kaiyala *et al.* 1995), the well-established effects of glucocorticoids in animal models of obesity (Bray & York, 1979), and the actions of OB protein on the hypothalamic– pituitary–adrenal axis in fasted mice (Ahima *et al.* 1996), suggested that OB protein may interact with the glucocorticoid system. In a series of experiments in which OB protein was administered centrally in adrenalectomized and sham-operated lean rats, a marked shift of the OB protein dose–response curve for the reduction of food intake to the left was observed in adrenalectomized rats (Zakrzewska *et al.* 1997). These preliminary results provide support for a potentially important interaction between OB protein and the glucocorticoid system (Campfield *et al.* 1996*a*, 1997*a*).

OB protein is not only linked to the regulation of energy balance but also may have a number of additional roles, such as maintaining the normal neuroendocrine activity that is important in the adaptation to starvation and controls stress responses and functions such as reproduction. Starved mice and OB protein-deficient *ob/ob* mice were found to have similar neuroendocrine abnormalities, including an activated hypothalamic–pituitary–adrenal response and depressed thyroid function. In addition, male mice had low levels of testosterone and delayed ovulation was observed in female mice. When fasted mice were treated with OB protein, these neuroendocrine abnormalities were reversed (Chehab *et al.* 1996). Administration of OB protein to female *ob/ob* mice restored reproductive function to near normal, and the mice became pregnant and successfully carried litters to term (Chehab *et al.* 1996).

It has been demonstrated that OB protein could modulate synaptic transmission when applied to hypothalamic slice preparations from rat brain. This report suggested another mechanism to explain the behavioural and metabolic actions of OB protein (Glaum *et al.* 1996). Direct administration of OB protein on glucose-receptive ATP-sensitive K channels resulted in hyperpolarization of hypothalamic neurones (Spanswick *et al.* 1997). Direct application of OB protein to ventromedial hypothalamus and luteinizing hormone neurones resulting in both stimulation and inhibition of firing rates have been reported (Yokosuka *et al.* 1997). Future neurophysiological research will help evaluate if OB protein has a neuromodulatory action.

### *Role of the OB protein pathway in the brain*

One possible role for OB protein is that of a modulator of gene expression, and the resultant synthesis of one or more neurotransmitters and/or neuropeptides within the brain. Much of the research conducted on the OB protein pathway has been focused on the identification of downstream genes that are regulated by OB protein. Three such genes are *npy*, *crh* and *pomc*, since OB protein has been shown to decrease the mRNA for NPY and increase POMC mRNA and the mRNA for CRH in different areas of the hypothalamus (Campfield *et al.* 1996*a*, 1997*a*).

Another possible role for OB protein is that of a modulator of synaptic transmission within the brain. This could involve alteration of the release or post-synaptic action of one or more neurotransmitters and/or neuropeptides and/or states of one or more presynaptic and/or post-synaptic ion channels. Since recent electrophysiological studies have demonstrated this hypothesis is correct (Glaum *et al.* 1996; Spanswick *et al.* 1997), then behavioural and metabolic responses to the administration of OB protein may be expected to depend on the 'background' amounts of these neurotransmitters and/or neuropeptides in synapses or nerve terminals. Since the amounts of these neurotransmitters and neuropeptides change with time, variability in either behavioural and/or metabolic responses to administration of OB protein would be expected. However, in our experiments with sequential administration of OB protein and NPY (described previously), we were impressed with the very reproducible, rather than variable, nature of these interaction data (Smith *et al.* 1996).

Another alternative role for OB protein would be a 'co-ordinator' or 'organiser' of the seemingly disparate neurotransmitter and neuropeptide effects on, and responses to, ingestive behaviour and body energy balance. The recent demonstration of 'co-ordinated' response of NPY, POMC and CRH gene expression (inhibition of NPY mRNA and increased POMC mRNA in arcuate nucleus and increased CRH mRNA in the paraventricular nucleus in response to central administration of OB protein) provides support for this concept. These responses were 'co-ordinated' in both space (two different anatomical locations) and time (both changes were seen at a common time point that was correlated with the behavioural response; Campfield *et al.* 1996*a*, 1997*a*).

These three possible roles do not have to be, and are probably not, mutually exclusive. Indeed, the 'co-ordinator' or 'organizer' function of OB protein within the brain may emerge from at least two distinct regulatory components mediated by OB-R as shown in Fig. 3: (1), the action of OB protein to regulate gene expression within areas of the brain critical for regulation of energy balance (Fig. 3) can be considered responsible for the 'long-term' or 'chronic' biological effects (e.g. critical molecules which determine the brain sensitivity to OB protein, 'settling point' for body fat

