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Perspective

Pharmacological Treatment of Obesity: Therapeutic Strategies

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Introduction

Obesity is now a common disorder in the industrialized world.[1] Much has been reported during the past several years in the popular press about the increasing incidence of obesity and the comorbidity of associated conditions. In addition, there have recently been fundamental advances in our understanding of factors which mediate hunger and satiety and those involved with energy utilization and storage. In this Perspective, we summarize the present state of research in the field of therapeutics targeted for obesity, including promising new approaches for the future.

Obesity is a chronic condition characterized by an overabundance of adipose tissue and can be measured in a variety of different ways.[2] Excess energy is stored in the form of triglycerides in adipose tissue, and increased adipose tissue mass can occur through increases in cell size, cell number, or both. Increased adipose cell size alone results in hypertrophic obesity which is relatively mild. Increased fat cell number, however, causes hyperplastic obesity characteristic of a more severe condition. While the volume of lipid an individual adipocyte can accumulate is finite, the capacity of adipose tissue to expand is virtually limitless.

The most common obesity measure is the body mass index or BMI, defined as weight in kilograms divided by (height in meters)^2. BMI values are easy to calculate and correlate well with rankings obtained from more sophisticated techniques and the cormorbidities associated with obesity. It is difficult to set a definitive BMI level threshold to define obesity, particularly for women in which excess weight is often found in the pelvis and not in the abdomen. For example, the BMI scale does not take the distribution of body fat or lean muscle mass into account. Nevertheless, obesity is defined by the World Health Organization as a BMI of 30.0 kg/m^2 and above, with a BMI of 25.0−29.9 kg/m^2 classified as overweight or preobese.[3]

A large body of epidemiological data correlates increased body weight with risks such as high blood pressure, coronary heart disease, diabetes, altered steroid metabolism, gallstones, and certain forms of cancer.[4] In one study, involving a cohort of >115 000 female nurses over a period of 14−16 years, increased risks of cardiovascular disease and cancer were observed with increasing BMI. Excluding smokers from all groups, there was a 100% greater risk of death from all causes for a BMI of >29.0 kg/m^2 relative to the risk of death for slender women (BMI of <19.0 kg/m^2) during the course of the study.[5] On the basis of an increasing awareness of the public health risks associated with excess weight, the American Heart Association has recently reclassified obesity as a "major, modifiable risk factor for coronary heart disease".[6] Second only to smoking, obesity is a major threat to the public health with ca. 300 000 deaths attributed annually in the United States.[1,7]

Our understanding of the prevalence of obesity in the U.S. population has come largely from the four cycles of the National Health and Nutrition Examination Surveys, conducted by the National Center for Health Statistics.[8] In 1994, 55% of the population was considered overweight and 22.5% were obese. These alarmingly high levels represent a marked increase from previous years and are accompanied by a worldwide

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increase in weight in both industrialized and developing nations. Children as well as adults have increased dramatically in weight during the past decade.

Considering the large numbers of people who are overweight or obese, which of these are suitable candidates for drug treatment? Although there are no absolute indications for drug therapy, contraindications include pregnancy and lactation, unstable cardiac disease, uncontrolled hypertension, severe psychiatric disorder or anorexia, and other drug therapies if incompatible.[9] Since obesity is a chronic condition and drug therapy is likely to occur over a prolonged period of time, it is important that the therapeutic index of new agents be as high as possible. The primary medical goal of any program of therapy to reduce weight is to lessen the risk of associated disorders such as coronary heart disease. The U.S. Food and Drug Administration (FDA) guidelines indicate that obesity drugs may be considered for those with a BMI of >30 kg/m^2 without comorbidities and a BMI of >27 kg/m^2 with comorbidities.[10] Clinical guidelines on the identification, evaluation, and treatment of obesity have recently been published.[11]

Obviously, there is a tremendous potential market for drugs of this type. Total costs in the United States for all obesity-related health problems are estimated at >$200 billion per year. Conservative estimates for the obesity drug market in the United States are >$5 billion by the year 2005 and >$10 billion by 2010, if suitable drugs are available during that period.

Obesity and Feeding

The major environmental factor associated with the rising prevalence of obesity has been an increasingly sedentary lifestyle, compounded by greater levels of caloric intake.[12] In addition, it is estimated that genetic factors are responsible for 40−70% of the variation in obesity-associated phenotypes in the general population, as biochemical and metabolic differences between lean and obese individuals have been well-documented.[13] Weight gain can be a function of defective energy output as well as excessive caloric intake,[14] and each of the 24 human chromosomes except the Y chromosome appears to contain obesity-related genes.[15] To facilitate genetic studies of obesity, several multiplex panels of candidate genes for obesity that are suitable for fluorescent genotyping have been assembled.[16] These mutliplex panels are composed of microsatellite markers linked tightly to 16 human gene products that are of potential importance in the control of body weight, including agouti-related transcript, neuropeptide Y (NPY) and its receptors (Y5 and Y6), proopiomelanocortin (POMC), uncoupling protein-2 (UCP-2), leptin and its receptor, and peroxisome proliferator-activated receptor (PPARγ).

The consumption of food is closely linked to maintenance of a constant level of adiposity.[17] Hormonal messages are integrated with the size of fat stores and regulate the subjective feelings of hunger and satiety. The regulation of food intake is redundant and complicated in humans by social factors, habits, and time of day. The control of feeding involves the central nervous system (CNS), adrenals, gastrointestinal tract, and adipose tissue (Figure 1). In general terms, this control

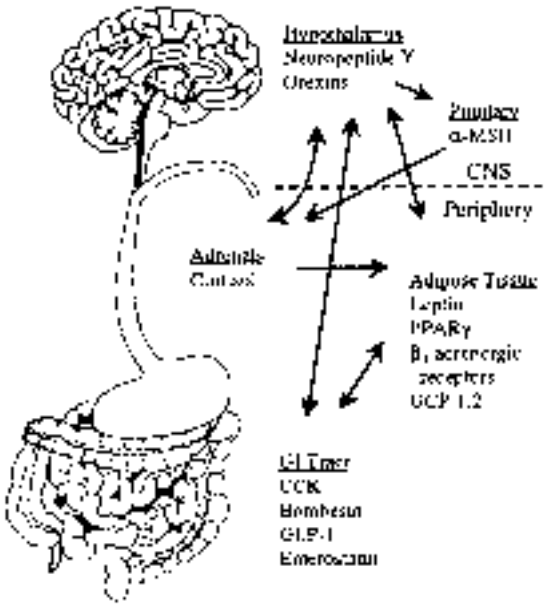


Figure 1. Control of feeding in humans illustrating the redundant and interconnected nature of central and peripheral mechanisms.

can be separated into those factors emanating from the CNS and those from the periphery.

A variety of peptides termed satiety factors are released from the gut during feeding which reduce meal size upon exogenous administration, including cholecystokinin (CCK), bombesin, gastrin-releasing peptide, neuromedin B, and glucagon. These peptides influence the brain through vagal afferent fiber stimulation as well as by central mechanisms. Even though satiety factors decrease the size of individual meals, they do not generally alter body weight after chronic administration in rats, due to an increase in the number of meals eaten. Therefore, there are other internal factors, such as those related to the size of fat stores, which control feeding patterns.

A key peptide involved in the control of feeding is leptin, the product of the *ob* gene. The levels of circulating leptin in humans correlate with fat mass, and women have ca. 2−3 times higher levels than men.[18] When leptin levels fall below a certain point as a consequence of reduced fat mass, there are changes in energy expenditure and feeding which are directed at restoring the previous state of adiposity. Chronic administration of leptin decreases food intake by selectively reducing meal size in rats,[19−21] and it decreases the in vivo synthesis of NPY.[22] Since NPY, synthesized in the hypothalamus, potently increases feeding, the inhibition of NPY secretion is a means by which leptin decreases food intake. Modulation of the hypothalamic pituitary adrenal (HPA) axis is keenly involved in the control of hunger and energy metabolism, and the hypothalamus is the primary feeding center in the brain. Hormonal regulation of the levels of circulating hormones such as adrenocorticotropic hormone (ACTH) affects the production of glucocorticosteroids in the adrenals, altering metabolism and energy storage. Some of the individual components controlling feeding listed in Figure 1 will be discussed separately as potential targets for drug discovery.

Table 1. Mouse Genetic Models of Obesity

model	defect	observation	mechanism
obese (*ob*/*ob*)	defective leptin (166 aa) production; synthesis of truncated leptin; recessive mutation	hyperphagia, decreased energy expenditure, infertility, stunted growth	leptin signal from fat to brain and other organs impaired
diabetes (*db*/*db*)	expression of leptin receptor defective; splicing defect; recessive mutation	similar to *ob*/*ob* mice	impaired leptin receptor
yellow (A^y)	overexpression of agouti-signaling protein (131 aa); dominant mutation	maturity-onset obesity, pigmentation effects, insulin resistance, increased tumor frequency	chronic antagonism of MC-4 receptors; competes with MSH for receptors
fat	missense (Ser-Pro) mutation in carboxypeptidase E; recessive mutation	progressive adult-onset obesity, hyperinsulinemia, infertility	abolishes enzyme activity in neuroendocrine tissues; propeptides not cleaved
tubby	carboxy-terminal deletion in tubby protein; recessive mutation	blindness, deafness, maturity-onset obesity	not known; possible malfunction of signaling pathways in satiety center

Mouse Genetic Models of Obesity

Rodents have been used extensively in preclinical studies because they have proven to be predictive of activity in humans and are also easy to work with in the laboratory. In this Perspective, we will discuss the known genetic models of obesity in mice, although other animal models of obesity exist. There has been remarkable progress in understanding the genetics of obesity in mice.[23] There are five monogenic mouse models of obesity that have been widely studied (Table 1). The genes implicated in these models have been isolated and characterized, and this information provides direct evidence for specific pathways involved in body weight regulation and obesity in mice.

