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5 Orally Bioavailable β_3-Adrenerg Receptor Agonists as Potential Therapeutic Agents for Obesity and Type-II Diabetes

BAIHUA HU and LEE L. JENNINGS

Chemical Sciences, Wyeth Research, P.O. Box 42528, Philadel PA 19101, USA

INTRODUCTION

Although obesity is now recognized as a common medic industrialized societies, it remains an inadequately treated di recognized as a major risk factor for serious health complications diabetes, high blood pressure, cardiovascular disease, altered bolism, and cancers of the breast and uterus. Obesity is esti

30 million deaths per year in the United States [2]. However, health-care professionals generally use drugs to treat the complications of obesity rather than the underlying condition because of the small number of treatment options available for managing the disease.

Obesity arises from an imbalance between energy intake and energy expenditure. The major life-style factors contributing to an increase in the incidence of obesity are an increasingly sedentary lifestyle and increased caloric intake. However, clinical studies indicate that genetic factors also contribute to the disease. For instance, biochemical and metabolic differences between lean and obese individuals have been described calling into question the widely held opinion that obesity is modifiable by behavioural changes alone [3]. The public health issues associated with obesity justify the development of new medications for its treatment. In parallel with the rapid evolution of our understanding of the molecular mechanisms that cause obesity, there has been a corresponding increase in efforts to discover and develop new anti-obesity medications. β_3-Adrenergic receptor (β_3-AR) agonists are one of a number of promising categories of drugs that are under investigation. For recent reviews, see Refs. [4–10]. This review will focus on recent progress in the development of potent, selective and orally bioavailable β_3-AR agonists for the treatment of diabetes, and more particularly, of obesity.

β_3-ADRENERGIC RECEPTOR: STRUCTURE AND ANTI-OBESITY ACTIVITY

As obesity arises from the storage of excess energy, especially in the form of triglycerides (TGs), weight reduction requires a period of negative energy balance, either by reducing food intake or by increasing energy consumption. However, most marketed anti-obesity drugs are appetite suppressants. An alternative mechanism for altering body fat composition is through increased energy expenditure, either by an increase in physical activity or by accelerating the metabolic processing of food and/or fat.

The β_3 receptor is found primarily in adipose tissue, where fat is organized, and is known to mediate a variety of metabolic functions, including fat mobilization (lipolysis) from white adipose tissue (WAT), increased fat oxidation (thermogenesis) in brown adipose tissue (BAT), improved sensitivity to insulin, and relaxation of urinary bladder detrusor tissue. (For review on structure and function of the β_3-AR, see Ref. [11]). A number of recent studies indicate that the receptor is present in the human heart, skeletal muscle, gall bladder, gastro-intestinal (GI) tract and prostate, in addition to adipocytes [12]. The β_3 receptor is composed of a single 408 amino acid residue peptide chain that belongs to the super family of G-protein-coupled receptors. As expected, it has seven hydrophobic stretches of about 22–28 residues forming s transmembrane spanning domains that form the catecholamin The glycosylated N-terminus is extracellular, whereas the intracellular. In contrast to the related β_1 and β_2 receptors, the the β_3 receptor contains no serine- and threonine-rich regions tha for protein kinase A phosphorylation. The absence of phosphoryl explain the resistance of the β_3 receptor to down regulate foll stimulation, a feature that distinguishes it from the β_1 and β_2 amino acid sequence of the human β_3-AR is about 50% identical the human β_1 or β_2 receptor, respectively [13]. Comparison of the other species with that of human reveals a high degree of sequenc approximately 80–90% between human, bovine, rodent, and canin monkey, and bovine β_3 receptors are more similar to each other th rodent (rat, mouse, and hamster) sequences. The human β_3 rece from the rodent sequences in several segments, a major one being t spanning domain 1 (TM1) where a (Val-Ala-Leu) deletion was c rodents but not in higher species.

A naturally occurring polymorphism in the amino acid sequenc in humans (Trp64Arg) has been identified. Interestingly, this varia in humans the arginine residue present at this position in anim mutation has been associated with an increased propensity for several populations, a feature of insulin resistance and early d type-II diabetes [14–16]. One functional study on white fat cells s mutant receptor is as responsive to the lipolytic effects of the noradrenaline as the wild-type [17]. However, it is yet to be estab β_3-AR agonists optimized for the wild-type β_3-AR are effe treatment for obesity in individuals carrying this mutation.