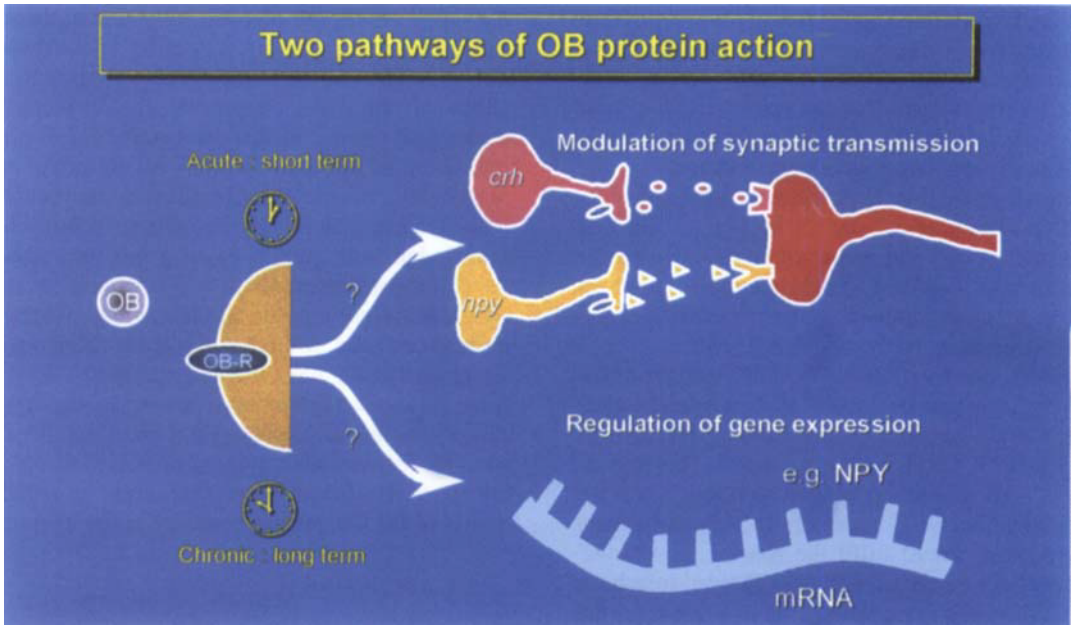


**Fig. 3.** Schematic representation of two pathways of OB protein action. (OB), OB protein; (OB-R), OB protein receptor. OB protein action is a combination of acute actions mediated by modulation of synaptic transmission and chronic actions mediated by regulation of gene expression. CRH, *crh*, corticotrophin-releasing hormone; NPY, *npy*, neuropeptide Y. (Reprinted by permission from Campfield *et al.* 1997a.)

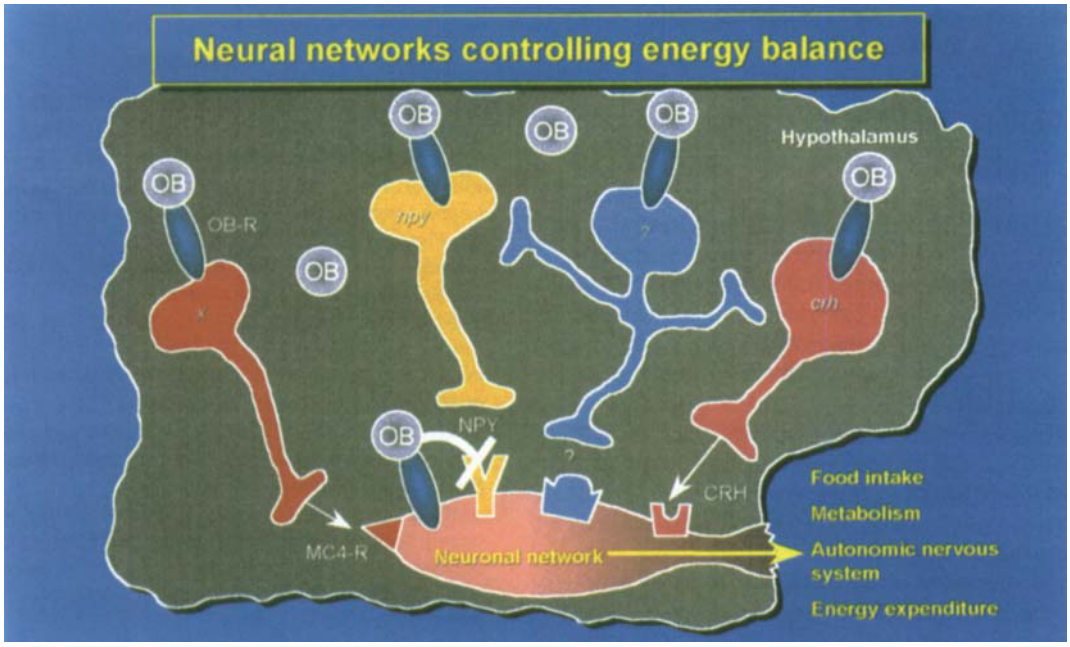


**Fig. 4.** Schematic diagram of hypothetical neural networks controlling energy balance. Several 'classes' of parallel neural pathways are represented by the four model neurones depicted. Each neurone is assumed to have the gene, synthesize and release one dominant type of neuropeptide: neuropeptide Y (NPY, *npy*), corticotropin-releasing hormone (CRH, *crh*), X (pro-opiomelanocortin; POMC), ?. Each neurone has the long form of the OB protein receptor (OB-R; ⬭) and the 'final common pathway', the neuronal network controlling ingestive behaviour, metabolism and energy balance, has distinct receptors for OB, NPY, CRH, X(POMC) and ?. (OB), OB protein. Note that OB protein can express its biological action through five parallel paths, each mediated by a different neuropeptide: NPY, CRH, POMC, or '?'. The ability of OB protein to inhibit the actions of released NPY is also shown. MC4-R, melanocortin 4 receptor. (Reprinted from Campfield *et al.* 1997a.)

