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TEACHERS' TOPICS

ß$_3$-Adrenergic Receptor Agonists and Other Potential Anti-obesity Agents

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Obesity is reaching epidemic proportions in America and rising at an alarming rate throughout the world. The morbidity of cardiovascular disease, diabetes, osteoarthritis, and some cancers increases in proportion with increased obesity. Numerous anti-obesity agents are being developed that produce effects through diverse mechanisms. This paper discusses various targets for the pharmacotherapy of obesity with special attention to ß$_3$-adrenergic receptors (ß$_3$-AR) in obesity and the mechanisms by which ß$_3$-AR agonists mediate their anti-obesity effects.

This topic is a part of 2 courses: *Autonomic Pharmacology* (PTM 704) and *Pharmacology/Medicinal Chemistry-I* (PH 133). Obesity continues to be an increasingly critical health concern and pharmacy students should have a good understanding of the mechanisms of diverse anti-obesity drugs. The topic of obesity and anti-obesity agents also provides an excellent opportunity for students to integrate the principles that they learn in physiology, biochemistry, pharmacology, and medicinal chemistry.

Keywords: obesity, anti-obesity drugs, adrenergic receptor agonists

INTRODUCTION

Obesity has been measured in several ways such as skin-fold thickness, waist-to-hip circumference ratio, percent of body fat, and weight adjusted for height. The most commonly used measure of weight adjusted to height is the body mass index (BMI), which is defined as weight in kilograms divided by height in meters squared (kg/m^2). The BMI is highly correlated to body fat, hence it is almost universally used among epidemiologists. The BMI can be quite accurately used to estimate the body fat percentage in adults using the following equation:

Body fat % = 1.2 (BMI) + 0.23 (Age) - 10.8 (Gender) - 5.4

where gender = 1 for men and 0 for women.[1] The National Institutes of Health, the American Health Foundation, and the World Health Organization (WHO)[2] have defined healthy weight as a BMI below 25 kg/m^2, overweight as a BMI between 25 kg/m^2 and 30 kg/m^2, and obesity as a BMI greater than 30 kg/m^2.

According to the National Health and Nutrition Examination Survey (NHANES) 1999–2000 data, 64% of Americans are overweight or obese, compared with 56% in NHANES III (1988–1994) and 47% in NHANES II (1976–1980).[3] Increased adiposity occurs when food intake exceeds energy expenditure. Therefore, an increase in food intake or a decrease in energy expenditure, or a combination of both could cause one to gain weight. Evolutionarily, food scarcity has led to our genetic predisposition to effectively store energy in times of food abundance. Abundantly available high-calorie foods and an accompanying sedentary lifestyle are major contributing factors to the obesity epidemic. Examination of trends over the past 6 decades in England shows no relationship between either total food intake or fat consumption and the prevalence of clinical obesity, while proxy measures of physical inactivity (television viewing and car ownership) are closely related.[4] This by no means implies that the amount and type of food intake have no bearing upon obesity, but it does imply that over a large period of time, energy expenditure seems to be the more important variable in the food intake and energy expenditure balance. There is now extensive evidence that links excessive body weight with overall mortality. The relationship between BMI and mortality is a J-shaped curve with an acceleration of mortality risk above a BMI of 30 kg/m^2.[5] Recent evidence shows that a loss of more than 9 kg in women is associated with a 25% reduction in all causes (diabetes, cardiovascular, and cancer) of mortality and this is most marked for cancer (40%-50% reduction) and diabetes (30%-40% reduction).[6]

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Table 1. Targets for the Treatment of Obesity

Treatment With Agents Targeting:	Mechanism of Action
Adipose Tissue	
$ß_3$-AR agonists	Enhance fat breakdown, increased energy expenditure through thermogenesis, increased insulin sensitivity.
α_2-AR antagonists	Enhance fat breakdown by increasing responsiveness to norepinephrine.
Thyroid hormones	Increase energy expenditure through thermogenesis.
PPARγ2 ligands	Increase insulin sensitivity.
PPARγ2 antagonists	Decrease adipocyte differentiation.
Altering adipocyte phenotype	Reversing adipocyte differentiation to preadipocytes.
Adipocyte antibodies	Decrease number of adipocytes and body weight.
Gastrointestinal Tract	
Pancreatic lipase inhibitors	Decrease absorption of fat as a result of which most of it passes undigested.
Glucagon like peptide -1 agonists	Slow gastric emptying and boost insulin levels resulting in a satiated feeling.
Enterostatin analogs	Decrease food intake.
Amylin analogs	Decrease food intake, enhanced glucose homeostasis, decreased gastric emptying.
Surgical treatment	Jejunoileal shunts and/or gastroplasty result in reduced gastric volume and increased sense of fullness.
Cholecystokinin agonists	Stimulate cholecystokinin receptors causing a reduction in food intake.
Cholecystokinin-inactivating peptidase inhibitors	Blockade of an enzyme responsible for the breakdown of cholecystokinin resulting in a reduction of appetite.
Central Nervous System	
Leptin or leptin agonists	Decrease food intake and increased energy output; if sufficient concentration is attained in the cerebrospinal fluid.
Neuropeptide Y inhibitors	Decrease the appetite stimulant effect of Neuropeptide Y. Antisense NPY is also being considered.
Serotonergic drugs	Have an anorexic effect by releasing serotonin from nerve endings and partially inhibiting the reuptake of serotonin.
Adrenergic drugs	Have an anorexic effect by releasing norepinephrine and/or acting as sympathomimetics.
Melanocortin mimicing compounds	Act as agonists on the melanocortin receptor-4 in the brain and decrease food intake.
Galanin antagonists	Act in the hypothalamus to reduce food intake.
Bombesin analogs	Decrease food intake.
Orexin analogs / agonists	Decrease food intake.
Mahogany receptor agonists	Decrease food intake.
H_3-receptor antagonists	Increase histamine release in the hypothalamus and decrease food intake.