The obese or *ob*/*ob* mouse is a widely studied animal model of obesity.[24] Mice homozygous for the *obese* gene mutation display hyperphagia and effects characteristic of perceived starvation, such as decreased energy expenditure, reproductive deficiency, and stunted growth. The *obese* gene product is the protein leptin, and different *ob*/*ob* mouse strains have mutations leading to the synthesis of truncated leptin or defective leptin production. When leptin is injected into *ob*/*ob* mice, food intake is reduced, body weight decreases, energy expenditure increases, and reproductive function is repaired.[25−27] In addition, the NPY5 receptor is downregulated in this model.[28]

Diabetes mice (*db*/*db*) do not produce functional leptin receptors and exhibit a phenotype similar to that of *ob*/*ob* mice.[29,30] As expected, when leptin is injected into these mice, there is little or no weight loss or decrease in food consumption.

The agouti or yellow mouse exhibits a complex phenotype which includes late-onset obesity, pigmentation defects (yellow coat), insulin resistance, and increased frequency of tumors.[31] The characteristics observed in these mice are caused by an overexpression of the agouti-signaling protein, which is an antagonist of α-melanocyte-stimulating hormone (α-MSH) at the melanocortin-1 (MC-1) and melanocortin-4 (MC-4) receptors.[32] The yellow coat color is due to the inability of α-MSH to stimulate brown-black pigment (eumelanin) synthesis mediated by the MC-1 receptor. MC-4 receptors are expressed in the hypothalamus and are involved in feeding (see below).[33]

The fat mouse phenotype, produced by an autosomal recessive mutation, displays a range of abnormalities, including adult-onset obesity, hyperinsulemia, and reproductive dysfunction.[34] The gene defect responsible for the fat mouse is a missense mutation in the carboxypeptidase E (CPE) gene.[35] CPE is required for proteolytic processing of a variety of peptides known to act on melanocortin receptors and is expressed in the CNS.[36]

Tubby mice are blind and deaf and display adult-onset obesity.[34] The condition is associated with expression of a novel protein that is highly expressed in the retina and brain. The normal function of the protein is unknown; however, since it is found in the hippocampus and hypothalamus, it is thought that obesity in these mice stems from a malfunction of signaling pathways in the satiety center.

Therapeutic Treatment of Obesity

Since obesity is the storage of excess energy, to reduce body fat there must be a period of negative energy balance such as by reducing food intake or by increasing energy consumption. Most marketed antiobesity drugs are appetite suppressants.[2,10] However, energy intake may also be decreased by increasing satiety or altering the relative dietary preferences for complex carbohydrates relative to fat.

Another major mechanism for altering body fat composition is thermogenesis, or increased energy expenditure. There are three main areas in which thermogenesis can be addressed: resting metabolic rate, following food intake, and physical activity.[2] Obesity drugs may either stimulate an increase in activity or increase metabolic rate directly.

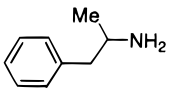
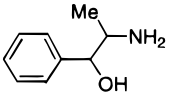
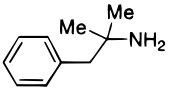
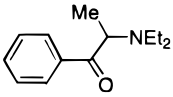
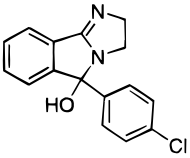
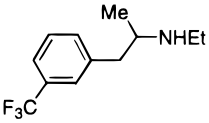
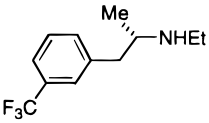
A third mechanism for treating obesity is interfering with energy utilization or nutrient partitioning. Ingested nutrients are guided toward a specific goal such as protein growth, lactation, or fat storage. The degree of partitioning differs among individuals who overeat, which suggests that agents modifying this partitioning have potential value for the treatment of obesity.

A further method to interfere with energy intake is to reduce the absorption of nutrients from the gastrointestinal tract. The inhibition of digestive lipases prevents the intake of energy-rich fat. In like manner, the inhibition of glycosidases prevents the digestion of dietary polysaccharides.

In the following sections we will discuss some of the strategies for discovering antiobesity drugs in more detail.

Adrenergic Agents. The appetite suppressants or anorectics act directly on the CNS and decrease food consumption by activating central adrenergic or serotonergic systems (Table 2).[37] Early anorectics such as amphetamine (1) and related compounds are no longer

Table 2. Sympathomimetic Amines That Have Been Marketed as Anorectic Agents

system	mechanism	drug	structure
adrenergic	stimulates norepinephrine release	amphetamine	 1
	α_1 agonist	phenylpropanolamine	 2
	stimulates norepinephrine release	phentermine	 3
	stimulates norepinephrine release	diethylpropion	 4
	blocks norepinephrine reuptake	mazindol	 5
serotonergic	stimulates 5-HT release	fenfluramine	 6
	stimulates 5-HT release	dexfenfluramine	 7

recommended for use because of their stimulant and reinforcing properties.[38] In particular, amphetamines as a class are abused because the euphoric feelings that emerge upon administration promote chronic readministration and chemical dependency. The anorectic agents that are marketed for the treatment of obesity are phenylpropanolamine (2), phentermine (3), diethylpropion (4), and mazindol (5). With the exception of 5 which is a norepinephrine reuptake inhibitor, they are β-phenethylamine derivatives that stimulate the release of norepinephrine. Although these sympathomimetics have some stimulant effects, they have little or no abuse liability.[38] Their CNS excitation is manifested as insomnia, anxiety, or irritability. Blood pressure and heart rate may be elevated as well.

Phenylpropanolamine (2) is a mixture of pseudoephedrine and norpseudoephedrine which acts by modulation of α_1-adrenergic receptors located in the paraventricular nucleus of the hypothalamus.[39] It is a constituent of many nonprescription cough and cold medicines and is marketed over-the-counter as an appetite suppressant. The efficacy of phenylpropanolamine has been confirmed in numerous clinical trials, but the therapeutic effect is tolerated with repeated use.[2,40] The major safety concern with phenylpropanolamine is the potential for hypertension and toxicity at higher doses.

Phentermine (3), unlike amphetamine, activates the adrenergic system selectively with little or no effect on dopaminergic neurotransmission thus minimizing the risk of euphoria.[41] Phentermine is rarely used as a single agent due to the presence of stimulatory side effects but is commonly used in conjunction with fenfluramine (6) (see below).

Diethylpropion (4) is widely used as an appetite suppressant and is considered to be one of the safest anorexigenic drugs for people with mild to moderate hypertension.[42,43]

Mazindol (5), while structurally distinct from other adrenergic appetite suppressants, is a norepinephrine reuptake inhibitor and exhibits a similar pharmacological profile as phenethylamines 2−4.[44] Mazindol has moderate stimulant effects and is not recommended for use in patients with cardiovascular disease.

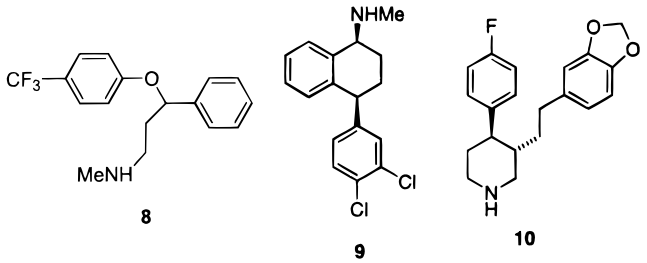
Serotonergic Agents. In recent decades, the importance of serotonergic neurotransmission in the control of appetite has become evident. Serotonergic antiobesity drugs increase satiation, the process which brings a period of eating to an end, and satiety, the absence of hunger after eating.[45] Anorectics of this class can promote the release of serotonin in the CNS, prevent its reuptake, or act as serotonin receptor agonists.[46,47]

Fenfluramine (6) is a racemate that is chemically related to the adrenergic drugs; however, the trifluoromethyl group on the phenyl ring alters its activity dramatically because of the added serotonergic activity. Fenfluramine has been used extensively for the management of obesity in conjunction with phentermine. Due to the finding that patients taking fenfluramine had a high incidence of valvular heart disease,[48] it was withdrawn from the U.S. market in September 1997. It is not presently understood why fenfluramine causes valvular heart disease.

Dexfenfluramine (7) is the therapeutically active dextrorotatory stereoisomer of fenfluramine.[49] While used widely throughout the world for many years, the U.S. FDA approved this drug only in April 1996. The reduction of body weight and the suppression of appetite with dexfenfluramine are thought to be mediated by 5-HT_{1B} or 5-HT_{2C} receptor modulation, but the exact underlying mechanisms are unclear.[50] Dexfenfluramine is more selective for serotonin release and reuptake, relative to norepinephrine and dopamine, than fenfluramine. Dexfenfluramine reduces food consumption in several rat-feeding models.[51]

Dexfenfluramine reduces overall caloric intake, lowers snack or meal size in human clinical trials, and produces a selective bias for the inhibition of the consumption of fatty foods.[52,53] Body weight continues to diminish even after significant weight loss has already been achieved.[54] As with fenfluramine, the largest weight reduction occurs in the first 6 months of treatment, and this loss is maintained over an additional 6 months.[55] After withdrawal of the drug, body weight increases slowly, indicating that the drug has residual effects even after 12 months of administration. Dexfenfluramine was withdrawn, along with fenfluramine, in September 1997.

Because of the role that serotonergic modulation can play in the regulation of food consumption, selective serotonin reuptake inhibitors (SSRIs) have been evaluated as antiobesity agents. Fluoxetine (8) has been associated with weight loss, unlike the tricyclic antidepressants which generally cause an increase in weight.[56] The mechanism of weight loss for fluoxetine is thought to be due to increased satiety. When higher doses of fluoxetine (60 mg/kg po) than those typically used for antidepressant therapy (typically 10−20 mg/kg po) were given in a human clinical trial once daily for 14 days, food consumption and body weight were reduced.[57] However, weight loss was not maintained for extended periods. Fluoxetine has not been approved for the treatment of obesity.

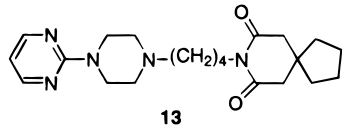


8 9 10

11 12

In addition to fluoxetine, a variety of other SSRIs have been investigated for their effects on weight lost. Sertraline (9) in rats enhances satiety and reduces body weight in freely feeding *ob*/*ob* mice following chronic administration.[51,58,59] The exact mechanism of action of sertraline on reducing food consumption remains controversial. Clinical trials with paroxetine (10) in obesity have been unsuccessful,[60] as have similar trials with citalopram (11).[61] However, the SSRI fluvoxamine (12) is associated with weight loss in the clinic.[62] The differences in the activities of the SSRIs are probably due to variations in their pharmacological profiles.

