Chapter 10
Obesity Study: Animal Models

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Introduction

Body weight and adiposity are maintained at near-constant levels through matching food intake to energy expenditure over long periods. Obesity is defined as a high body mass index with a large amount of adiposity resulting from chronic excess of energy intake over energy expenditure. Recently, the number of obese people increased in the world, and obesity is becoming a world-wide problem because obesity is a risk factor in increased induction of type II diabetes, cancer, hyperlipidemia and hypertension, so-called metabolic syndrome [1]. Moreover, clinical studies have revealed that obesity is comorbid with several forms of mental disorder [2–4]. Epidemiological studies have demonstrated that the incidence of depression and cognitive impairment is higher in obese subjects than normal body weight subjects [5, 6]. There is the possibility that mental disorder acts as a trigger of the development of obesity. Also, such mental disorder might cause further progression of obesity. After findings on the hypothalamus as the center of energy regulation in 1940's, the central nervous system came to the forefront of attention in the pathophysiology of obesity. Genetic and environmental factors play a role in the development of obesity, and diet is one of the main environmental factors that contribute to this disease [1, 7]. Human studies have shown that increased fat intake is associated with body weight gain, resulting in obesity and other related metabolic diseases. It is therefore important to understand the basics of the controls of food intake and how they might relate to obesity. Animal rodent models are useful tools for studying obesity, and are important in the development of treatments for obesity. This chapter shows representative animal obese models that are used to analyze pathophysiological properties of obesity.

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Energy Regulation

Energy homeostasis including food intake and energy consumption has been demonstrated to be predominantly regulated by orexigenic and anorexigenic systems in the hypothalamus (Fig. 10.1) [8–10]. The blood-born signals, such as leptin, insulin and ghrelin, mainly interact with receptors in the hypothalamus to regulate food intake and energy expenditure [9, 10]. In the arcuate nucleus (ARC), orexigenic neuropeptides, such as neuropeptide Y (NPY) and agouti-related protein (AgRP) increase food intake and decrease energy expenditure. On the other hand, anorexigenic neuropeptides, α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART) decrease food intake and increase energy expenditure. Activities of these neuropeptides in the ARC are regulated by leptin, insulin and ghrelin. These neuropeptide-containing neurons project to lateral hypothalamic area (LHA), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH) and to paraventricular nucleus (PVN). Moreover, the LHA, orexigenic neuropeptides, such as orexin (ORX) and melanin-concentrating hormone (MCH), project to mesolimbic dopamine system, the so-called reward system. Oxytocin (OXT) neurons project to the brain stem and modulate activity of sympathetic and parasympathetic nervous systems. Recently, several lines of evidence have indicated that energy regulation is also modulated by extra-hypothalamic brain areas originally related to regulation of emotion and cognition, such as the nucleus

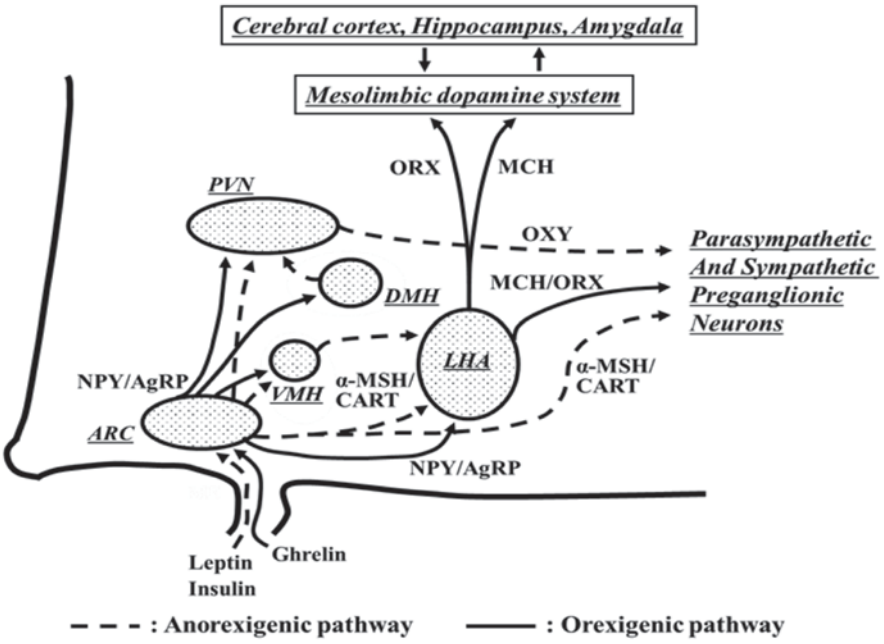

Fig. 10.1 Energy regulation in the central nervous system

accumbens, amygdala, hippocampus and cerebral cortex [9]. These findings suggest that maintaining energy homeostasis and regulating emotion and cognition share common brain regions, as well as bidirectional interaction between energy regulation and emotional/cognitive functions.