The role of the β_3 receptor in adipocytes is now well understoo [4–6]. Like the β_1 and β_2 receptors, the β_3 receptor is fully stimulatory G-protein that activates adenylate cyclase in the plas to generate intracellular cAMP. Measurement of an increase in c Chinese hamster ovary (CHO) cells expressing the β_3 receptor widely used screening assay for β_3 agonists [18–20]. The cAM activates protein kinase A that in turn activates hormone sensi phosphorylation. The resulting lipase-induced lipolysis converts WAT into free fatty acids (FFAs). In brown adipocytes, FFA is o uncoupling protein 1 (UCP1) into carbon dioxide and water. U proton transport across the inner mitochondrial membrane withou ATP, thus 'wasting' energy as heat. The overall effect is a loss body at the expense of more oxygen consumption. Thus, measuren consumption is the mostly commonly used *in vivo* model for β_3

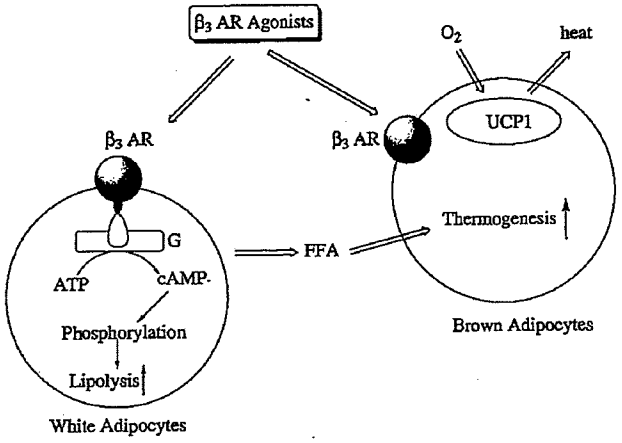


Figure 5.1 Proposed mechanism underlying the anti-obesity effect of β_3-AR agonists: FFAs, the breakdown products of β_3-AR mediated lipolysis of white adipocytes, stimulate a thermogenesis response in brown adipocytes via the UCP1.

Mice treated with a selective β_3 agonist can double oxygen consumption, which demonstrates the remarkable capacity of this thermogenic mechanism [22].

In contrast to β_1 and β_2 receptors, which are primarily localized in the heart or on vascular, uterine, or airways smooth muscle, β_3-ARs are expressed abundantly and predominantly on BAT. The amount of adipose tissue in neonates is high relative to that in adults. However, with increasing age, the amount of BAT in lean humans declines, so it has been argued that the amount of BAT (and hence the amount of β_3 receptors) in adult humans may not be enough to produce satisfactory thermogenesis by the activation of β_3 receptors. However, evidence from a number of studies suggests that BAT can he restored in adult humans following chronic treatment with catecholamines. Other studies suggest that, in addition to BAT, skeletal muscle is another tissue where the oxidation of FFAs occurs. Skeletal muscle represents up to 40% of total body weight and is endowed with significant capacity for thermogenesis. A recently reported clinical study demonstrated that treating young lean volunteers with a selective β_3 agonist induced an increase in plasma FFA concentrations, 24 h fat oxidation, and stimulated glucose disposal [23]. These new findings suggest that the expression level of β_3 receptors is high enough in humans (at lean subjects) to achieve the desired β_3 agonist mediated metabo

UCP1, which oxidizes FFA into carbon dioxide and water, is specifically expressed in BAT. This would imply that the thermo β_3 agonism would be limited in the body to BAT where U However, two homologues of UCP1 have been recently discover specific for BAT: UCP2, expressed in most tissues at varying leve expressed mainly in skeletal muscle, WAT and BAT. Several st that these UCPs also have proton transport capacity. Given that U are highly expressed in adult human tissues, this could mean tha than BAT, such as WAT and skeletal muscle, could contribute s energy expenditure and fat oxidation on stimulation of Experiments have shown that chronic stimulation of the β_3 rec animals resulted in reduced adiposity, associated with an increased UCP1. β_3 Agonists also up-regulate UCP2 and UCP3 in skeletal m yellow KK mice. These results suggest that the anti-obesity effects are attributable to increased thermogenesis, not only by UCP1, but and UCP3 [24–27].

