A Selective Human β_3 Adrenergic Receptor Agonist Increases Metabolic Rate in Rhesus Monkeys

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Abstract

Activation of β_3 adrenergic receptors on the surface of adipocytes leads to increases in intracellular cAMP and stimulation of lipolysis. In brown adipose tissue, this serves to up-regulate and activate the mitochondrial uncoupling protein 1, which mediates a proton conductance pathway that uncouples oxidative phosphorylation, leading to a net increase in energy expenditure. While chronic treatment with β_3 agonists in nonprimate species leads to uncoupling protein 1 up-regulation and weight loss, the relevance of this mechanism to energy metabolism in primates, which have much lower levels of brown adipose tissue, has been questioned. With the discovery of L-755,507, a potent and selective partial agonist for both human and rhesus β_3 receptors, we now demonstrate that acute exposure of rhesus monkeys to a β_3 agonist elicits lipolysis and metabolic rate elevation, and that chronic exposure increases uncoupling protein 1 expression in rhesus brown adipose tissue. These data suggest a role for β_3 agonists in the treatment of human obesity. (*J. Clin. Invest.* 1998. 101:2387–2393.) Key words: obesity • lipolysis • brown fat • uncoupling protein • energy metabolism

Introduction

Obesity constitutes a major risk-factor in the development of non–insulin-dependent diabetes and cardiovascular disease. This condition is difficult to control by food restriction alone because compensatory decreases in metabolic rate follow reductions in body weight imposed by reduced caloric intake (1). Thus, to offset this metabolic resistance to weight loss, an increase in energy expenditure (stimulation of metabolic rate) would be a desirable component of antiobesity therapy. A unique β adrenergic receptor subtype has been identified on the surface of rat adipocytes. Activation of this receptor, subsequently termed β_3, leads to increases in cellular cAMP and stimulation of lipolysis (2). In brown adipose tissue (BAT),[1] these serve to up-regulate and activate the mitochondrial uncoupling protein (UCP1), which mediates a proton conductance pathway that uncouples oxidative phosphorylation from fatty acid β oxidation, leading to a net increase in energy utilization (3). There is an ongoing debate as to whether UCP1 acts directly as a proton transporter, or as a fatty acid anion transporter where the free fatty acids function as cycling protonophores (3, 4). A number of rat-selective β_3 agonists have been discovered (2, 3, 5, 6) and shown to cause increases in metabolic rate, weight loss, and an improvement in glucose tolerance in dogs and rats (5–8). The observed weight loss resulted from a decrease in body lipids with no decrease in muscle mass (8). However, the relevance of this mechanism of energy metabolism in primates, which have much lower levels of BAT, has been questioned (3). Studies with these compounds in humans were inconclusive at best, and were complicated by side-effects of tremors and tachycardia, presumably via stimulation of β_2 and β_1 receptors, respectively (9, 10). The reason for these failures became clear with the identification and cloning of a human β_3 receptor (11, 12) and the demonstration of pharmacological differences between the rat and human β_3 receptors (13). Our own unpublished results show that all of the β_3 agonists tested in the clinic to date are only weak partial agonists of the human β_3 receptor, and are not selective for the β_3 receptor over the β_1 and β_2 receptors in humans. Thus our research focused on the discovery of β_3 agonists selective for the human receptor.

Several groups have utilized CGP12177, a β_1 and β_2 adrenergic receptor antagonist and a weak β_3 adrenergic receptor agonist, to demonstrate that β_3 adrenergic agonists will stimulate lipolysis in humans and some species of nonhuman primates (14, 15). As is generally observed with agonists of G protein–coupled receptors, the efficacy and potency of CGP12177 as a β_3 agonist varies dramatically with the level of expression of the β_3 receptor (16). Accordingly, we have assessed β_3 agonist activity in cell lines expressing low levels (< 100 fmol/mg) of the β_3 receptor, which mimic the pharmacology observed in human adipocyes.

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1. *Abbreviations used in this paper:* BAT, brown adipose tissue; RQ, respiratory quotient; UCP, uncoupling protein; WAT, white adipose tissue.