Other than the 5-HT_{1B} and 5-HT_{2C} receptors, there is little evidence linking additional 5-HT receptor subtypes with effects on feeding.[47] Although 5-HT_{1A} agonists induce feeding behavior in experimental models,[63] a low incidence of weight abnormalities have been seen in the widespread clinical use of the 5-HT_{1A} partial agonist buspirone (13),[64] and the 5-HT_{1A} antagonist WAY-100635 (14) did not significantly reduce food intake in freely feeding rats.[65]



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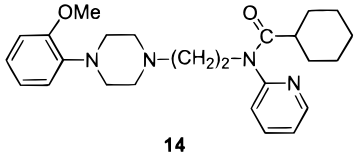

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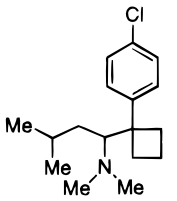
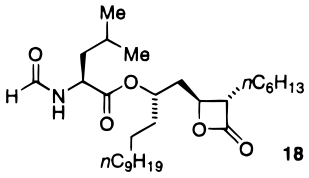
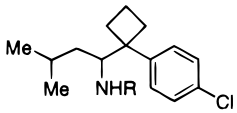
The effects of activation of the 5-HT_{1B} receptor on feeding in rodents are difficult to interpret, partly because of the lack of highly subtype-selective agents and the marked species differences observed. Nevertheless, it appears that agonists inhibit feeding in rats although this effect is diminished upon chronic treatment.[47] The 5-HT_{2C} receptor is implicated as a pharmacological target for obesity, given the activity seen with dexfenfluramine. Feeding studies in this area are also somewhat contradictory, as 5-HT_{2C} agonists, such as *N*-(*m*-chlorophenyl)piperazine, induced weight loss in humans in addition to causing nausea.[47] Some 5-HT_{2C} antagonists were hyperphagic in rodents, but others have not shown that effect.[47]

Combination Therapy. Combinations of fenfluramine and phentermine (fen−phen) have been investigated. A 4-year study showed that the combination was effective when diet alone was not.[66] No serious health problems were reported, although primary pulmonary hypertension (PPH) was a concern.[67] Use of the fen−phen combination was widespread during 1996−1997 in the United States, even with the report of a 23-fold increase in the risk of PPH after more than 3 months of treatment.[68] This combination enjoyed significant popularity because it was particularly effective in decreasing weight and the paucity of available therapy. The U.S. FDA estimated that ca. 30% of those taking fen−phen combinations suffered from some form of valvular disorder, although less than 5% of an age- and weight-matched control population had similar abnormalities.[69] The U.S. FDA withdrew fenfluramine and dexfenfluramine in September 1997. Phentermine (3) was not withdrawn because it is not believed to be responsible for these cardiovascular effects. Phentermine and fluoxetine (phen−pro) combinations[70,71] have been used by some weight loss clinics without U.S. FDA approval.

Table 3. Newer Antiobesity Drugs

drug	structure	mechanism	status
sibutramine (Meridia, Reductil; Knoll Pharmaceutical)	 **15**	monoamine reuptake inhibitor: serotonin, norepinephrine receptor affinity	launched in U.S. (Feb 1998), Brazil, Mexico; preregistered in Canada, Germany, Spain, U.K., France
orlistat (Xenical, Zenical; Hoffman-LaRoche)	 **18**	gastric lipase inhibitor	launched in certain European countries (France, Germany, U.K.), New Zealand, plus others; preregistered in U.S. (July 1997), Canada, European Union

Newer Antiobesity Drugs. Sibutramine (15; Table 3) was launched in the United States in February 1998.[72] Originally evaluated in the clinic as an antidepressant, 15 is a β-phenethylamine that is a dual serotonin and norepinephrine reuptake inhibitor.[73,74] The reuptake inhibition for sibutramine is relatively weak, but it undergoes rapid and extensive metabolism resulting in demethylated amines 16 and 17 which have greater pharmacological activity.[75]



16 R = Me
17 R = H

Sibutramine has minimal direct affinity for serotonergic, adrenergic, dopaminergic, muscarinic, glutamate, and benzodiazepine receptors and has little or no effect on monoamine oxidase.[76] Sibutramine reduces food consumption by both the inhibition of monoamine reuptake and enhanced thermogenesis, perhaps via an indirect activation of the β_3-adrenergic system in brown adipose tissue.[77] In human clinical trials, sibutramine produced dose-dependent weight loss.[78] This was also observed with patients who suffer from non-insulin-dependent diabetes.[79] Sibutramine even reduces food intake in obese subjects not trying to lose weight.[80] Although there are no reported cases of PPH with 15, the drug does increase blood pressure and heart rate in healthy volunteers.[81] In addition, sibutramine has the potential to cause dependency if it is abused.

Orlistat (18; Table 3) is a natural product from *Streptomyces toxytricini* that is a hydrogenated derivative of lipostatin, a naturally occurring lipase inhibitor.[82] Orlistat is a potent inhibitor of pancreatic, gastric, and carboxylester lipases and phospholipase A_2, which are required for the hydrolysis of dietary fat in the gastrointestinal (GI) tract into fatty acids and monoacylglycerols.[83] Orlistat reacts with specific nucleophiles, such as Ser-152 for pancreatic lipase, to form esters which hydrolyze so slowly that the inhibition is essentially irreversible (Figure 2). Since carboxylester lipase is also involved in the hydrolysis and absorption of fat-soluble vitamin esters, it is recommended that multivitamin supplements be taken daily during therapy. Orlistat decreases systemic absorption of dietary fat,

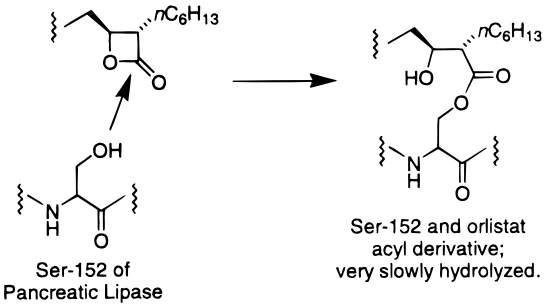


Figure 2. Mechanism of action of orlistat for the inhibition of pancreatic lipase.

leading to a reduction in body weight and lowering of plasma cholesterol.

Orlistat reduced the absorption of dietary fat in human clinical trials by 30%.[84] Additionally, it decreased total cholesterol and lipoprotein levels without affecting the HDL/LDL ratio.[85] Due to increased levels of fat in the stools, orlistat caused an increased frequency and urgency of defecation, fecal incontinence, flatulence, and abdominal pain. These effects were more prevalent in the first year of treatment and then were tolerated in the second year due to increased patient compliance.[83]

Orlistat has now been approved for marketing in several European countries (e.g., France, Germany, and United Kingdom), New Zealand, and additional countries in South America. The New Drug Application for orlistat in the United States was resubmitted by Hoffmann-La Roche in November 1997. The biggest concern in the early clinical trials was an increased risk of breast cancer in female patients, which was found to be statistically significant. Since many of the cases of breast cancer were found within 6 months of initiating therapy, the company has concluded that this effect was incidental to orlistat treatment.[83]

Modulators of Adipose Tissue

***β*$_3$-Adrenergic Receptor Agonists.** The β_3-adrenergic receptor is found primarily in adipose tissue and mediates a variety of metabolic functions, including lipolysis, thermogenesis, and motility in the GI tract.[86] β_3-Adrenergic receptors induce both catecholamine-stimulated lipolysis in white and brown adipose tissue and thermogenesis in brown adipose tissue (BAT); it appears that BAT thermogenesis is primarily respon-

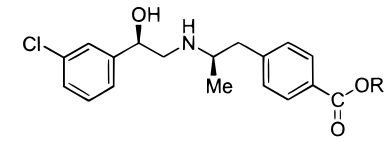
sible for the removal of excess fat in animal models.[87] Rodents and hibernating species of mammals maintain large stores of BAT, which is their primary site of thermogenesis. There is a strong correlation between abnormalities in BAT function and obesity.[88] Individuals with a mutation causing an Arg replacement for Trp-64 in the β_3-adrenoceptor have been identified as having an increased propensity to gain weight.[89] Unlike rodents, humans lose most of their BAT during the transition from newborn to adult. Infants contain relatively high levels of BAT because their metabolic rates increase in response to cold or adrenergic stimulation.

The β_3-adrenoceptor is a seven-transmembrane G-protein-coupled receptor (GPCR) that activates adenyl cyclase.[90] Stimulation of this receptor produces a cAMP-dependent activation of lipase, greater production of uncoupling protein-1 (UCP-1) in BAT, and higher insulin sensitivity. These activities result in a reduction of body weight and ameliorate diabetic symptoms in various animal models of obesity and diabetes. In addition, it has been hypothesized that β_3-adrenergic agonists reduce food intake via central β_2- or β_3-adrenoceptor activation. A recent study supports this proposal. Food intake was decreased when β_3-adrenergic agonist BRL-37344 was injected intraperitoneally into lean and obese Zucker rats.[91] The lessened food intake was attenuated when propranolol, a nonspecific β-adrenergic antagonist, was injected into the third cerebral ventrical of the brain.

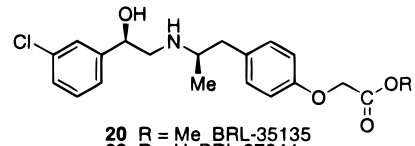
The key to success in this area is the discovery of selective β_3-adrenergic receptor agonists that lack cardiovascular or other effects mediated by β_1- or β_2-adrenergic stimulation.[92,93] There is some pharmacological evidence for a fourth β-adrenoceptor (β_4), but this remains controversial.[94] A large number of β_3-adrenoceptor agonists have been prepared and evaluated, and these fall under either the arylethanolamine or aryloxypropanolamine chemical families.[95] In this Perspective, we describe many of the compounds with this type of activity, but not all. We apologize to those researchers whose series we have omitted for the sake of brevity.