There are various hormones, receptors, proteins and other mediators that affect either food intake or energy expenditure, deviations in the balance of which cause shifts from "normal" body weight to obesity. All these, in one way or another, provide a potential target for anti-obesity therapies. One can treat or prevent obesity either by decreasing food intake or by increasing energy output. Decreasing food intake can be mediated either through central mechanisms (mostly in the hypothalamus, enhancing satiety) or alternatively, by interfering with the absorption of nutrients from the gastrointestinal tract. Sibutramine (*Meridia*) is a serotonin and norepinephrine reuptake inhibitor and acts in the hypothalamus to enhance satiety, thereby reducing food intake; whereas Orlistat (*Xenical*) is a pancreatic lipase inhibitor and acts by interfering with the digestion and absorption of fat. On the other hand, increases in energy output can be mediated by thermogenesis (which is divided into basal metabolic rate, diet-induced thermogenesis, and physical activity). Therefore, there are 3 major target areas for anti-obesity drugs: the brain, adipose tissue, and the gastrointestinal tract. Some of the currently available treatments for obesity and promising future targets for anti-obesity drugs are briefly discussed in the subsequent section and summarized in Table 1.

CENTRALLY ACTING ANTI-OBESITY DRUGS

The majority of the drugs on the market today for the treatment of obesity are centrally acting anorectic agents intended to reduce food intake. Many of the adrenergic agents in this category can be addictive and have an abuse potential and other unwanted side effects. Novel anorectic agents are currently at various stages of development, and these may have safer profiles than the currently available drugs.

Adrenergic Agents

Among the earliest used appetite suppressants were amphetamine and its derivatives. The earlier derivatives had abuse potential, primarily due to their effects on dopaminergic transmission with an associated risk of euphoria. The currently available adrenergic agents include phenylpropanolamine, phentermine, and diethylpropion, which are norepinephrine releasing agents; and mazindol, which is a norepinephrine reuptake inhibitor. These compounds are not used in patients with cardiovascular disease.

5-Hydroxytryptamine Agents

Following intrahypothalamic injections, 5-hydroxytryptamine (5-HT, serotonin) suppresses food intake in freely feeding or food-deprived rats and these effects have been localized to the hypothalamic paraventricular nucleus and the ventromedial hypothalamus and are mediated by postsynaptic 5-HT1B receptors.[7] Selective 5-HT reuptake inhibitors, like fluoxetine and sibutramine, and amphetamine-like compounds that both block 5-HT reuptake and stimulate 5-HT release, such as fenfluramine, are effective in reducing food intake, but many subjects do not reduce body weight. In humans, it has been suggested that both 5-HT and norepinephrine are required for a reduction of food intake in humans. The monoamine reuptake inhibitor, sibutramine (*Meridia*[7]), elevates both 5-HT and norepinephrine concentrations and inhibits eating.

Combination Therapy

One of the most commonly used combination regimens for the treatment of obesity was the combination of fenfluramine (an agent causing the release of serotonin and preventing its reuptake) and phentermine (an agent causing norepinephrine release). This combination (fen-phen) decreased weight and was commonly used in the early 1990s. However, its continuous use led to an increased risk of primary-pulmonary hypertension. Subsequently, Connolly et al[8] reported the observation of valvular heart disease in 24 patients who had taken the combination of fenfluramine and phentermine. Shortly thereafter, on September 15, 1997, the Food and Drug Administration announced the withdrawal of fenfluramine and dexfenfluramine. Recently, other similar combinations have been used for the treatment of obesity. One of these is "herbal fen-phen,"in which fenfluramine is replaced with hypericum (St. John's Wort) extract and phentermine is replaced with ephedra (Ma Huang) extract. Another example is "phen-pro" in which phentermine is retained and fenfluramine is replaced with fluoxetine (*Prozac*,[7] a selective serotonin reuptake inhibitor). However, these combinations have not been tested clinically, and the combinations might lead to unexpected side effects, as were noticed with the fenfluramine-phentermine combination.