Methods

Unless otherwise noted, all chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Functional assays. The human β_3 receptor was obtained from Dr. J. Grannemann (Wayne State University, Detroit, MI), and other receptors were cloned as previously described (17, 18). Human and rhesus monkey β_1, β_2, and β_3 receptors were expressed in mammalian cell lines for the primary screening assays. CHO cells, stably transfected with the cloned β-adrenergic receptors were harvested in enzyme-free dissociation media (Specialty Media, Lavallette, NJ) 3 d after plating. Cells were counted and distributed in the assay tubes, after being resuspended in ACC buffer (75 mM Tris, pH 7.4, 250 mM sucrose, 12.5 mM $MgCl_2$, 1.5 mM EDTA), containing the antioxidant sodium metabisulfite at a concentration of 0.2 mM and a phosphodiesterase inhibitor (0.6 mM IBMX). The cAMP production reaction was initiated by mixing cells with 20 μl of a 6× stock of the ligand to be tested. Tubes were shaken at 275 rpm for 45 min at room temperature, and the reaction stopped by boiling the tubes for 3 min. The cAMP produced in response to the ligand was measured in the lysate by competing against [^{125}I]-cAMP for binding to a cAMP-directed antibody using an automated RIA machine (ATTOFLO; Atto Instruments, Baltimore, MD). The cAMP level was determined by comparison to a standard curve.

Binding assays. CHO cells expressing the cloned human and rhesus β receptors were grown in selective media for 3 d and membranes prepared by hypotonic lysis in 1 mM Tris, pH 7.2. Receptor binding assays were carried out in a final volume of 250 μl containing 5–10 μg of membrane protein, the radioligand [^{125}I]-cyanopindolol at a concentration of 45 pM, and the compound of interest at various concentrations. Binding reactions were carried out for 1 h at 23°C, and terminated by filtration over GF/C filters using a 96-well cell harvester from Inotech (Lansing, MI).

Lipolysis assays. Rhesus adipose tissue was obtained by surgical biopsy of subcutaneous adipose depots and used immediately. Adipose cells were harvested from the tissue after digestion with collagenase D (Boehringer Mannheim, Indianapolis, IN) as described by Rodbell (19) or performed directly on minced tissue pieces (50–100 mg/assay). The incubation mixture was shaken gently in an incubator under 5% CO_2 atmosphere for 2 h. The infranatant was collected for glycerol determination. The glycerol content of the samples was determined by the glycerol kinase procedure, using the Sigma Chemical Co. kit 337A.

Western analysis. Antibodies were generated in rabbits after immunization with the peptide corresponding to residues 232–247 of the human UCP1. This sequence is homologous to the corresponding sequence in the rhesus protein. Tissue homogenates were electrophoresed using SDS-PAGE, and transferred to nitrocellulose before incubation with antibody. The signals were quantitated on a fluorimager (Molecular Dynamics, Sunnyvale, CA) using the substrate Attophos™ (JBL Scientific Inc., San Luis Obispo, CA). Western analysis using increasing concentrations of homogenized tissue produced a linear response. Analysis of each sample was performed on three separate gels. Data for samples on each gel were normalized to one post-treatment vehicle sample before averaging the three values for each sample. Protein concentrations were determined using the Bradford reagent. Data were analyzed using the GraphPad Prism program (San Diego, CA).

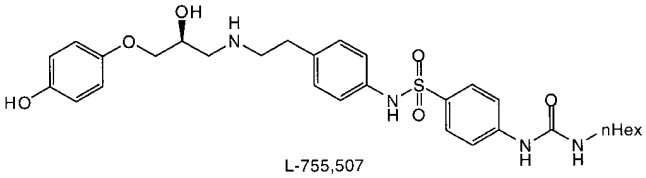
Measurement of lipolysis and heart rate in vivo. All animal procedures were reviewed and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Male lean rhesus monkeys (4–7 kg body weight; age 3–5 yr) were fasted for 24 h and were lightly anesthetized with ketamine (10 mg/kg, i.m.; Fort Dodge Labs, Fort Dodge, IA). A 22-gauge intravenous catheter (Becton Dickinson & Co., Sandy, UT) was placed in a saphenous vein for the administration of test compounds after which the animals were administered Nembutal (25 mg/kg, i.v.; Abbott Labs, North Chicago, IL). A 20-gauge angiocatheter, connected to a TNF-R pressure transducer (Ohmeda Medical Device Systems, Madison, WI), was placed in a femoral artery for monitoring blood pressure. ECG leads were connected for the continuous measurement of heart rate. Heart rate and blood pressure were monitored for ~ 30 min until stable baseline values were obtained, at which time animals were administered a series of rising dose infusions (0.1 ml/min) of agonists (isoproterenol, L-755,507), or an equivalent volume of vehicle over a 15-min period. Infusion periods were separated by an interval of ~ 20 s. Blood samples (2 ml) were collected from the femoral artery 1 min before the initiation of infusions and 14 min into each infusion period. Serum glycerol was measured using an enzymatic colorimetric assay and serum potassium was determined using an ion specific electrode.