1. Arylethanolamines. Beecham derivatives BRL-26830A (19) and BRL-35135 (20) were the first β_3-adrenoceptor thermogenic agents disclosed and are potent stimulators of metabolic rate in rodents, with minimal cardiovascular effects.[96] These esters are well-absorbed and rapidly converted to their pharmacologically active acid metabolites, BRL-28410 (21) and BRL-37344 (22), respectively. The carboxylic acids (21 and 22) are themselves more selective for the β_3- relative to β_1- or β_2-adrenoceptors than are the corresponding esters (19 and 20), resulting in increased functional selectivity for 21 and 22 (relative to 19 and 20).[97] Among the four diastereomers for 19−22, the *RR* isomer is the most active in all of the tissues and receptors studied.[98] In obese rats, treatment with 19 and 20 produced weight and adipose tissue loss. Both 19 and 20 have been evaluated in human clinical trials. Although they demonstrated modest clinical efficacy, the extent of weight loss and the presence of side effects such as muscle tremors have precluded further development.[95]

The first β_3-adrenergic agonist clinical candidates were chosen based upon a favorable therapeutic index



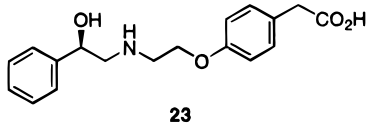
19 R = Me BRL-26830A
21 R = H BRL-28410



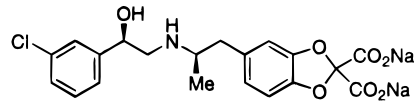
20 R = Me BRL-35135
22 R = H BRL-37344

in rodents. Despite high (80−90%) sequence homology between rodent and human receptors, agonists possess widely different functional effects at each receptor.[99] For example, 22 has comparable K_i values for rat and human β_3-adrenoceptors but is 38-fold less active in stimulating adenylate cyclase in CHO cells expressing human β_3-adrenoceptors when compared to those expressing the rat variants. Compound 22 did not stimulate lipolysis in human fat, even though it had marked activity in rat white adipocytes.[100] In addition, it has been difficult to predict the responses of β_3-adrenoceptor agonists in tissues with a high number of β_1- or β_2-adrenoceptors or a low number of β_3-adrenoceptors based on data obtained from cloned receptors.[101]

After evaluation of a series of related structures, ZD2079 (23) was chosen for human clinical trials.[95] It is a full agonist in human β_3-adrenoceptor assays but proved to have only limited efficacy in human clinical trials. It is reported to have only weak agonist activity in a human lipolysis assay in adipocytes.[102]



23



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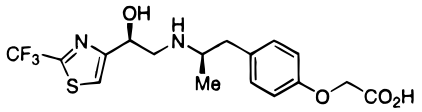
CL-316,243 (24) is a dicarboxylic acid derivative of 22 that also shows greater in vitro activity in rats when compared to experiments using human receptors.[103] Although the compound increased metabolic rate and reduced body weight in clinic trials, it displayed a poor pharmacokinetic profile which necessitated the use of high doses. Thiazole 25 (CP-114,271) is another β_3-agonist examined in the clinic which did not prove as effective in humans as expected earlier based on rodent data.[95]

Replacement of the phenyl ring of the phenoxyacetic acid portion of 22 with an indole led to AD 9677 (26).[104] This compound has a 0.06 nM EC_{50} in a human β_3-adrenergic receptor assay with >100-fold selectivity relative to β_1- and β_2-adrenoceptor subtypes. In a functional β_3-adrenergic test, it displayed 116% of the response of isoproterenol (β agonist reference) but only had ca. 25% of the isoproterenol effect in β_1 and β_2 functional assays.



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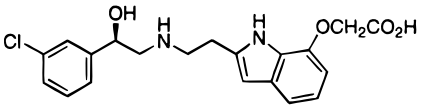

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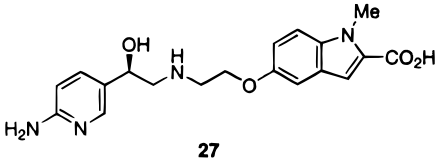
The acetic acid portions of 21−26 are a key structural feature for imparting β_3-adrenoceptor selectivity. Conformationally restricted bioisosteres for this group have been examined in an attempt to increase activity and selectivity. CP-331,679 (27) contains an indoleacetic acid and was found to be a potent (300 nM EC_{50}) full agonist at the human β_3-adrenergic receptor with >100-fold selectivity relative to other β-adrenoreceptors.[105] Compound 27 also has a 2-aminopyridine replacement for the 3-chlorophenyl ring of 19−22 and was subsequently shown to have poor bioavailability (<10% in rats).[95] Further efforts are ongoing to improve its in vivo properties.

FR-149175 (28) is one of a class of related structures in which the aryl ring of the phenoxyacetic acid moiety has been incorporated into a bicyclic framework connected back toward the amine nitrogen.[106] It has a <100 nM EC_{50} in a cAMP accumulation assay indicative of β_3-adrenoceptor functional activity and suppresses weight gain in both obese and lean rodent models of obesity. It is >900-fold selective relative to β_1- and β_2-adrenoceptor activity.



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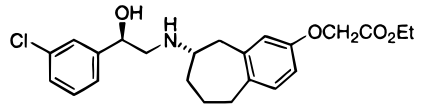

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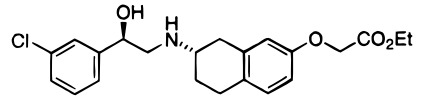

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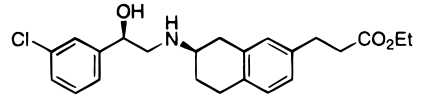
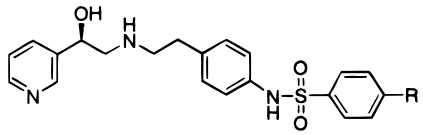


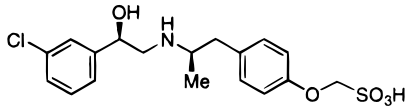
30

The phenylethanol aminotetralins were the first compounds which incorporated the bicyclic modification similar to that found in 28.[107] Ester 29 was the most potent and selective among a series of close analogues and stereoisomers.[108] It displayed the lipolytic and thermogenic responses expected for a β_3-adrenoceptor agonist and is presently undergoing clinical evaluation.[109] Compound 30 (SR-59062A) has subsequently been found to be 4-fold more potent and selective than 29 based on functional testing in various tissues.[110]

In addition to 27, a variety of other pyridylethanolamines are β_3-adrenoceptor agonists.[111] From one such series, 31 was highlighted because the β_3-adrenoceptor affinity was selective relative to β_1- and β_2-adrenoceptor binding, and it had a 6.3 nM EC_{50} for functional β_3 activity with 70% intrinsic activity.[112] When 31 was given to Rhesus monkeys ip, hyperglycerolemia was observed with a maximal effect similar to that of isoproterenol. Although the presence of 31 was not detected after oral administration to dogs, the related 4-iodophenylsulfonamide 32 exhibited an oral bioavailability of 51%. The in vitro biological activity for 32 was very similar to that of 31. Both 31 and 32 contain the sulfonamide group as a bioisostere for the carboxylic acid found in 22 and related compounds. The synthesis of a large number of such sulfonamides as β_3-adrenoceptor agonists has just been reported.[113−115]



31 R = $NHC(O)NHC_6H_{13}$
32 R = I



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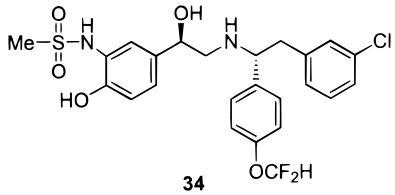
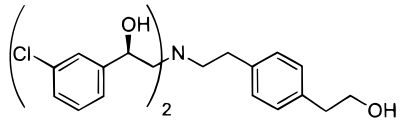


34

In addition to esters and sulfonamides, other prodrugs of 22 and carboxylic acid surrogates have been explored. These include alcohol, amide, hydroxamic acid, malonic acid, phosphinic acid, phosphonic acid, phosphonic acid ester, and sulfonic acid.[116] Of these, sulfonic acid 33 (BMS-187413) is among the most potent and selective with a 60 nM EC_{50} in a functional β_3-adrenoceptor assay and an intrinsic activity of 0.57 that of isoproterenol. A structure−activity relationship (SAR) study of a series of sulfonamides resulted in BMS-210285 (34).[117] This compound has a 9 nM K_i at the human β_3-adrenoceptor, and a β_3 intrinsic activity of 83% relative to isoproterenol.

Although most β_3-adrenoceptor agonists contain a secondary amine as a key structural feature, there are two exceptions. The first are tertiary amines in which two arylethanolamine units, which are reported to have thermogenic activity in animal models,[118] are attached to the amine nitrogen as in 35. The second are derivatives in which the benzylic hydroxyl and the amine nitrogen are connected together to form potential prodrugs of the parent structures. For example, oxazolidine 36 was reported to increase oxygen consumption, an indirect calorimetry measure, by 135% after oral administration in rats of 1 μmol/kg.[119] Several additional

cyclic derivatives have recently been reported, such as the hemiketal 37.[120]



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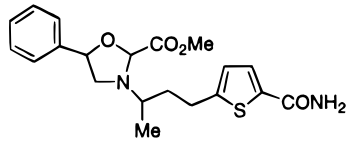

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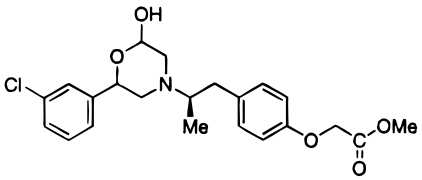
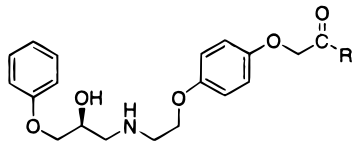