Animal Models

Genetic Obese Models

Lethal Yellow Mutant Mouse (Ay)

An agouti mouse was first described more than a century ago. Agouti gene is the first obesity gene characterized at the molecular level [11]. The agouti protein transiently expressed in the follicular melanocyte stimulates production of the red/yellow pigment and inhibits production of the black/brown pigment [12, 13]. The red/yellow pigment production is especially regulated by antagonism of the binding of α-MSH to melanocortin 1 receptor (MC1R) [12]. The lethal yellow mutant mouse (Ay) is one of five dominant agouti mutations and has been found to be an excellent mouse model of obesity [11]. The Ay mutation is characterized by the deletion of 120–170 kb genomic DNA that places it under the control of a ubiquitously expressed promoter, resulting in ubiquitous agouti expression due to loss of the tissue-specific control promoter element [14–16]. The Ay mouse has a complex phenotype including yellow coat color, mature-onset obesity, type II diabetes, hyperleptinemia, increased linear growth, higher tumor susceptibility, and infertility. Transgenic mice expressing ubiquitous agouti exhibited yellow coat color, obesity, hyperinsulinemia, and hyperglycemia similar to Ay mouse [15], revealing the molecular mechanism of agouti in mouse phenotype. However, agouti expression in skin has little effect on the obesity phenotype, because transgenic mice overexpressing agouti in skin do not become obese [17]. Mice with adipose tissue-specific agouti overexpression exhibit an overgrowth of adipose tissue without alteration of food intake, suggesting that increased fat in this model is due to changes in energy metabolism [18]. The adipose tissue agouti overexpression model could be relevant to human obesity because agouti gene expression is found in human adipose tissue [19, 20] and is increased in the adipose tissue of type 2 diabetic subjects [21]. The obesogenic role of agouti is considered to be tissue dependent [22]. The molecular categorization of agouti was responsible for elucidation of the melanocortin system's involvement in energy regulation, due to its mimicry of AgRP activity in the hypothalamus. The melanocortin receptor family comprises five G-protein–coupled proteins, MC1R to MC5R, which demonstrate tissue-specific patterns of expression. Especially, MC4R in the hypothalamus plays a key role in the regulation of feeding and metabolism and is normally antagonized by AgRP [23]. Novel expression of agouti in the brain results in the chronic antagonism of MC4R, disrupting its function.

Ob/ob Mouse (Lepob/Lepob mouse)

In 1950, Ingalls et al. described a spontaneous mutation in a V mice colony, which provoked severe obesity. This mutation, named ob [24], was subsequently introduced into C57BL/6J to obtain ob/ob mice, which are obese and exhibit hyperinsulinemia, insulin resistance, hyperglycemia, infertility, hypothyroidism, hypercorticoidism, low sympathetic activity, impaired thermoregulation and lowered physical activity [25, 26]. In 1966, Hummel et al. described a spontaneous mutation in a C57BL/Ks mice colony that provoked moderate hyperphagia and obesity, accompanied by severe fasting hyperglycemia increasing with age and a marked increase in plasma insulin concentration [27, 28]. Parabiosis studies with ob/ob mice and normal mice indicated that the mutation causes a deficiency in a circulating lipostatic factor [26]. In 1994, Zhang et al. [29] identified the product of the gene mutation in ob/ob mice that was responsible for their obesity and was also the presumed circulating factor. This protein, leptin, has the structure of a long chain helical cytokine [30] and is expressed in adipose tissue in proportion to adipocyte size [31, 32]. Leptin is an adipocyte-derived blood-borne satiety factor that is involved in the regulation of food intake and body weight acting in the hypothalamus. Ob/ob mice exhibit a mutation located on the +105 codon, replaces an arginine by a stop codon (CGA→TGA) [29], resulting in a deficiency of biologically functional leptin. It was reported that leptin recovers the decreased metabolic rate, body temperature, and locomotor activity in ob/ob mice [33]. The discovery of leptin has opened up a whole new field of studies on regulation of food intake, energy expenditure and obesity. They have been used in many studies of the effects of antiobesity and antidiabetogenic drugs.