Measurement of metabolic rate in vivo. Male lean rhesus monkeys (4–8 kg body weight; age 3–6 yr) were fasted and prepared as described above. An endotracheal tube was inserted into the trachea and a 6-mm diameter vacuum line was attached to the outlet of the endotracheal tube in order to sample exhaled air. Exhaled air was drawn into the mixing chamber of a respiratory analyzer (Oxyscan model OXS-1RM O_2/CO_2 respiratory gas analyzer; Omnitech Electronics Inc., Columbus, OH) at a rate of 1 liter/min. Energy expenditure was calculated from the volume of O_2 consumed and the volume of CO_2 generated. L-755,507 was administered after a stabilization period of 30–40 min after anesthesia, and metabolic rate was monitored for an additional 60 min. Heart rate was recorded continuously, and blood samples were collected from the femoral artery at the times indicated for determination of serum glycerol.

Results

In vitro potency and selectivity of β_3 receptor agonist L-755,507. Benzenesulfonamide derivative L-755,507 is a partial agonist

Table I. Activity of L-755,507 at the Cloned Human and Rhesus β Adrenergic Receptors



L-755,507

	β_3		β_1		β_2	
Species	EC_{50} (% act)	IC_{50}	EC_{50} (% act)	IC_{50}	EC_{50} (% act)	IC_{50}
	nM	*nM*	*nM*	*nM*	*nM*	*nM*
Human	0.43±0.31 (52±16)	13±7.2	580±300 (25±7)	200±40	> 10000 (2)	190±110
Rhesus	2.2±1.0 (68±14)	130±27	> 10000 (33±20)	2300±920	> 10000 (0)	1500±960

The receptors were expressed in CHO cells at receptor densities of 46–88 fmol/mg (*β_3 receptors*) or 300–500 fmol/mg (*β_1 and β_2 receptors*). Agonist potency (EC_{50}) and efficacy (*% act*) were assessed by measurement of intracellular cAMP levels, and the latter is expressed relative to the maximum response evoked by isoproterenol in each cell type. Binding affinities were quantified as inhibition of [^{125}I]-cyanopindolol binding in each cell type. Data are mean±SD of ≥ 3 determinations, and are expressed as nanomolar values. The sequence for the rhesus β_3 adrenergic receptor that we have cloned and utilized is identical to that recently deposited in GenBank (U63591 and U63592). The homology between the human and rhesus β_3 receptors is 99% within the transmembrane spanning regions, and between the human and rhesus β_1 and β_2 receptors the homology is 100% within this same region.

for the human β_3 receptor, with maximal activation 52% of that evoked by isoproterenol. L-755,507 activates the human β_3 receptor with an EC_{50} of 0.43 nM and inhibits ligand binding to β_1 and β_2 receptors with IC_{50}'s of 200 and 190 nM (Table I). It has weak agonist activity at the human β_1 receptor, but is > 1,000-fold selective for activation of the β_3 receptor versus activation of the β_1 receptor. L-755,507 has no measurable β_2 agonist activity. In contrast, CGP12177 activates the β_3 receptor in these cells with an EC_{50} of 5,300 nM and inhibits binding to β_1 and β_2 receptors with IC_{50}'s of 2.6 and 2.9 nM. Thus, under these conditions of receptor expression, L-755,507 is > 10,000-fold more potent than CGP12177 as a β_3 agonist. Moreover, L-755,507 is > 400-fold selective for the β_3 receptor (as reported by activation of the β_3 receptor versus activation of or binding to β_1 and β_2 receptors), while CGP12177 is a potent β_1 and β_2 receptor antagonist.

L-755,507 is also a potent and selective β_3 partial agonist in rhesus monkeys as assessed by its affinity for the cloned β adrenergic receptors from this species (Table I). It activates the rhesus monkey β_3 receptor with an EC_{50} of 2.2 nM with maximal activation 68% of the maximal activation of isoproterenol. It has weak activity at the rhesus monkey β_1 receptor with 33% of the maximal activity of isoproterenol at the highest concentration tested (10 μM), and it has no measurable activity at the rhesus monkey β_2 receptor. Thus, although the compound is fivefold less potent at the rhesus monkey β_3 receptor than at the human β_3 receptor, its selectivity for activation of β_3 receptors versus binding to or activation of β_1 and β_2 receptors is > 400-fold in both species. In addition, L-755,507 stimulates lipolysis in rhesus adipocytes in vitro with an EC_{50} = 3.9 nM, and a maximal effect 23% of that of isoproterenol (Fig. 1).