Neuropeptide Y Antagonists

Neuropeptide Y (NPY) is a 36-amino-acid peptide synthesized throughout the brain in rodents and humans, with particularly high levels in the hypothalamus, which is a crucial region in the regulation of appetite. Injections of NPY into the cerebral ventricles or directly into specific hypothalamic regions produces potent and sustained hyperphagia within 10–15 minutes with a combined reduction in thermogenesis.[9] All these actions cause a shift towards positive energy balance, causing increased food intake and storage of excess calories primarily as fat through the lipogenic actions of insulin. NPY concentrations are elevated during starvation, food restriction, and insulin-deficient diabetes. Moreover, the blockade of NPY by infusion of monoclonal antibodies into the third ventricle reduces food intake when starved rats are refed, indicating a physiological role of NPY in the compensatory hyperphagia seen in conditions of negative energy.[10]

Six G-protein-coupled receptors have been discovered that bind to NPY with high affinity, and of these, the Y5 receptor influences feeding and is found mostly in the hypothalamus.[11] Since NPY is the most potent hyperphagic agent known, its blockade could be an important anti-obesity target. This could be achieved by the development of either a selective NPY5 receptor antagonist or NPY antisense oligonucleotides.

Leptin

Elegant parabiotic experiments done by Coleman accurately predicted that the genetically obese ob/ob mouse (a monogenic rodent obesity model that has a mutation in the leptin gene) lacked a lipostatic factor, while the db/db mouse (a monogenic rodent obesity model that has a mutation in the leptin receptor) was unable to respond to it, suggesting a defect in the receptor.[12] Homozygosity for the ob gene results in reduced brown adipose tissue (BAT)

activity and energy expenditure, hyperphagia, and hyperinsulinemia. Using positional cloning, the ob mutation was located within a gene encoding the 167-amino-acid protein of 16 kDa, now known as leptin.[13] Leptin is expressed only in adipose tissue and is secreted into the circulation. Injection of leptin into ob/ob mice leads to decreased food intake, increased energy expenditure, and a subsequent loss of body fat content without any reduction in lean body mass. The actions of leptin in suppressing food intake and stimulating thermogenesis are apparently mediated in part by inhibiting NPY neurons in the hypothalamus and reducing both synthesis and transport of NPY.[14]

Melanocortin Agonists

The agouti mouse has yellow fur and is obese. It was discovered that α-melanocyte stimulating hormone (α-MSH) is involved in pigmentation and obesity. The agouti-gene-related peptide antagonizes α-MSH at the melanocortin-1 receptor (MC-1 is involved in pigmentation) and the melanocortin-4 receptor (MC-4 is involved in the brain in obesity).[15] The melanocortin-4 receptor knockout mice are obese. Therefore, melanocortin agonists may be potential appetite suppressants.

Other Peptides

In addition to the main neuropeptides involved in obesity described above, there are others that have similar properties. When injected into the hypothalamus of satiated rats, galanin, an amino acid peptide, stimulates food intake, with a preference for intake of dietary fat.[16] Bombesin, a tetradecapeptide that is administered either centrally or peripherally, inhibits food intake in animal models and in mice lacking the bombesin-3 receptor, and has been associated with mild obesity and impairment of glucose metabolism.[17] Enterostatin is a peptide produced from the gastric mucosa and mucosal epithelia of the small intestine. When administered chronically, it reduces food intake, body weight, and body fat, but its mechanism(s) of action remains unclear.[18] Orexins A and B are novel neuropeptides, which upon central administration to rats, reduce food intake. Two orexin receptors have also been discovered that are involved in the mediation of the effects of orexins.[19] Recently, a novel protein, mahogany, a large, single-transmembrane-domain receptor-like molecule, has been discovered, and it has been shown that the expression of mahogany can suppress diet-induced obesity in mice.[20]

ANTI-OBESITY DRUGS ACTING AT THE GASTROINTESTINAL TRACT

Cholecystokinin Agonists

Cholecystokinin (CCK) is synthesized in the gut wall and released into the portal circulation in response to the presence of nutrients (especially fatty acids) within the gut. It acts as a satiety signal. In rats, peripheral or central injections of CCK reduce food intake. Therefore, CCK agonists may be important in decreasing food intake. This effect is mediated through 2 receptors: CCK-A receptors (present primarily in the gastrointestinal tract) and CCK-B receptors (present in the central nervous system). The relative importance of these receptors and studies with selective agonists needs to be clarified. The CCK-A receptor is involved in feeding behavior and several agonists have been developed.