37

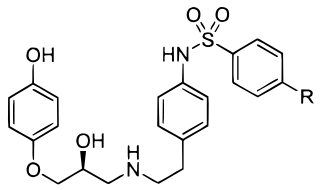
2. Aryloxypropanolamines. The aryloxypropanolamines were first described as β_3 agonists in the mid-1980s.[121,122] As a class, aryloxypropanolamines are partial agonists or antagonists at β_2- and β_3-adrenoreceptors, which contributes to the selectivity of their in vivo biological effect. Compounds 38 and 39 are potent β_3-adrenoceptor agonists chosen from a large number of related derivatives.[123,124] However, amide 39 (ZD7114) proved to be ineffective in human clinical trials.[95] ZD7114 has now been reported to not induce lipolysis in human omental adipocytes.[102]

As with the phenethanolamines, aryloxypropanolamines with sulfonamide-containing side chains including 40 and 41 have been designed and investigated. Compound 40 showed a 6.3 nM EC_{50} for the stimulation of human β_3-adrenoreceptors and an intrinsic activity of 51% compared to isoproterenol; 40 was selective relative to human β_1- and β_2-adrenoreceptors.[125] Urea 41 (L-755,507) emerged from an effort to prepare acylamino derivatives in the sulfonamide series and is among the most potent β_3-adrenoceptor agonists reported, with a 0.43 nM EC_{50} for β_3-adrenoceptors.[126] Although 41 displays low oral bioavailability (ca. 1%), additional research involving bioisosteric replacements for the phenolic group led to aminopyridine 42 which was reported to have 4% and 47% bioavailability in rat and dog, respectively.[127] Additional modifications to this series led to improved pharmacokinetic properties.[128] L-749,372 (43) and L-750,355 (44) are selective partial agonists of the human β_3-receptor, with 33% and 49% activation, respectively. Both compounds were found to stimulate lipolysis in Rhesus monkeys with ED_{50} values of 2 and 0.8 mg/kg iv, respectively, and have minimal effects on heart rate. Oral bioavailability in dogs is improved to the levels of 41% and 47% for 42 and 43, respectively.

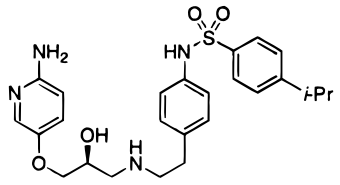
Heterocyclic replacement of the phenoxyacetic acid group of compounds related to 38 led to thiazole BMS-187257 (45), which has comparable biological activity to that of 22.[129] Compound 46 is a benzimidazolone derivative that has considerable in vivo selectivity



38 R = OH
39 R = $NH(CH_2)_2OMe$



40 R = H
41 R = NHC(O)NH-*n*-C_6H_{13}



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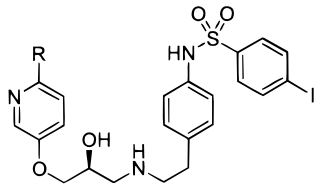
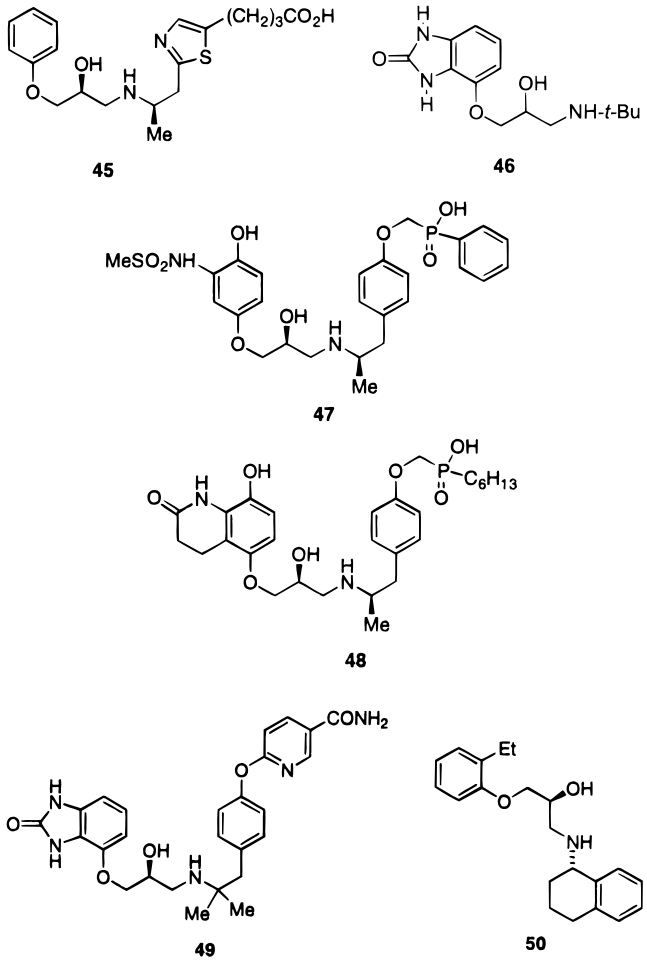


43 R = H
44 R = NH_2

because of antagonist properties at β_1- and β_2-adrenoceptors.[130] The benzimidazolone moiety was incorporated as the key component of a template for compounds of the phosphonic and phosphinic acid series, leading to derivatives 47 and 48.[131] These compounds have <300 nM EC_{50} values in functional β_3-adrenoceptor assays and excellent selectivity relative to β_1- and β_2-adrenoceptors. Further, their intrinsic activity relative to isoproterenol is >0.8. Related structure 49 was discovered using a combination of parallel synthesis and traditional medicinal chemistry and is a full and selective agonist with a 30 nM EC_{50} in the functional β_3-adrenoreceptor assay.[132]

Aminotetralin 50 (SR 59230A) is the first functionally selective β_3-adrenergic antagonist to be reported.[133] When administered orally, it inhibited colonic motility and the thermogenic response in BAT of 22 and 29, both effects mediated by β_3-adrenoceptor activation. This compound is a useful pharmacological tool to probe the role of β_3-adrenoceptors in vivo.

Clinical evaluation of early β_3-adrenoceptor agonists was disappointing, largely due to pharmacological differences between human β_3-adrenoceptors and the rodent assays used in drug discovery programs. Although many excellent compounds were developed for the treatment of obesity in mice, human clinical trials demonstrated poor efficacy and substantial β_1- and β_2-mediated side effects. The present generation of compounds selective for activation of human β_3-adrenocep-



tors offers considerable promise for the treatment of obesity, and they are in various stages of preclinical and clinical development.

Uncoupling Proteins. Energy expenditure is comprised of the amount required for basal physiological functioning and that related to the performance of specific functions. Uncoupled mitochondrial respiration in BAT allows rapid access to energy stores as needed. Uncoupling protein-1 (UCP-1) is an integral component of the mitochondrial inner membrane of brown adipocytes and is central to BAT function (Figure 3).[134] UCP-1 transports fatty acids into the mitochondria by an active process involving protonation.[135] UCP activation causes the electrochemical gradient generated along the inner mitochondrial membrane during respiration to dissipate. As a result, substrate oxidation is uncoupled from ATP generation, producing heat instead of chemical energy.[136]

UCP-1 expression and activity is regulated by the sympathetic nervous system, and signaling via the β_3-adrenergic receptor has been implicated in UCP-1 activation.[137−139] Uncoupling activity is regulated by an increase both in UCP-1 abundance and in UCP-1 activity resulting from cAMP-induced elevations in intracellular free fatty acid concentrations. Inhibitors of UCP-1 activity exist, such as purine nucleotides, but their role in physiological regulation of UCP-1 activity is not well defined. While humans express UCP-1 in BAT, the abundance of BAT in most adult humans is quite small. Consequently, a role for UCP-1 activation

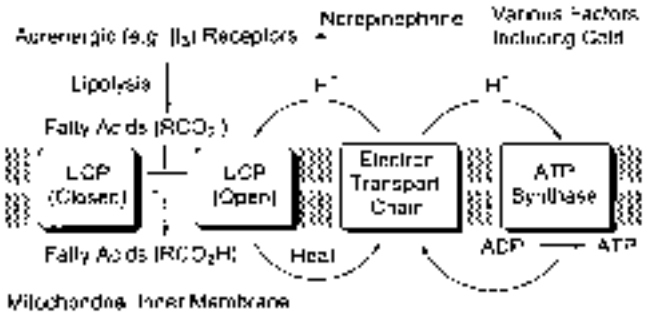


Figure 3. Proton transfer across the mitochondrial inner membrane mediated by uncoupling proteins, supplementing the normal ATP synthase pathway.

in regulating energy expenditure in humans is not firmly established at this time.

Two other members of the UCP gene family, UCP-2 and UCP-3, have been identified in humans. UCP-2 and UCP-3 are structurally homologous to UCP-1 and also share functional properties. While UCP-1 is solely expressed in BAT, UCP-2 and UCP-3 are expressed in other tissues as well. UCP-2 is found in white adipose tissue, lung, liver, spleen, and macrophages,[140,141] while UCP-3 is primarily expressed in muscle and BAT.[142,143]

The expression of UCP-2 is modulated by diet and not by the sympathetic nervous system.[140] The consumption of a high-fat diet selectively upregulates UCP-2 expression in white fat and UCP-1 expression in brown fat.[144,145] Resistance to obesity is correlated with an early and selective induction of UCP-1 and UCP-2 but is not associated with changes in the expression of UCP-3. Augmented expression of certain UCPs may provide defense against high-fat-induced obesity.

The physiological importance of the uncoupling proteins may be determined by where and when they are expressed. UCP-1, found solely in BAT, may primarily modulate cold-induced thermogenesis for the purpose of maintaining body temperature. UCP-2, which is widely distributed, may be important for determining basal metabolic rate and, possibly, resistance to obesity. UCP-3, found abundantly in skeletal muscle, a tissue which has been implicated in thermogenesis as well, may also be essential for maintaining basal metabolic rate.[146] Presently, it is unclear how direct UCP modulation can be used effectively in drug therapy for obesity. It is possible that UCP agonists or inducers may have a useful role in raising metabolic rate.