In addition to their anti-obesity effects, β_3 agonists also exer effects, including enhancement of insulin sensitivity and im insulin-mediated glucose uptake. Chronic treatment with β_3 ag hyperglycemia even at doses that do not cause weight loss. Th underlying the anti-diabetic effect of β_3 agonism are cu examination and readers interested in this aspect are referred t in-depth discussions [5–7, 28].

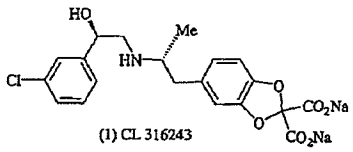
BIOLOGICAL ASSAYS

With the recognition of the differentiation between the roder adrenergic receptors, researchers have come to rely on the use of β_3 receptor assays for the identification of β_3 agonists [18–20]. activities are assessed *in vitro* by measuring the accumulation of c cells expressing human-cloned β_3-, β_2-, and β_1-ARs. The resu functional assays are reported in terms of potency (EC_{50}) an intrinsic activity (IA) which is defined as a fraction of the maxim caused by the non-selective full agonist isoproterenol). Howeve with low cAMP functional activity at the β_1- and β_2-ARs may antagonist activity that may cause unwanted side-effects [5–10]. affinities (K_i) of the compounds to membranes prepared fro expressing human-cloned β_3-, β_2-, and β_1-ARs are determined, an used to assess the selectivity of the agonist or antagonist.

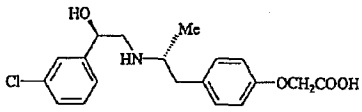
A number of *in vivo* assays have been developed or adapted to assay the anti-hyperglycemic, anti-obesity and/or anti-diabetic activity of β_3-AR agonists in animals [9, 21]. Potent and selective human β_3-AR agonists have usually been evaluated *in vivo* in db/db mice, a model of type-II diabetes and obesity, for their anti-hyperglycemic properties (such as lowering plasma glucose or change in TG levels). Another *in vivo* assay measures changes in metabolic thermogenesis by measuring changes in oxygen consumption in transgenic mice expressing the human β_3-AR. However, the thermogenesis assay proved to have low sensitivity and necessitated using high doses. A lipolysis assay that measures the transformation of TGs to glycerol and FFAs has the advantage of greater sensitivity over the thermogenesis model.

ORALLY BIOAVAILABLE β_3-AR AGONISTS AS THERAPEUTIC AGENTS

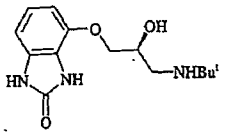
CL-316243, BRL-37344, and CGP-12177A (compounds 1–3) are representative of the first generation of β_3 agonists that were optimized for activity and selectivity between β-AR subtypes by using rodents as a model for the modulation of adipose tissue in humans [4–10]. These compounds have shown effects attributable to β_3 receptor stimulation, such as the mobilization of fat from WAT deposits, increased thermogenesis, and increased fat oxidation in rodents. In addition to their anti-obesity effects, they exhibit potent anti-diabetic effects (such as an increase in insulin secretion and improvement in insulin-mediated glucose uptake) in the rodent model type-II diabetes. However, human clinical trials with these early β_3 agonists were disappointing because of a lack of selectivity and insufficient anti-obesity effects. In the late 1980s, important progress was made in the cloning and sequencing of the rat and human β_3 receptors. With the human β_3-AR now available for the first time, it was soon apparent that these early clinical candidates were only partial agonists of this receptor and selectivity for the β_3-AR over β_2- and β_1-ARs in humans was actually a lot lower than that observed in rats. Many groups recognized that a cloned human receptor assay would offer major advantages over rodent models for the identification and optimization of future β_3 agonists. Continued research effort led to a number of so-called second-generation compounds that are showing promising results in both primates and in humans. A large number of β_3 agonists have been prepared and evaluated, and these fall basically into three structural classes, i.e., arylethanolamines, aryloxypropanolamines, and tetrahydroisoquinolines. In the following discussion, we summarize progress in the discovery and optimization of orally bioavailable β_3-AR agonists as agents for the treatment of obesity and diabetes.