A serine peptidase responsible for inactivating CCK also has been found and an inhibitor of this peptide, butabindide, exhibits a satiating effect in starved mice and to significantly reduce food intake in rats.[21] Both CCK-A receptor selective agonists and CCK peptidase inhibitors appear to be promising targets for future anorectic agents.

Glucagon-like Peptide-1 Agonists

Glucagon-like peptide-1(GLP-1) is a 29 amino-acid peptide, with its amino-acid sequence highly conserved in mammals, implying its importance. GLP-1 is important in glucose homeostasis and a synthetic version-insulinotropin, is in phase II clinical trials for obesity-related diabetes.[22] GLP-1 has an anorectic effect on a short-term basis, and this effect is blocked by the GLP-1 receptor antagonist exendin. However, the long-term effects of GLP-1 agonists require further investigation.

Amylin Agonists

Amylin is a 37-amino-acid pancreatic peptide released from the ß-cells, and is similar to calcitonin and cacitonin gene-related peptide (CGRP). Both amylin and cacitonin gene-related peptides reduce food intake, although amylin is more potent than cacitonin gene-related peptide.[23] Other properties of amylin include maintenance of glucose homeostasis, decreasing gastric acid secretion, and decreasing gastric emptying.[24]

Orlistat

Orlistat is a pancreatic lipase inhibitor and it acts locally in the gastrointestinal tract to prevent the absorption of fat. It has been approved for the treatment of obesity (*Xenical*[7]). Even though it inhibits other lipases, the systemic absorption, which is negligible, is not required for its anti-obesity effects.[25]

ANTI-OBESITY DRUGS ACTING ON ADIPOSE TISSUE

An interesting approach to the treatment of obesity is the use of antibodies to adipocytes. Recently, passive

immunization of rats with sheep polyclonal antiserum raised against plasma membranes of adipocytes produces a significant reduction in both the number of adipocytes (30%-40%) and in body weight (10%). If a human monoclonal antibody that is specific for the cell surface antigens is developed, anti-obesity immunization therapy might be a viable option for the treatment of obesity.[26]

Peroxisome proliferator activated receptor-γ (PPARγ) is a member of the peroxisome proliferator activated receptor subfamily of nuclear hormone receptors. It appears to function as both a direct regulator of many fat-specific genes and also as a "master regulator" that can trigger the entire program of adipogenesis. PPAR-γ ligands, such as the new insulin-sensitizing drugs, the thiazolidinediones (troglitazone, pioglitazone, rosiglitazone), have antidiabetic properties and increase the transcription of proteins that are actively involved in enhancing energy metabolism, as will be discussed in subsequent sections. But PPAR-γ ligands also induce adipocyte differentiation, implying a "pro-obesity" property. The concept of developing PPAR-γ antagonists as anti-obesity agents is attractive, as this would theoretically interfere with adipocyte differentiation. However, it remains unclear whether antagonizing PPAR-γ will exacerbate diabetic symptoms, or possibly even induce them.

Adipocytes are responsible for the storage of fat and its breakdown is primarily controlled by the sympathetic nervous system. Activation of α_2-adrenergic receptors decreases lipolysis and activation of the $ß_3$-adrenergic receptor ($ß_3$-AR) increases lipolysis. α_2-adrenergic receptor antagonists are being investigated for their anti-obesity potential, but may possess many unwanted side effects. The following sections discuss in detail the primary adrenergic receptor involved in adipose metabolism (initiating lipolysis and increasing energy expenditure), the $ß_3$-AR and the other related modulators involved in energy metabolism.

$ß_3$-ADRENERGIC RECEPTORS

The ß-adrenergic receptors (ß-ARs) were divided into $ß_1$-(responsible for cardiostimulation and lipolysis) and $ß_2$-(responsible for bronchodilation and vasodepression) adrenergic receptors by Lands et al.[27] Lands, with remarkable foresight, mentioned that the receptors responsible for lipolysis had dual ß-AR characteristics. Subsequent studies correlating the pA2 values of separate ß-ARs antagonists in various tissues indicated that the lipolytic receptor was a hybrid and was eventually termed the atypical ß-AR.

In 1983, Tan and Curtis-Prior[28] conducted studies in rat adipocytes and found that propranolol (a nonselective ß-AR antagonist) showed a greater potency in inhibiting glycerol release than did $ß_1$- and $ß_2$-AR specific antagonists. Observing that the lipolytic response did not appear to be $ß_1$- or $ß_2$-ARs mediated, they were the first to propose the existence of an atypical ß-AR in rat adipose tissue and to call it the $ß_3$-AR. In 1984, studies with 3 ovel ß-agonists developed by SmithKline Beecham suggested that the rat lipolytic receptor did not fit the $ß_2/ß_1$ classification and represented a third class of ß-ARs, for which these compounds were selective.[29] Since then, many studies have been conducted demonstrating that the lipolytic effects are mediated through an atypical ß-AR.