Peroxisome Proliferator-Activated Receptor. Expansion of adipose tissue mass necessitates the differentiation of new adipocytes from precursor cells. Controlling or reversing adipocyte differentiation could lead to the treatment of obesity and associated disorders.[147] Adipocyte differentiation from adipoblasts, which are adipose precursor cells, is orchestrated by a set of interdependent transcription factors (Figure 4).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which modulate gene expression. They mediate the effects of fatty acids upon gene expression. In mammals, three distinct PPARs have been identified (α, γ, and δ).[148] PPARγ exists as two isoforms, γ_1 and γ_2, which are derived from the same gene by alternative promoter splicing and differ only at their N-termini.[149] While PPARγ_1 is expressed in many tissues, PPARγ_2 is

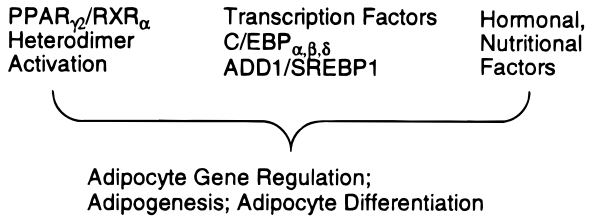
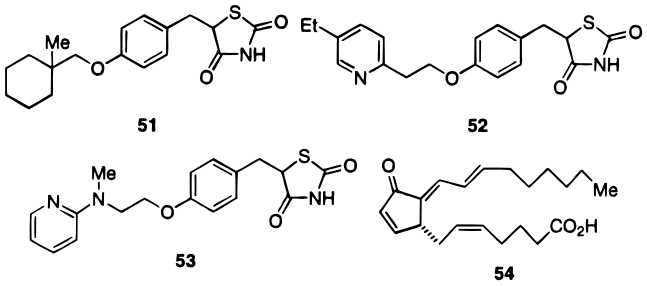


Figure 4. Various factors promoting adipogenesis and adipocyte differentiation.

expressed at high levels only in adipocytes.[150] PPARγ_2 appears to have several functions related to adipogenesis.[151] As a lipid-activated transcription factor, PPARγ activation connects systemic lipid metabolism and adipocyte differentiation and directly regulates the expression of many fat-specific genes triggering the entire program of adipogenesis.[152]

PPARγ is an obligate heterodimer with the retinoic acid receptor (RXR) which binds to DNA at DR-1-like sequences, the direct repeat of hormone response elements separated by one base. Most adipocytes contain binding sites for the PPARγ/RXR heterodimer. The ability of PPARγ to increase transcription requires the binding of specific ligands. Early PPAR activators were eicosinoids which functioned by stimulating the intracellular production of endogenous ligands.[150]

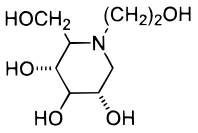
The thiazolinediones such as ciglitazone (51), pioglitazone (52), and rosiglitazone (53) were found earlier to increase insulin sensitivity in models of insulin resistance in patients with non-insulin-dependent diabetes. These compounds display high affinity for PPARγ.[153] Rosiglitazone is significantly more potent than the others, and it is hoped that this will translate into a greater safety margin in the clinic relative to the liver toxicity which has plagued this structural class. Certain prostaglandins such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (54) also bind to PPARγ.[154]



PPARγ_2 induces an adipocyte phenotype in fibroblasts and muscle cells when infected with a retroviral vector overexpressing mPPARγ_2.[155] Pioglitazone (52) induced >95% of the fibroblasts to be differentiated to fatty tissue. Several key factors form a regulatory loop that controls adipogenesis. Transcription factors C/EBPβ and -α are elevated early in the process and may increase the expression of PPARγ. Additionally, ADD1/SREBP1 promotes the transcriptional ability of PPARγ. Maintaining this regulatory loop requires the generation of an endogenous PPARγ ligand. The development of specific antagonists of PPARγ may provide new therapeutics for the treatment of obesity.

Mechanisms Targeting the Gastrointestinal Tract

Inhibition of the Absorption of Dietary Carbohydrates. Inhibition of the breakdown of dietary polysaccharides slows or inhibits their absorption from the GI tract. This is accomplished by a variety of agents, most notably the aza sugars such as *N*-(2-hydroxyethyl)deoxynojirimycin (55, miglitol).[156] Metformin (56) delays the absorption of dietary carbohydrates by inhibiting the glucose transporter found on the brush border of the epithelial lining of the intestines.[157] In support of this mechanism, metformin is more active at enhancing glucose tolerance when glucose is administered orally than when it is administered parenterally.[158] Metformin is marketed for the treatment of non-insulin-dependent diabetes.



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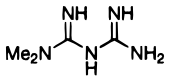

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Glucagon-like Peptide-1 Agonists. Glucagon-like peptide-1 (7−36) amide (GLP-1) is a 29-amino acid peptide prepared by differential posttranslational processing of the preglucagon gene product.[159] The sequence of GLP-1 is highly conserved in mammals suggesting that this peptide may have an important physiological role. GLP-1 appears to be an endogenous factor in the regulation of food intake when acting on specific receptors in the CNS. GLP-1 also plays a role in the periphery and is secreted from the distal gut in response to the presence of mixed nutrients in the GI tract.[160] GLP-1 is important in glucose homeostasis after the ingestion of meals comprised of carbohydrates via modulation of gastric emptying and alteration of the secretion of insulin and glucagon from the pancreas.[161] A synthetic version of the hormone GLP-1, insulinotropin, is currently in phase II trials for obesity-related diabetes. This drug may slow stomach emptying and boost insulin levels and thus may be useful for both obesity and NIDDM.

In the CNS, receptors for GLP-1 are concentrated in the paraventricular nucleus of the hypothalamus, the amygdala, and regions of the brain stem.[162] Several reports have suggested that GLP-1 may act as an anorectic agent and reduce food intake when injected intracerebroventricularly in rats.[163,164] This effect was blocked by pretreatment with the specific GLP-1 receptor antagonist exendin. Thus, GLP-1 may be a natural regulator of food intake and adiposity. While intraventricular GLP-1 reduced short-term feeding, it had no effect on either long-term feeding or body weight in lean or obese rats. More research is needed to fully clarify the role of GLP-1 in feeding.

Cholecystokinin-A Agonists. Cholecystokinin (CCK) is a peptide hormone found in the CNS and GI tract. While a variety of biologically active forms of CCK have been identified, the truncated C-terminal octapeptide CCK-8 (57) retains full biological activity. CCK mediates many diverse functions, both hormonal and neuromodulatory, through the action of the CCK-A and CCK-B receptor subtypes.[165] CCK-B receptors are located primarily in the CNS where CCK is involved in anxiety

and pain. CCK-A receptors are found mainly in the periphery and also in the brain.[166]

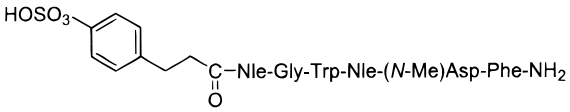
Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

57

CCK has received considerable attention as a possible modulator of food intake in animals. CCK aids the digestion of nutrients by inducing gallbladder contraction, pancreatic secretion, delayed gastric emptying, and satiety.[167] The release of CCK-8 from the small intestine following nutrient ingestion triggers these effects at CCK-A receptors on vagal afferents which relay the satiety signal to the brain.[168,169]

Intraperitoneal administration of CCK-8 decreased meal size in rats,[170] lean humans,[171] and obese humans.[172] However, daily food intake in rats was not affected suggesting that the animals eat more in subsequent meals to compensate for the lower caloric content of the first meal.[173] This observation underscores the probable limited role that satiety factors such as CCK will have in the treatment of obesity.

The human clinical data on CCK-8 spawned numerous programs to develop novel and selective CCK-A agonists. The poor pharmacokinetic and metabolic profiles associated with CCK and CCK-8 have been addressed with chemical modifications.[174] Initially, most CCK agonists were peptide derivatives;[175] for example, A 71378 (58) was found to be a potent and selective CCK-A agonist.[176] Studies on CCK-8 analogues indicated that Asp N-methylation is responsible for CCK-A receptor selectivity. The duration of the anorectic action of 58 in vivo was greater than that of CCK-8.[177]



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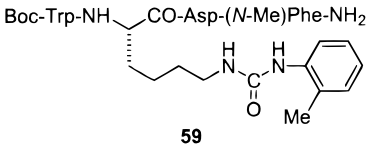

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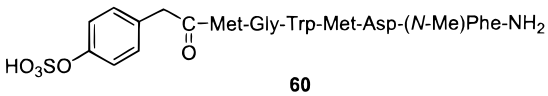
Replacement of the methionine residue of Boc-CCK-4 (Boc-Trp-Met-Asp-Phe-NH_2) with side-chain-substituted Lys derivatives resulted in A 71623 (59).[178] This tetrapeptide is a potent and selective agonist at the CCK-A receptor and is functionally equivalent to CCK-8 with enhanced metabolic stability. It also exhibited potent anorectic activity upon intraperitoneal administration in rats, dogs, and monkeys.[179]

Several additional hexapeptide CCK-A agonists have been reported.[180] Compound ARL 14294 (60) potently inhibited feeding in rats and dogs and was 18-fold selective for the CCK-A versus CCK-B receptor subtype.[181] Further research led to the discovery of ARL 15849 (61) which was 6600-fold selective for CCK-A versus CCK-B with improved stability and longer duration.[182] Compound 61 inhibited food intake with nanomolar potency following intraperitoneal administration in fasted rats. In addition, it produced weight loss in rats when administered for nine consecutive days. Unfortunately, 61 was not orally active.