Db/db Mouse (Leprdb/Lepdb Mouse)

The db (stands for “diabetes”) mutation is an autosomal recessive trait. The obese mouse was identified initially in 1966 by researchers in the Jackson Laboratory [34]. In 1995, the mouse leptin receptor gene was cloned [35] and the db gene encodes for a G-to-T point mutation of the leptin receptor, leading to abnormal splicing and defective signaling of the adipocyte-derived hormone leptin [36, 37]. This mutation affects the alternative splicing of the ob-Rb subtype of leptin receptors, the only subtype with a functional C-terminal intracellular domain to transmit the signal. Leptin can bind to the receptors but the signal transduction system is not functional. Lack of leptin signaling in the hypothalamus leads to persistent hyperphagia, hyperlipidemia, insulin resistance and diabetes [37, 38]. Following the onset of overt diabetes, db/db mice develop progressive kidney disease, which has similarities to human diabetic nephropathy. Therefore, db/db mice are now regularly used to examine the mechanisms of renal injury, which result from type 2 diabetes.

SHROB (Spontaneously Hypertensive-Obese) rat

SHROB rats were first described in 1973 [39]. They result from crossing a spontaneously hypertensive female rat and a Sprague–Dawley male rat, which leads to a spontaneous mutation, first designated f for fatty [40, 41] and then fak [42]. This mutation is recessive and is located on the +763 codon of the leptin receptor gene in a sequence coding for the extracellular domain of the leptin receptor and common to all subtypes. This mutation is non-sense, replacing a tyrosine with a stop codon (TAT→TAA), and does not affect the leptin receptor mRNA. However, the protein is not expressed [43–45]. They are obese because of the accumulation of fat, in particular at the sub-cutaneous, retroperitoneal and mesenteric level. These animals exhibit hyperlipidemia, hyperglycemia and insulin resistance. Arterial pressure in SHROB rats increases with age up to 180 mmHg for systolic blood pressure. The life span of male SHROB is quite short (10–11 months in average) and the principal causes of death are kidney, urinary tract or vascular pathologies [40, 41].

Zucker Fatty Rat and Zucker Diabetic Fatty Rat

In 1961, Zucker et al. observed an obese phenotype due to a spontaneous mutation in their 13 M rat colony [46, 47]. The mutation, named fatty or fa, is an autosomal recessive mutation in the fatty (fa) gene and affects the leptin receptor gene, but is different from the fak mutation of SHROB rats [42]. Located on the +269 codon, it replaces a glutamine with a proline, leading to production of a truncated protein [43, 44, 48, 49]. This mutation affects the extracellular part of the leptin receptor. In experiments using cells expressing wild-type or mutated leptin receptors, mutated receptors have shown weaker affinity for leptin, and altered signal transduction [50, 51]. Zucker fatty rats required much higher doses of leptin than normal rats to produce a similar effect [52]. These rats are characterized by hyperphagia and early-onset obesity corresponding to an accumulation of subcutaneous and retroperitoneal fat. Zucker fatty rats exhibit hyperlipidemia with age. They are slightly insulin-resistant but do not exhibit fasting hyperglycemia. Nevertheless, obesity in Zucker fatty rats is not as marked as in SHROB rats. Zucker fatty rats can present moderate arterial hypertension, but only when old [46, 47, 53]. In addition, Zucker diabetic fatty (ZDF) rats were derived from substrain of obese Zucker fatty rats and display early deregulation of glucose metabolism [54, 55]. ZDF rats constitute a model of metabolic syndrome with type 2 diabetes.

OLETF Rat (Otsuka Long-Evans Tokushima Fatty Rat)

OLETF rats were developed by the selection of spontaneously type 2 diabetic rats from the outbreeding of Long Evans rats in a closed colony of Charles River at the Tokushima Research Institute of Otsuka Pharmaceutical in Japan. [56]. OLETF rats are hyperphagic beginning several weeks after birth, with increasing body weight

eventually progressing to frank obesity [56] These rats exhibit obesity, hyperglycemia, hypertriglyceridemia, hyperinsulinemia and chronic diabetes mellitus [56, 57]. In 1995, Otsuki et al. demonstrated that pancreatic acini of OLETF rats are insensitive to the amylase actions of cholecystokinin (CCK) due to low expression or absence of expression of CCK-1 receptor mRNA [58, 59]. Other studies showed that this lack of expression of CCK-1 receptors is due to a deletion including the promoter region and the first and second exons [60]. Because CCK plays an important role in satiation, OLETF rats are a valuable animal model to study deregulated control of eating and obesity.