The absence of highly selective $ß_3$-AR antagonists has precluded the attempt to pharmacologically demonstrate that selective antagonists can block the observed effects of $ß_3$-AR agonists. Nevertheless, anti-obesity and anti-diabetic actions of putative $ß_3$-AR agonists such as disodium (RR)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxazole-2,2-dicarboxylate (CL 316243) have been conclusively demonstrated using $ß_3$-AR knockout mice to be exclusively mediated by the $ß_3$-AR.[30] CL 316243-mediated lipolysis enhanced the adenylate cyclase activity, increased serum levels of free fatty acids, increased oxygen consumption, increased insulin levels, decreased glucose, and decreased food intake in wild-type mice, while all these effects were abolished in the $ß_3$-AR knockout mice. The above-mentioned effects were completely restored in the knockout mice with transgenic re-expression of $ß_3$-AR in white and brown adipocytes,[31] indicating that all the observed effects were mediated by the presence of $ß_3$-AR in white and brown adipose tissue. The mechanisms of $ß_3$-AR-mediated reduction of food intake and increases in insulin levels remain unknown since there is no evidence of $ß_3$-AR expression on insulin-secreting ß-cells of the pancreas or brain centers involved in appetite regulation.[31]

The Human $ß_3$-Adrenergic Receptor

The existence of an atypical ß-AR was postulated in humans through pharmacological studies, but the protein had not been identified as a distinct entity. In 1989, a human gene that encoded a third ß-AR was isolated.[32] The human $ß_3$-AR was later transfected into Chinese hamster ovary (CHO) cells and exposure of these cells to epinephrine and norepinephrine led to increased cyclic adenosine-3',5'-monophosphate (cAMP) and the pharmacological profile of this expressed protein showed clear atypical ß-AR properties. The effects mediated by this receptor on human adipocytes are similar to the atypical ß-AR of the rat adipocyte.

The existence of the $ß_3$-AR in humans has been

demonstrated in a couple of studies. In 1993, $ß_3$-AR mRNA in human omental fat was detected by polymerase chain reaction (PCR) and Northern blot analysis, thereby showing that the $ß_3$-AR is expressed in human white adipose tissue.[33] RNAse protection assays and reverse transcriptase/polymerase chain reaction (RT-PCR) have identified human $ß_3$-AR mRNA in the gall bladder, stomach, intestine, prostate, left atrium, brown adipose tissue, and white adipose tissue.[34] Recently, more conclusive evidence of the existence of the $ß_3$-AR in humans has been demonstrated immunohistochemically by using a monoclonal antibody to the human $ß_3$-AR.[35] This study demonstrated the presence of $ß_3$-ARs in intact human adipocytes and in the ventricular myocardium.

A missense mutation of the $ß_3$-AR that results in the replacement of tryptophan by arginine at position 64 (Trp64Arg) has been associated with an earlier onset of non-insulin dependent diabetes mellitus and a decreased resting metabolic rate in the Pima Indians. Subsequent studies have shown the presence of this mutation in almost all populations of the world, many of them showing associations with body mass indices (BMI, a measure of obesity) and/or non-insulin dependent diabetes mellitus.[36] As mentioned earlier, energy expenditure is a major determinant in weight control. A major part of energy expenditure involves thermogenesis. The different types of thermogenesis, associated proteins, and the role of the $ß_3$-AR in affecting energy expenditure are discussed in the sections below.

THERMOGENESIS

Thermogenesis is defined as heat production above basal metabolic rate in the resting state and is divided into obligatory thermogenesis and facultative thermogenesis. Obligatory thermogenesis refers to heat generated as a byproduct of essential cellular metabolic processes, such as growth, movement, digestion, and the production of ionic gradients. Obligatory thermogenesis is controlled by the levels of circulating thyroid hormones. Facultative thermogenesis is the heat production to maintain homeostatic energy levels and thermoneutrality. This part is governed by the sympathetic nervous system and is subdivided into

1. Exercise-induced thermogenesis (skeletal muscle, under CNS and somatic nervous system control);
2. Thermoregulatory thermogenesis subdivided into cold-induced shivering thermogenesis (skeletal muscle, under CNS and somatic nervous system control) and non-shivering thermogenesis (brown adipose tissue, under direct sympathetic innervation); and
3. Diet induced thermogenesis (brown adipose tissue, under direct sympathetic innervation).