content); (2), the action of OB protein to modulate synaptic transmission within the brain by altering the release or post-synaptic action of one or more neurotransmitters and/or neuropeptides and/or states of one or more presynaptic and/or post-synaptic ion channels (Fig. 3) can be considered responsible for the 'short-term', 'acute' or 'immediate' behavioural (suppression of food intake) and metabolic effects (decreased serum concentrations of insulin and glucose). This 'dual-function' mechanism of action, regulation of gene expression and modulation of ongoing cellular function such

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as neurosecretion, is a common and classical property of hormones. If additional experiments support this 'dual-function' mechanism of action for OB protein, it would provide additional evidence for the concept that OB protein is a hormone that plays a critical role in the regulation of brain mechanisms involved in the regulation of energy balance.

One representation of this hypothesis at the molecular level in the hypothalamus is shown in Fig. 4. Several 'classes' of parallel neural pathways are represented by the four model neurones depicted. Each neurone shown is assumed to have the gene, synthesize and release one dominant type of neuropeptide, NPY, CRH, X(POMC), ?. Each neurone has OB-$R_L$ (the long form of OB-R), and the 'final common pathway', the neuronal network controlling ingestive behaviour, metabolism and energy balance, has distinct receptors for OB, NPY, CRH, X(POMC) and ?. The demonstrated ability of OB protein to inhibit responses to NPY is shown, blocking the NPY receptor. In this theoretical construct, OB protein would fulfil the role of 'conductor' integrating the distinct 'sections' of the 'orchestra' to behave as one while it plays the 'symphony'. In this analogy, the 'symphony' is the integrated behavioural, neuroendocrine and metabolic response of an individual, the 'orchestra' is the spatially-distributed neural network controlling ingestive behaviour, metabolism and energy balance, and the 'sections' would be the elements and subsystems of this neural network. This attractive hypothesis must await experimental testing in future studies.

## The future of the neurobiology of OB protein

The rapid characterization of the OB protein signal pathway within the brains of laboratory rodents and human subjects has been significant for several reasons. OB protein has also demonstrated that the neurobiology and neuroendocrinology of ingestion, metabolism and energy balance have moved to the front of the research agenda. The unravelling of the brain mechanisms underlying the behavioural and metabolic actions of OB protein and responsible for the sensitivity of the brain to OB protein has become a major objective of neuroscience, neuroendocrinology and obesity research. The rapid evolution of the OB protein pathway has provided a series of important advances in the knowledge base of this field. This increasing knowledge base should help to prise open and shine a bright light onto the very dark 'black box' that contains the mechanisms and decision rules or algorithms used in the brain for determining the level at which body fat content is regulated in the brains of man and other animals. When these mechanisms are understood at the molecular level, they will provide novel targets for discovery and development of new, safe, effective pharmacological treatment for obesity as adjuncts to diet and exercise in the future. It is hoped that these new therapeutic agents will reduce and maintain body fat at reduced levels and, therefore, increase metabolic fitness, reduce risk factors and promote improved health of obese individuals.

The numerous papers on OB protein published in the last 3 years, some, but not all, of which are discussed and specifically cited in the present review, clearly demonstrate the intense interest within the global biomedical community in the rapidly emerging field of the OB protein neurobiology, the OB protein pathway, and its potential therapeutic applications for the treatment of obesity. These research findings also demonstrate the enthusiasm, excitement, intensity and talent of the basic scientists and clinical investigators throughout the world that are committed to unravelling the regulation of the secretion and the hormonal actions of OB protein and, eventually, illuminating the mechanisms in the brain responsible for the regulation of body fat. This new knowledge will greatly expand our understanding of the development, maintenance and treatment of obesity, and it should certainly provide additional hope for the many obese individuals who struggle on a daily basis to maintain the weight loss they have worked so hard to achieve. We continue to await experimental testing of the hypotheses presented here in our laboratories, as well as those of others, and we look forward with great anticipation to the surprises and unexpected twists and turns that surely lie ahead as the elucidation of the OB protein pathway in the brain continues.

## Acknowledgements

We thank Jack F. R. Curtis for assistance with the conceptualization and execution of the artwork. We also thank our departmental colleagues and many collaborators for stimulating discussions and suggestions, and Dr Paul Burn and Dr Denis Loh for their interest and support.

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https://doi.org/10.1079/PNS19980062 Published online by Cambridge University Press