Diet-Induced Model

High-Fat Diet-Induced Obese Model

The development of obesity and metabolic syndrome in human is mostly linked with increased caloric intake and lack of physical activity, in addition to genetic predisposition. It would be of interest to study the pathogenesis of metabolic syndrome induced by greater food intake in general. A high-fat diet is often used in obesity research as a non-leptin-deficient model. In animal models, although there is no consensus about the definition of a low- or high-fat diet, and a wide variety of diets are used in animal experiments, in general, standard diets contain less than 10% of calories from fat whereas high-fat diets or very-high-fat diets contain 30–50%, and more than 50% of calories from fat, respectively. From a nutritional perspective, diets with 60 kcal% fat are commonly used to induce obesity in rodents since animals tend to gain more weight more quickly thereby allowing researchers to screen their compounds after a shorter period of time [61, 62]. Many high-fat diets used in laboratory animal research contain more saturated fat such as lard, beef tallow, or coconut oil and are quite capable of inducing metabolic disorders such as obesity, insulin resistance or glucose intolerance in susceptible strains [63–65]. Addition of sucrose aggravates the metabolic consequences of enriched diets and induces severe dyslipidemia [66, 67]. Moreover, high-fat diets rapidly and specifically reduce the central actions of insulin and leptin, most likely due to a post-receptor effect [68–72]. This effect is rapid, occurring after a few days of HF exposure.

An appropriate diet in an appropriate strain is necessary to induce the development of a specific metabolic problem because there are mouse strain-specific differences in responses to the high-fat diet [73]. Among the various strains, C57BL/6J mice are the most widely used for high-fat diet -induced obesity because they exhibit abnormalities similar to human metabolic syndrome when fed with high-fat diet [74]. Interestingly, within the C57 mouse strain, there are significant differences among substrains in response to the high-fat diet. For instance, whereas C57BL/6J mice exhibit high-fat diet -induced obesity, hyperinsulinemia, and insulin resistance that closely parallel the progression of human disease, C57BL/KsJ mice display a weak phenotype [73]. For example, murine strains C57BL/6 and AKR both develop

obesity on a high-fat diet, but (for the same weight gain) C57BL/6 mice also exhibit fasted hyperglycemia, and glucose intolerance due to a decreased insulin concentration, whereas AKR mice also exhibit hyperinsulinemia but with normal fasted glucose [75]. Other strains, such as A/J mice, are resistant to obesity induced by a high-fat diet [66]. Rat models including Sprague-Dawley and Wistar rats are popular strains to study obesity as they readily gain weight on high-fat diets. Sprague Dawley or Long Evans rats are also used for non-mouse rodent models of high-fat diet -induced obesity [76].

These reports indicated that the high-fat diet-induced obesity animals could be a good model for the experimental therapy and the translational research aiming to discover a novel therapeutic strategy for obesity epidemic.

Cafeteria Diet-Induced Obese Model

Another experimental rodent diet model that exists, which more accurately reflects the variety of highly palatable, energy dense foods that are prevalent in Western society and associated with the current obesity pandemic: the "cafeteria diet". The cafeteria diet foods include cookies, cereals, cheese, processed meats, crackers, etc. Rats become obese when offered a varied and palatable diet that mimics the so-called Western diet of humans (cafeteria diet) [77–79]. Cafeteria diet-induced obesity mainly results from hyperphagia with increased average meal size as well as increased meal frequency that is partly compensated by increased energy expenditure, in particular diet-induced thermogenesis due to sympathetic activation of brown fat. This contrasts with overeating of palatable diets with no choice of foods, which mainly influences meal size.

Genetically Modified Obese Model

As the central and peripheral pathways involved in food intake and energy expenditure are elucidated, a wide range of genes affecting these processes is being identified. Accordingly, many genetically modified obese mice have been created with genes either overexpressed or deleted. In particular, genes of the melanocortin system have been examined using this approach because melanocortin system is a major player in regulation of energy homeostasis (Fig. 10.1).

POMC (Proopiomelanocortin)-Knockout Mouse

POMC-expressing neurons in the arcuate nucleus of the hypothalamus are direct targets of leptin. POMC is the precursor of several biologically active peptides including α-MSH. In the brain, α-MSH is a potent anorexigenic neuropeptide that reduces food intake and increases energy expenditure by activating MC3R and MC4R

in the paraventricular nucleus of the hypothalamus and elsewhere. Mice lacking POMC overeat and develop marked obesity that is exaggerated on a high-fat diet [80, 81]. Heterozygous mutants develop an intermediate phenotype, implying that a functional POMC gene is necessary to maintain normal energy homeostasis. Although treatment with leptin is ineffective in POMC-knockout mice, the obesity in POMC-knockout mice can be markedly reduced when these mice are treated with α-MSH or other agonists for the MC4 receptor, such as MT II. POMC deficiency has also been reported in rare cases of human obesity [82].