Nonshivering thermogenesis and diet-induced thermogenesis take place in the brown adipose tissue. Brown adipose tissue differs from white adipose tissue in several ways. Upon maturation, white adipocytes have a single droplet of fat, whereas brown adipocytes contain multilocular fat droplets. The brown color of brown adipose tissue is due to the presence of a larger number of mitochondria compared with white adipose tissue. While white adipose tissue is mostly involved in energy storage, brown adipose tissue is involved in energy expenditure. In humans, brown adipose tissue is expressed abundantly in newborn infants, but disappears after a few weeks. However, in people working in extremely cold conditions, and in people with pheochromocytoma, brown adipose tissue is expressed in perirenal, intrascapular, and supraaxial locations, as in the newborns.[37] This suggests that undifferentiated brown adipose tissue cells may be present interspersed among white adipose tissue in adults and could be reactivated in the right conditions. Rodents, unlike humans, maintain sufficient amounts of brown adipose tissue throughout their life. A characteristic feature of brown adipose tissue is its expression of uncoupling protein-1, which plays a major role in thermogenesis.

Uncoupling Proteins

Uncoupling proteins (UCP) are members of the mitochondrial carrier family and are integral proteins located in the inner mitochondrial membrane. At least 4 uncoupling proteins: UCP-1, -2, -3, and -4, have been identified. These proteins act as transporters that dissipate the electrochemical proton gradient, which is normally required for the activity of ATP-synthase and the subsequent production of ATP. The dissipation of the gradient decreases the production of ATP, dissipating the stored energy as heat. The proton transport is facilitated by fatty acids.

Uncoupling protein-1 (UCP-1) is the key and rate-limiting component of thermogenesis in brown adipose tissue. It allows for regulatory dissipation of the electrochemical proton potential gradient generated across the inner mitochondrial membrane by the respiratory chain enzymes during the Kreb's cycle (Figure 1). UCP-1, therefore, uncouples respiration from ATP synthesis. It is upregulated during exposure to cold, thyroid hormone, and catecholamines, the action of which is mediated through ß-AR.[38]

$ß_3$-AR agonists recently have been demonstrated to increase uncoupling protein mRNA levels in adipocytes of adult humans.[39] Moreover, in rats, white adipose tis-

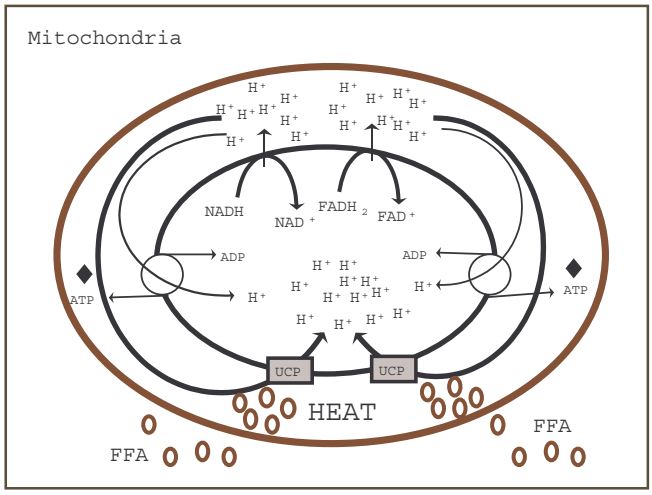

Figure 1. A proton gradient is generated in the mitochondrial inter-membrane space during oxidation of the reducing equivalents formed during Kreb's cycle. The proton gradient normally drives the synthesis of ATP. The presence of uncoupling proteins in the inner mitochondrial membrane dissipates the proton gradient resulting in the generation of heat, and few ATP are synthesized. Free fatty acids (FFA) increase the activity of the uncoupling proteins.