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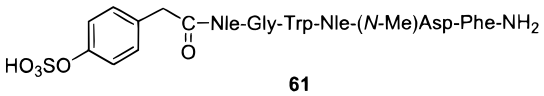

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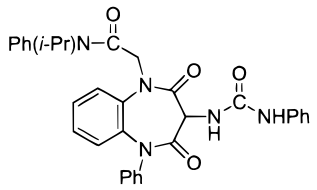

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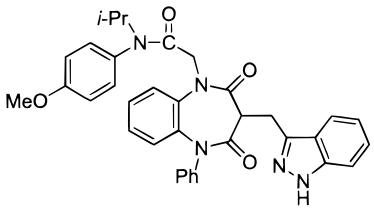

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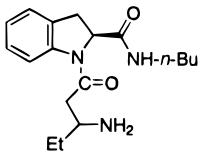
Despite success in the design of metabolically stable, receptor-selective ligands, oral activity has been difficult to obtain. The first nonpeptide CCK agonist reported was 1,5-benzodiazepine 62.[183] This compound was discovered by screening a library of selected compounds against a CCK-A agonist functional assay. Subsequently it was found that these benzodiazepines were not selective for CCK-A versus CCK-B receptors and had no oral activity in rodent-feeding models. The *N*-isopropylanilide moiety of 62 is possibly the key to its agonist properties, as incorporation of this structural feature into known CCK antagonists converted them into agonists.[184]

Continued investigation of the 1,5-benzodiazepine series replaced the C-3 phenylamide of 62 with an indazolylmethyl group leading to GW 5823 (63).[185] Compound 63 is the first CCK-A agonist to demonstrate oral activity in a rat-feeding model. It has modest selectivity for the CCK-A receptor subtype relative to CCK-B (50-fold). Unfortunately, 63 displayed a relatively low oral bioavailability in rats (ca. 8%) due to delayed gastic emptying and rapid metabolic clearance.[186]

CCK-Inactivating Peptidase Inhibitors. A CCK-inactivating serine peptidase has recently been identified.[187] It is a membrane-bound isoform of tripeptidyl peptidase II, whose function was not previously known. The peptidase was found in neurons that responded to CCK as well as in nonneuronal cells. It inactivates CCK-8 in two steps that corresponded to CCK-8 → CCK-5 (Gly-Trp-Met-Asp-Phe-NH_2) → (Gly-Trp-Met). Both fragments are biologically inactive.

Inhibitors of the peptidase have been designed in a rational manner resulting in the discovery of butabindide.[188] Butabindide (64) inhibits the CCK-inactivating peptidase competitively and selectively (7 nM K_i) without activity in CCK receptor binding assays. When 64 was administered intravenously to starved mice, a significant satiating effect was observed that was selectively blocked by devazepide, a CCK-A receptor

antagonist. Butabindide was also shown to significantly reduce food intake in rats.



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Leptin. Leptin is produced in adipocytes and secreted in concentrations proportional to the amount of adipose tissue.[189−192] Our understanding of leptin is growing so quickly that this review will be outdated as it is published. The realization that adipose tissue functions as an important endocrine gland, and not merely as a storage depot, has renewed interest in the older lipostatic theory of body weight control.[193] Leptin is a 167-amino acid protein that contains an amino-terminal secretory signal sequence of 21 amino acids. This signal sequence is functional and effects translocation of leptin into microsomes with subsequent removal of the signal peptide.[194] The leptin that circulates in the blood is a protein of 146 amino acids with an apparent molecular weight of 16 000 Da. The amino acid sequence of the rat *ob* gene cDNA is 96% identical to that in the mouse.[195] Human leptin is 83−84% homologous to mouse and rat leptin.

Tertiary structure modeling of human leptin predicts that leptin is a globular protein similar to hemopoietic cytokines such as interleukin-2 and growth hormone.[196] The probable structure is comprised of four α-helices, two short β-sheets, and a single disulfide bond located at the C-terminal end. An X-ray diffraction structure generated with leptin-E100, a 1-amino acid mutant, provided evidence in support of this structure.[197]

The leptin receptor was cloned from rat choroid plexus cDNA and found to be similar to gp 130.[198] Currently, there are five variants of the leptin receptor that are known in mice and rats, generated by alternative splicing of a common mRNA precursor.[199,30] The long receptor isoform, termed OB-Rb, is the most abundantly expressed in the hypothalamus. Its structure includes a large extracellular domain, a short hydrophobic transmembrane domain, and a fairly short intracellular portion containing two sequences for binding of Janus protein tyrosine kinase (JAK) and signal transducers and activators of transcripion (STAT) signaling pathways.[200] The long receptor isoform appears essential for leptin signaling, as the *db*/*db* mouse possesses a mutation that prevents its expression.[201]

Three short forms (OB-Ra, OB-Rc, and OB-Rd) of the leptin receptor include the extracellular, transmembrane, and short intracellular domains and lack only the sequence for JAK binding. Another short form, OB-Re, lacks both the transmembrane and intracellular domains and may be a soluble form of the receptor. These isoforms, expressed abundantly in the choroid plexus, may mediate uptake of leptin across the blood-brain barrier. They also do not appear capable of activating the JAK−STAT pathway.

Adipocytes secrete leptin, and the levels of leptin produced correspond to the extent of adipose tissue. Leptin reduced food intake by lowering meal size in rodents.[18−20] When leptin was administered to *ob*/*ob* mice, which are leptin-deficient, a sharp decrease in weight occurred via reduced food intake, increased oxygen consumption, and body temperature elevation.[202,203] No weight loss was observed when leptin was administered to *db*/*db* mice (leptin receptor-deficient). Obesity in humans is generally associated with high leptin levels.[204] Obese humans have increased serum leptin concentrations and *ob* mRNA concentrations in adipose tissue as compared to lean controls. This suggests that human obesity corresponds more to the *db*/*db* mouse leptin-resistant state than to the *ob*/*ob* mouse leptin-deficient state. One notable difference between obese and lean individuals is that the majority of leptin in plasma is bound to plasma proteins in lean subjects and is free in obese subjects.[205] Obese subjects also have a decreased CSF/serum leptin ratio, implying a lower efficiency of leptin uptake into the CNS.[206] This suggests that a saturatable transport mechanism for leptin uptake into the CNS does exist. Leptin is being evaluated by Amgen in limited clinical trials. Small-molecule leptin receptor agonists that are orally active may be important therapeutics for the treatment of obesity.

Recently, another anorectic peptide affecting the leptin system has been described. The hypothalamic peptide cocaine- and amphetamine-regulated transcript (CART) has been shown to be a satiety factor that is closely associated with the actions of leptin and NPY.[207] In animal models of obesity with disrupted leptin signaling, CART mRNA was almost absent from the arcuate nucleus of the hypothalamus. Peripheral administration of leptin in obese mice stimulated CART mRNA expression. When recombinant CART was injected intracerebroventricularly into rats, both normal and starvation-induced feeding were inhibited and the feeding response induced by NPY was completely blocked.

Leptin has been converted into a "pegylated" derivative by covalent attachment with poly(ethylene glycol).[20,21] The resulting modified leptin has a prolonged serum half-life in rats (>48 h) and exhibits the same profile of activities as does leptin in binding to the long form of the OB receptor and in in vivo models.

Amylin. Amylin,[208] a 37-amino acid peptide, is a hormone which is released by the β-cells in the pancreas along with insulin. It is similar in structure to calcitonin and the calcitonin gene-related peptides, and these peptides have similar effects on carbohydrate metabolism. Amylin inhibits food intake in a variety of rodent-feeding models, which provides support for the role of amylin as a peripherally acting satiety factor.[209]

Neuropeptide Y. Neuropeptide Y (NPY) is a 36-amino acid C-amidated peptide which is a member of the pancreatic polypeptide family.[210] It is highly expressed in several regions of the brain and is released into the circulation from neuronal stores in times of stress. In the CNS, NPY has been implicated in obesity and feeding,[211] anxiety and depression, endocrine function, and metabolism. NPY is a powerful stimulant of food intake when administered directly into the hypothalamus. Recent discoveries have revealed six GPCRs that bind with high affinity to NPY. The most likely candidate for the NPY receptor which most influences feeding is the Y5 receptor, found primarily in the hypothalamus.[212] Food intake is inhibited by antisense oligodeoxynucleotides to the NPY5r.[213] In addition, in obese Zucker rats, which are characterized by reduced

hypothalamic NPY receptor density, intracerebroventricular injections of a weak but selective NPY5 receptor agonist ([D-Trp^{32}]NPY) did not stimulate feeding whereas it did in lean rats.[214] The agonist also did not affect the feeding response to hNPY. These results indicate a down regulation of the NPY "feeding" receptor in these obese rats and suggests that it is the Y5 receptor. The Y5 receptor from mouse, rat, dog, and humans has now been cloned and pharmacologically characterized.[211,215] The sequences of the four species homologues is highly conserved, with 88−97% amino acid identity between any two species.

A recent study on the feeding effects of the Y1-selective antagonist BIBP 3226 suggests that both the NPY1 and NPY5 receptors in the paraventricular nucleus (PVN) of the hypothalamus are involved in the regulation of food intake.[216] In addition, BIBP 3226 had no effect on the consumption of regular chow but significantly reduced the intake of a highly palatable diet and the food intake stimulated by fasting. However, BIBP 3226 did not block the orexigenic effect of the Y5 receptor agonist PYY_{3-36} injected into the PVN. This indicates that the stimulatory effect of exogenous NPY is probably mediated through a Y5 receptor; however, the Y1 receptors may mediate the effect of exogenous NPY on intake of a highly palatable diet.

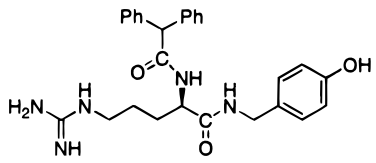
NPY is secreted upon leptin stimulation in the hypothalamus. When transgenic mice lacking NPY were mated with *ob*/*ob* mice (leptin-deficient), the offspring were markedly less obese than the *ob*/*ob* parental strain.[217] They also had lower serum glucose and insulin levels and were more fertile than the *ob*/*ob* parental strain.

NPY-induced feeding in rats may be influenced by the histaminergic system. Thioperamide maleate, a specific histamine H3 receptor antagonist, reduced the feeding response to NPY by 52% and had no effect on food intake in sated rats.[218] This suggests that thioperamide may have a specific effect on NPY receptor-mediated neuronal systems related to feeding.

Considerable progress has been made in the design of antagonists at the NPY1r, which may play a role in feeding and obesity. The first potent nonpeptide structure (65, BIBP 3226) was designed using the C-terminal region of NPY and modified into a nonpeptide structure.[219] Compound 65 exhibited low nanomolar affinity for the Y1 receptor and blocked NPY-induced inhibition of adenylate cyclase activity in vitro. It was also highly selective for the Y1 receptor versus other receptor subtypes. The data reported for effects on feeding for this compound are inconsistent.