(1) CL 316243



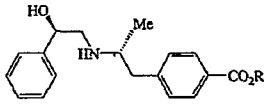
(2) BRL 37344



(3) CGP 12177A

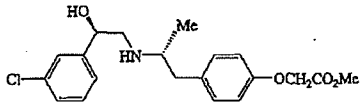
ARYLETHANOLAMINES

The phenethanolamine derivatives BRL-26830A (4) and E synthesized at Beecham Research Laboratories (now GlaxoSm the first β_3-AR agonists to be examined in rodents. For reviews, se These esters are well absorbed and rapidly metabolized i corresponding acids. *In vitro* the acids BRL-28410 (5) and I were shown to have potent effects on rat lipolysis (β_3 effec selectivity over atrial (β_1) and tracheal (β_2) effects. BRL-37344 is and selective agent of the two, exhibiting 400-fold selectivity ove versus β_2. The esters (4) and (6) were evaluated in a number o A slightly greater weight loss compared to placebo was obse further clinical trials were halted due to poor results and the occur β_2-mediated side-effects.



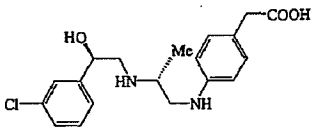
(4) R = Me BRL-26830

(5) R = H BRL-28410

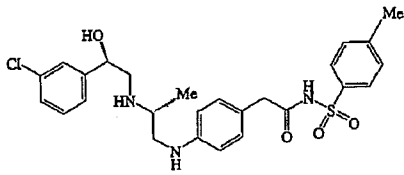


(6) BRL-35135

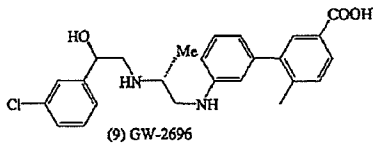
Researchers at Glaxo (now GlaxoSmithKline) explored a series of aniline-based phenethanolamine β_3 agonists in the 1990s [31, 32]. The parent compound (7) (GR-9803) was found to be a potent full agonist of the human β_3-AR ($EC_{50} = 9$ nM) but with low selectivity over β_1 and β_2 receptors. Varying the size and acidity on the right-hand side of the phenyl substituent of (7) led to the acylsulphonamide derivative (8) and biphenyl derivative (9) (GW-2696). Acylsulphonamide (8) has an EC_{50} value of 1 nM and shows modest selectivity over the β_1 and β_2 receptors (500-fold over β_1 and 60-fold over β_2) [31]. Although it has a pharmacokinetic half-life of less than 2 h, it does show low clearance in the dog. The biphenyl analogue is a very potent and selective human β_3 agonist ($EC_{50} = 1$ nM, 375-fold over β_1 and 750-fold over β_2) [32]. This compound induces no significant stimulation of β_1 and β_2 receptors. GW-2696 has a half-life of 4.4 h and 41% bioavailability in the dog. In the db/db mice, it reduced glucose levels by at least 50% at a dose of 10 mg/kg for 1 or 2 weeks (route of administration unknown).



(7) GR-9803



(8)



(9) GW-2696

CL-316243 (1), optimized by the Wyeth group against rodent [33], is an extremely potent stimulant of rat BAT lipolys $EC_{50} = 3$ nM) with more than 100,000-fold selectivity for the over the β_1 and β_2 receptors. Although in early clinical studies increased metabolic rate and reduced body weight without elicitir it had low oral bioavailability, which necessitated high doses (u A number of prodrugs of CL-316243 were synthesized in an eff the oral bioavailability. A 2–3-fold increase in bioavailability wa simple alkyl di-esters derivatives [34]. However, no clinical conducted on these prodrug forms.

Typical of β_3 agonists optimized for thermogenic activity i 316243 was subsequently found to be a weak partial agonist of receptor with much reduced potency and selectivity (β_3 EC_{50} $EC_{50} = 262$ μM; β_1 $EC_{50} = 111$ μM). The synthesis and activity series of compounds with improved potency and selectivity in the have been reported [35–44]. A piperidine analogue (10), pos thiazolidine moiety as a carboxylic acid replacement, was shown and selective human β_3-AR agonist (β_3 $EC_{50} = 10$ nM, IA = 1 selectivity for β_3 over β_1 and β_2) [38]. The therapeutic potential (10) for disorders related to obesity or type-II diabetes was demo *in vivo* procedure which compared thermogenesis in human β_3- mice (Tg mice) with β_3-AR knock-out mice (KO mice). Administ (i.p.) to Tg mice and KO mice compound (10) was acti

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