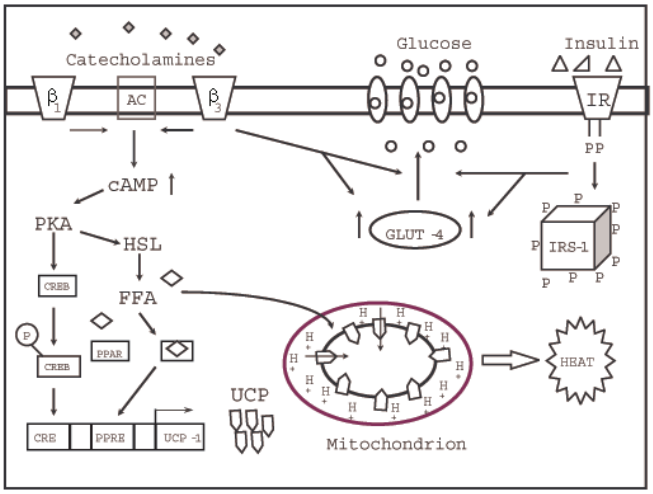

Figure 2. In brown adipose tissue, catecholamines cause lipolysis and this effect is mediated by the ß-adrenergic receptors, primarily the $ß_3$-adrenergic receptor ($ß_3$-AR). Activation of the receptor stimulates adenylate cyclase (AC) leading to the production of cyclic AMP (cAMP), which activates protein kinase A (PKA). PKA activates hormone sensitive lipase (HSL) which is responsible for the breakdown of fat. PKA also phosphorylates cAMP response element binding protein (CREB), causing it to bind to cAMP response elements (CRE) in the uncoupling protein-1 (UCP-1) gene and increase its transcription. The fatty acids (FFA) can also enhance UCP-1 transcription by binding to peroxisome proliferator activated receptors (PPAR) and the respective response elements (PPRE). Activation of the $ß_3$-AR may also cause translocation of the insulin-responsive glucose transporter (GLUT-4) to the cell membrane, aiding in glucose transport.

sue contains precursors to brown adipocytes and these can be stimulated by $ß_3$-AR stimulation.[40] This raises the possibility that similar abundant brown adipocyte precursors might be present in the white adipose tissue depots of adult obese humans, and this has been demonstrated in one study.[39]

The sympathetic control of uncoupling protein-1 expression is mediated through the activation of $ß_1$-, $ß_2$-, $ß_3$-, and α_1-ARs.[41,42] Triiodothyronine (T3) is also important for the full induction of UCP-1. Other potent activators of UCP-1 gene expression include retinoic acid and PPAR-γ agonists such as the thiazolidinediones,[43] a novel class of anti-diabetic agents that includes troglitazone, pioglitazone, and rosiglitazone. The promoter regions of the UCP-1 gene have been studied and shown to possess cyclic AMP response elements,[44] thyroid hormone response elements,[45] and peroxisome proliferator response elements, referred to as uncoupling protein regulatory element (URE1), thereby explaining the induction by cyclic AMP-elevating agents (ß-AR agonists), thyroid hormone, and PPAR-γ agonists, respectively. The potential of synergism or additivity as a result of stimulation of a combination of the response elements may represent efficient ways of significantly increasing UCP-1 gene expression.

In 1997, 2 more uncoupling proteins were cloned and named UCP-2 and UCP-3, respectively.[46] Uncoupling protein (UCP-2-UCP-3) polymorphisms have been associated with variations in metabolic rate in young Pima Indians. The contributions from UCP-2 or UCP-3 may contribute to overall body fat content later in life. PPAR-γ agonists have been shown to induce UCP-2 and UCP-3. $ß_3$-AR agonists increase UCP-2 and UCP-3 expression as well.[47] Therefore, the anti-obesity effects of $ß_3$-adrenergic receptor agonists may be partially mediated by an induction of various uncoupling proteins in tissues where both the $ß_3$-ARs and the uncoupling proteins are present.

The $ß_3$-ARs not only stimulate thermogenesis, but are required for thermogenesis. Strong evidence supporting this has recently been obtained by the production and study of mice lacking all 3 ß-ARs (ß-less mice). These mice are thermogenically inactive, have a reduced expression of UCP-1, and are completely resistant to cold-exposure induced increases in UCP-1.[48]

$ß_3$-ADRENERGIC RECEPTOR AGONISTS

The mechanism of action by which $ß_3$-AR agonists produce their effects is shown in Figure 2. In both white and brown adipocytes, the $ß_3$-AR agonists bind to the $ß_3$-

AR in the cell membrane, which is a Gs-protein–coupled receptor linked to adenylate cyclase. The stimulated adenylate cyclase produces cyclic AMP which activates protein kinase A, having 2 major effects. First, the protein kinase A phosphorylates hormone-sensitive lipase, which is the primary enzyme responsible for the breakdown of fat into fatty acids. Secondly, protein kinase A phosphorylate cyclic AMP response-element binding protein (CABP), which binds to the cyclic AMP response elements (CRE) present in the promoter region of uncoupling protein genes and increase their transcription levels. The fatty acids produced during the fat breakdown can directly stimulate uncoupling protein activity. Moreover, some fatty acids are also agonists for the PPARs and may also stimulate uncoupling protein production through the peroxisome proliferator response element (PPRE) present in the promoter region of the uncoupling protein genes. Many $ß_3$-AR agonists have anti-diabetic effects in rodents. The exact mechanisms involved are unclear. However, it is postulated that $ß_3$-AR agonists cause increased translocation of the insulin responsive glucose-transporter 4 to the cell membrane, thereby enhancing the cell's glucose-uptake capability (Figure 2).

The $ß_3$-AR agonists are remarkably effective in animal models of obesity and Type II (or NIDDM) diabetes. Several compounds found to be highly selective for the rodent $ß_3$-AR, have been used in human clinical trials. However, the pharmacological profiles of these rodent $ß_3$-AR selective compounds in humans have not been very successful. Their failures have been attributed primarily to (1) a poor pharmacokinetic profile, with problems in both bioavailability and extensive biotransformation; (2) major side effects such as tremors (a $ß_2$-AR mediated effect)[49] and tachycardia (a $ß_1$-AR mediated effect),[50] and (3) much lower intrinsic activities and varied potencies on the human $ß_3$-AR vs the rodent $ß_3$-AR.