MC4R and MC3R-Knockout Mouse

α-MSH and AgRP influence energy homeostasis via melanocortin receptors. The MC4 receptor subtype among melanocortin receptors in particular is involved in the control of food intake. These mice exhibit obesity, hyperphagia, hyperglycemia, hyperinsulinemia [83, 84]. Knockout of the MC4R gene in mice is observed to result in early-onset obesity, non–insulin-dependent diabetes and other obesity associated syndromes. As agouti peptide is an antagonist of MC4-R, these symptoms are a parallel to the yellow agouti mouse syndrome, indicating that agouti expression in the hypothalamus inhibits MC4R function, leading to obesity. In contrast to many other obesity models, MC4R-knockout mice do not have elevated circulating corticosterone levels. MC4-knockout mice do not respond to leptin, AgRP or α-MSH. Similar mutations of the MC4 receptor are often stated to be the most frequent genetic cause of obesity in humans. An MC4R knockout rat has recently been described [85]. It has many characteristics in common with MC4R-knockout mice (such as increased body weight, food intake and body length, and lower spontaneous activity). Targeted deletion of the MC3R gene also results in a late onset obesity phenotype, but regulation of appetite and metabolism appear to be intact [86].

Hypothalamic Lesion-Induced Obese Model

In the earliest obesity study with rodents, obese models in rats were induced by surgical or chemical lesions of the VMH as a satiety center and the ARC of the hypothalamus that results in hyperphagia, increased body weight and adiposity.

VMH Lesion-Induced Obese Model

Rats with VMH lesions often begin eating voraciously even before fully recovering from the effects of anesthesia [87, 88]. The overeating and obesity have traditionally been divided into two stages [88]: a dynamic phase of marked hyperphagia and rapid weight gain followed by declining food intake as body weight is maintained during the static phase of obesity. While the precise mechanisms underlying VMH-

lesion induced obesity are still unclear, it was detected a change in the tone of the sympathetic (decrease) and parasympathetic (increase) nervous systems that is associated with reduced energy expenditure contributes to the syndrome [89]. VMH lesion induces hyperphagia, resulting in hyperglycemia, hyperinsulinemia, insulin resistance, and reduction in physical activity, hyperlipidemia and obesity. Hyperphagia is probably due to the destruction of POMC neurons from the ARC and possibly of neurons producing brain-derived neurotrophic factor as anorexigenic factor in the VMH [90–92]. VMH lesion-induced obesity has been found in a wide variety of species including mice, ground squirrels, rabbits, cats, dogs, pigs, goats, chickens, sparrows, monkeys, and humans [90].

ARC Lesion-Induced Obese Model

The ARC is considered one of the most important hypothalamic regions involved in the regulation of energy homoeostasis. It is difficult to perform a selective surgical lesion of the ARC due to its anatomical shape and location. The repeated administration of monosodium glutamate (MSG), which is a neuroexcitatory amino acid that is harmful to the central nervous system (CNS), to neonatal rats within the first 10 postnatal days has been used to induce the relatively distinct lesion of ARC neurons projecting to the VMH and PVN. All features of metabolic syndrome, such as massive adipose tissue accumulation, insulin resistance, glucose intolerance, hypogonadism and dyslipidaemia are induced by neonatal MSG treatment [93–96]. It is important to note that systemic MSG treatment also lesions neurons in the circumventricular organs due to their open blood-brain barrier. MSG lesions are therefore not restricted to the ARC and interpretation of results must take this into account. ARC neurons can also be destroyed by local administration of goldthioglucose, resulting in a similar obesity phenotype [97–99].

Conclusion

There has been a growing body of literature using rodents as models of human obesity, even though there are many confounding factors including species, strain, age of the animals, type of diet, level of fat, and type of control diet. The development of the human obesity is multifactorial; it depends not only on particular environmental conditions (increased caloric intake, activity), but also on genetic predisposition, moreover has a strong component of individual dependence. This aspect is probably a limit of the use of animal models to investigate human obesity and metabolic syndrome. Therefore, intensive and continuous efforts should be made to establish novel obesity-associated animal models that mimic human health problems, as suitable animal models are fundamental to testing novel therapeutic strategies against obesity.

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