Cyclohexyl compound 66 was reported to have a 39 nM IC_{50} at the Y1 receptor.[220] A series of novel benzimidazole Y1 antagonists has recently been described, as an extension of related indolic compounds.[221] Benzimidazole 67 has a 1.7 nM IC_{50} at the Y1 receptor, without appreciable affinity at the Y5 receptor. No data for the effects on feeding have been reported for these compounds.

The first Y5-selective antagonists appeared in the patent literature in mid-1997.[222,223] Quinazoline sulfonamide 68 was found to have low nanomolar affinity at the Y5 receptor and inhibited food intake in Sprague−Dawley rats by 96% relative to controls at 30 mg/kg after intraperitoneal administration. Quinazoline 69 also had low nanomolar Y5 receptor affinity. Aminopyrazole 70 has been reported to have an 8.3 nM IC_{50} at the Y5 receptor.[224] A related compound (71) had a 2.3 nM IC_{50} at the NPY5r.[225] No in vivo data has been given for 70 or 71. Aminopyridine 72 was reported to have an IC_{50} value of 4.1 nM for NPY binding to NPY5 receptors.[226] The amide derivative 73 is the most potent NPY5 receptor antagonist described in the literature.[227] It is reported to have an IC_{50} value of 0.47 nM at the Y5 receptor and shows no significant affinity at NPY1, NPY2, and NPY4 receptors (IC_{50} values > 5 μM). Again, no in vivo data has been provided for this compound.



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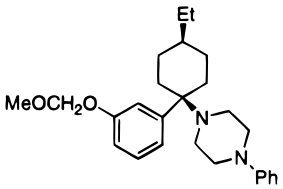

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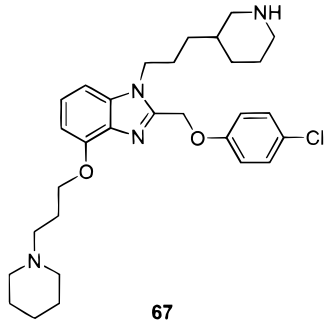

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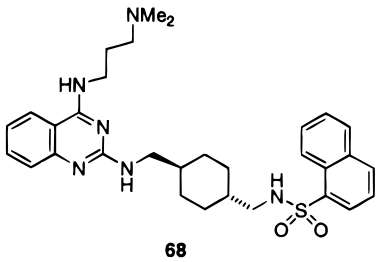
It is likely that a variety of new compounds will be described in the next several years that are potent and selective rNPY5 antagonists, given the level of interest in this target. With the advent of these compounds, we will be able to probe the merits of this approach for the treatment of obesity.

Melanocortin Receptor Activation. The gene proopiomelanocortin (POMC) is expressed in the pituitary gland, where it is processed to both adrenocorticotopic hormone (ACTH) and melanocyte-stimulating hormone (α-MSH).[228] α-MSH stimulates pigmentation and also has directs effects in the CNS which are independent of downstream effects mediated by the adrenals. There are five GPCRs which have been found to recognize α-MSH. The association of α-MSH with obesity was first appreciated because the agouti peptide stimulates obesity in mouse models and is also a high-affinity antagonist at MC-4 receptors.[229] Further, mice in which the MC-4 receptor expression is disrupted develop a state of obesity.[230] Studies in rats injected with a MC-4 receptor antagonist into the CNS indicate that MC-4 receptor signaling is important in mediating the effects of leptin on food intake and body weight.[231]

The physiological feeding response evoked by the antagonism of MC-4 receptors is influenced by NPY signaling. The NPY1 receptor antagonist 1229U91 was shown to significantly attenuate the orexigenic effect of HSO14, a selective MC-4 receptor antagonist.[232] Therefore, the MC-4 receptor is a further means by which leptin and NPY promote energy homeostasis.

Galanin. Galanin is a 29-amino acid neuropeptide with a variety of known functions, and it binds to at



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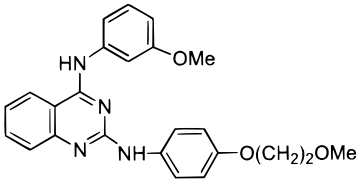

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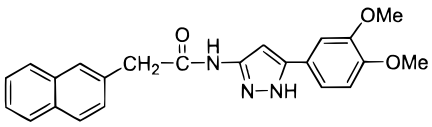

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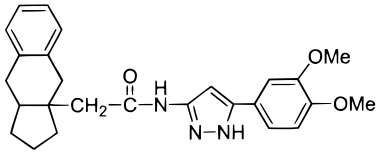

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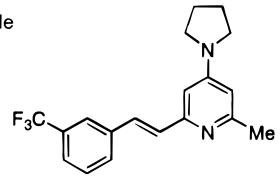

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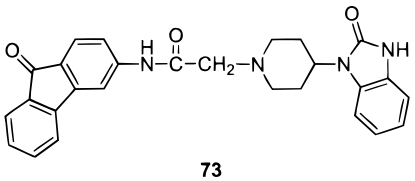

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least three GPCRs.[233] When galanin is injected into the hypothalamus of satiated rats, it stimulates food intake, with a preference for the intake of dietary fat.[234] Galanin also reduces energy expenditure and sympathetic activation of BAT. In normal rats given a choice of diet, galanin levels and expression are found to positively correlate with the ingestion of fat, but not of carbohydrate or protein. The discovery of selective galanin antagonists will address the question of whether compounds binding to a specific receptor subtype will decease feeding, especially the consumption of fat.

Bombesin. Bombesin is a tetradecapeptide originally isolated from the skin of the European frog *Bombina bombina*, and it is found throughout the mammalian CNS and GI tract.[235] Administration of bombesin, either centrally or peripherally, inhibits food intake in animal models. Bombesin and bombesin-like peptides bind to specific GPRCs, and it was recently reported that mice lacking the bombesin-3 receptor developed a mild obesity, associated with hypertension and impairment of glucose metabolism.[236] Thus, this receptor subtype is likely to be required for proper regulation of energy homeostatis and adiposity. Bombesin agonists acting at this site may be useful new therapeutics, perhaps for increasing metabolic rate in those individuals with a genetic propensity for a lower basal rate of energy expenditure.

Enterostatin. Entertostatin is a peptide formed in the intestines after feeding.[237] It selectively inhibits fat intake during both normal feeding and in experimental paradigms that involved dietary choice. Both peripheral and central sites of action have been proposed, but no specific receptors or binding sites have yet been identified. Small-molecule enterostatin agonists may find promise in the treatment for obesity.

Orexins. Two new novel neuropeptides, the hypocretins or orexins-A and -B, have been found in the hypothalamus of the adult rat brain.[238,239] When administered centrally to rats, these peptides stimulate food consumption, and the mRNA for the preproorexin is upregulated upon fasting. Two GPCRs which recognize the orexins have been identified, termed OX_1R and OX_2R.[239] OX_1R is structurally similar to certain neuropeptide receptors, most notably to the NPY2r (26% homology), and has a significantly higher affinity for orexin-A than for orexin-B. OX_2R binds with high affinity to both orexin-A and -B. Both of the receptors are found exclusively in the brain of rats. Due to their clear association with feeding in the rat in terms of both expression and localization, inhibition of orexin receptors is a reasonable new strategy in the design of drugs for the treatment of obesity.

Summary

Agents which reduce body weight have been actively sought after for many decades. Early animal-based feeding models were most commonly used to evaluate anorectic agents (appetite suppressants) or general metabolic stimulants. The amphetamine-type mechanism is now disfavored, because of the associated risks of abuse and chemical dependence. Activation of serotonergic systems either by direct activation of serotonin receptor subtypes or by inhibiting serotonin reuptake (SSRIs) has proved successful. Nevertheless, the exact receptor subtype profile that is required is not known, so that it is very difficult to design a drug discovery program based on this approach. It is more reasonable to explore obesity as a possible therapeutic indication for a serotonin-based compound developed for another purpose. In general, the adventitious finding of weight loss during the clinical trials of any compound should be considered as a possible opportunity for further development.

The valvular heart disease seen with fenfluramine was a disappointment. In general, extreme caution regarding possible cardiovascular complications is needed for patients already at risk based on their high BMI. Further, drugs for non-insulin-dependent diabetes need to be carefully monitored for their effects on body weight because of the high correlation of diabetes with obesity. Due to the chronic nature of treatment, antiobesity drugs must have a high margin of safety relative to side effects that may emerge.

The value of human β_3-adrenoceptor agonists will become apparent once data are available from relevant

clinical evaluations. These data will help researchers to determine whether the needed separation from cardiovascular side effects is possible. It is risky to start new human β_3-adrenoceptor drug discovery programs now unless these clinical results are favorable.

Modulation of satiety factors such as CCK or bombesin is not a prime strategy because the control of feeding is incredibly redundant. Inhibition of meal size is often compensated by an increase in the number of meals.

Newer approaches are attractive, partly because they have the allure that their deficiencies are not yet apparent. Nevertheless, we believe that there is great promise in several of these. Specifically, NPY receptor antagonists and MC-4 receptor agonists appear to act at a more fundamental level than satiety factors and might be useful as therapeutics. The challenge in modern drug discovery is not only obtaining high affinity receptor ligands but also achieving desirable pharmacokinetic properties such as high oral bioavailability and long half-life suitable for chronic use.

The uncoupling proteins are more likely to remain secondary mechanisms by which antiobesity compounds act in part and not themselves a primary target for intervention. PPARγ antagonists may prove useful, unless metabolic side effects such as hyperglycemia interfere.

There is a wealth of new research in the area of obesity which lends credence to the view that there will be a variety of effective and mechanism-based antiobesity drugs introduced into clinical practice in the coming decades. With the growing incidence of obesity due to an increasingly sedentary lifestyle, these drugs, when used properly and under responsible medical supervision, could increase the length and quality of life for many people. Given the large genetic component of obesity, one can imagine that phenotyping of individuals, both in clinical trials and in broad clinical practice, will offer the greatest chance for effective therapy. With increasing use of the tools of molecular biology, additional targets for obesity will be discovered such as orexin and its receptors. These new approaches may then be evaluated using high-throughput screening of large compound libraries and combinatorial chemistry or parallel synthesis, to obtain selective agents for further evaluation. There is every reason to believe that medicinal chemists will be able to make a significant contribution to the discovery of new and useful therapeutic agents for the treatment of obesity.

Biographies

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