Of the compounds tested in humans, (RR+SS)-(")-methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino]propyl]-benzoate, (E)-2-butenedioate (2:1) salt (BRL 26830) and (RR+SS)-(")-methyl 4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]-propyl]-phenoxyacetate hydrobromide (BRL 35135) have very short biological half lives due to their efficient clearance through the kidneys.[51] CL 316243 is a potent full agonist of the rodent $ß_3$-AR, but a weak partial agonist of lipolysis in human omental adipocytes (pD2=4, I.A.=57%). Both prototypical $ß_3$-AR agonists, (RR+SS)-(")-methyl 4-[2-(2-(3-chlorophenyl)-2 hydroxyethyl)amino)propyl]phenoxyacetate (BRL 37344) and CL 316243, are more potent than isoproterenol at the rat $ß_3$-AR. However, these compounds are much less potent than isoproterenol and exert low intrinsic activities on the human $ß_3$-AR.

The high potency and intrinsic activity of CL 316243 on the rodent $ß_3$-AR has been helpful in elucidating some of the properties that might be expected of $ß_3$-AR agonists. A study done on the effects of CL 316243 in Otsuka Long-Evans Tokushima Fatty rats demonstrated a significant reduction in body weight (27% in the fatty rats and 15% in the controls). The fat depots from all measured locations were reduced significantly, and both UCP-1 mRNA and protein levels were increased two- to 3-fold in brown adipose tissue in both control and fatty rats that were treated with CL 316243, as compared with untreated control and fatty rats, respectively. The CL 316243 treatment also increased the levels of glucose transporter-4 in both white and brown adipose tissue, and produced decreases in serum glucose and insulin levels. An interesting finding was that CL 316243 increased the UCP-1 protein levels not only in brown adipose tissue but also in white adipose tissue (generally considered devoid of UCP-1).[52] Significant amounts of brown adipose tissue (as measured by UCP-1 expression) appeared in the white adipose tissue of Zucker fa/fa rats following treatment with CL 316243. These data support the proposal that brown adipocyte precursor cells are present in white adipose tissue and can be reactivated under the appropriate conditions, and that potent $ß_3$-AR agonists are capable of causing this reactivation. Since agonist binding and cyclic AMP- signaling responses of $ß_3$-AR agonists on the rodent $ß_3$-AR differ from those on the human $ß_3$-AR subtype, in this case rodent models do not represent an ideal model for comparison and drug selection for humans. The nonhuman primates, because of their species proximity to humans, represent good models for preclinical investigations of $ß_3$-AR agonists, especially since the monkey, bovine, and human $ß_3$-AR are closer to each other than to any of the rodent sequences. Recently, a novel human $ß_3$-AR agonist, 4-(3-hexyl-ureido)-N-(4-{2-[(S)-2-hydroxy-phenoxy)-propylamino]-ethyl}-phenyl)-benzenesulfonamide (L-755507), a potent and selective partial agonist, has been shown to mediate lipolysis and increase uncoupling protein-1 and metabolic rate in rhesus monkeys.[53] L-755507 exhibited an EC_{50} of 0.45 nM in Chinese hamster ovary cells expressing the human $ß_3$-AR with an intrinsic activity of 52% that of isoproterenol. This drug was a thousand-fold selective for the human $ß_3$-AR compared with the human $ß_1$-AR (EC_{50} = 580 nM, intrinsic activity = 25%), with no agonist activity on cells expressing the human $ß_2$-AR. The compound also produced lipolysis in the adipocytes of rhesus monkeys, both in vitro (EC_{50} = 3.9 nM, intrin-

sic activity = 23%) and in vivo (ED_{50} = 0.03 mg/kg), and increased the basal metabolic rate, and produced tachycardia (peaking at a 15% increase in heart rate) after doses of 0.1 mg/kg. Also, UCP-1 levels in the monkeys increased after 2- and 4-weeks of treatment (3 mg/kg, iv, twice a day).[53] The effects are expected to be similar in humans.

CONCLUSIONS

The $ß_3$-AR agonists are attractive as potential anti-obesity and anti-diabetic drugs. The primary obstacles to their successful application include insufficient selectivity and problematic pharmacokinetic profiles, especially oral bioavailability. Numerous pharmaceutical companies have active programs for developing a marketable $ß_3$-AR agonist and some compounds are in phase I and phase II clinical trials. In the future, a $ß_3$-AR agonist may be a potent and effective tool in fighting the rising epidemic of obesity throughout the world.

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