L-755,507 was selected for further evaluation since this compound shows broad selectivity for the β_3 receptor versus other biogenic amine G protein–coupled receptors. L-755,507 is at least 300-fold selective for the human β_3 adrenergic receptor vs the human alpha 1a, alpha 1b, alpha 1d, alpha 2a, alpha 2b, alpha 2c, dopamine D3 and D4 receptors, and is 46-fold selective for the dopamine D2 receptor (data not shown). As expected, the structure activity relationship for affinity at the β_3 receptor varies independently of the structure activity relationship for these other G protein–coupled receptors, and optimization of this selectivity is both critical and challenging, but is attainable.

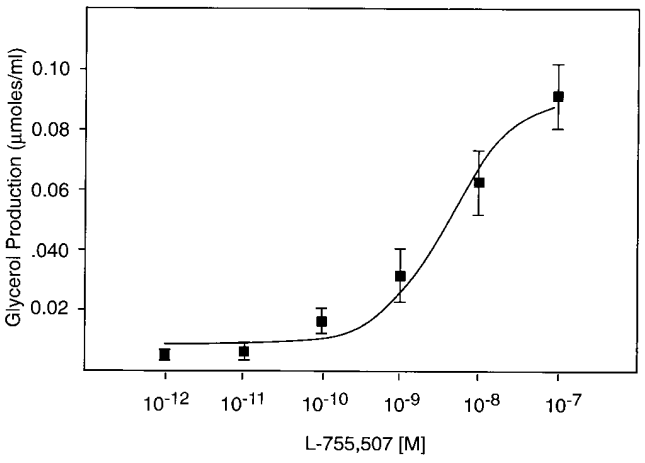


Figure 1. Stimulation of lipolysis in isolated rhesus adipocytes. Adipose tissue from subcutaneous depots was obtained from anesthetized rhesus monkeys by surgical biopsy. Adipocytes were isolated by collagenase digestion as described in Methods, and incubated with isoproterenol or L-755,507 for 2 h at 37°C before determination of glycerol levels in the incubation media.

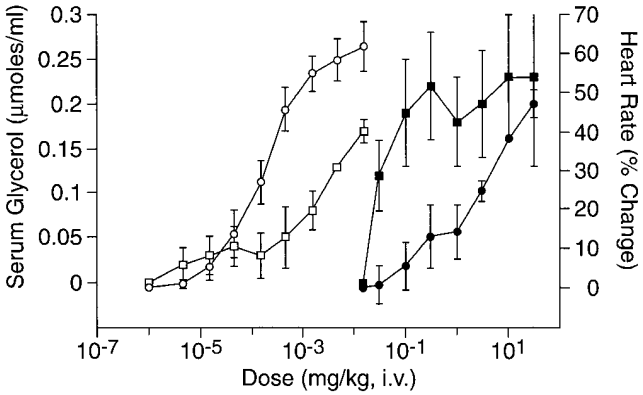


Figure 2. The effects of isoproterenol (*open symbols*) and L-755,507 (*closed symbols*), administered by intravenous infusion, on serum glycerol (*squares*) and heart rate (*circles*) were determined in anesthetized rhesus. Each point represents the mean of determinations in two to five animals and the vertical bars the standard error of the mean.

Effects of L-755,507 on lipolysis and heart rate in the rhesus monkey. L-755,507 and isoproterenol were administered as sequential rising dose infusions over 15 min into catheterized and instrumented barbiturate anesthetized monkeys (Fig. 2). Although these studies were conducted in male animals, preliminary studies indicated that the profile of activity of L-755,507 was similar in male and female rhesus. For L-755,507, the ED_{50} for glycerolemia (dose producing 50% maximal increase in serum glycerol) was 0.03 mg/kg and the ED_{50} for tachycardia (dose producing 50% maximal increase in heart rate, i.e., ∼ 40 bpm) was 2.5 mg/kg. For isoproterenol, the ED_{50} for glycerolemia was 0.003 mg/kg and the ED_{50} for tachycardia was 0.0002 mg/kg. No changes in mean arterial pressure or serum potassium were evident in L-755,507–treated animals, but at higher doses of isoproterenol (≥ 0.003 mg/kg), hypokalemia and hypotension were observed (data not shown).

Effects of L-755,507 on metabolic rate in the rhesus monkey. L-755,507 stimulates metabolic rate by ∼ 30% after acute bolus intravenous administration of 0.1 mg/kg to rhesus monkeys. The response peaked within 30 min and was sustained for at least 60 min, the duration of the monitoring period. Under these conditions, lipolysis and tachycardia accompanied the metabolic rate increase: lipolysis was maximal within 15 min of agonist administration, whereas the tachycardia, which occurred in the absence of changes in mean arterial pressure, was slowly developing and peaked (∼ 15% increase in heart rate) at 40 min after L-755,507 administration (Fig. 3). Note that the profile of the tachycardia evoked by L-755,507 is markedly different from that elicited by β_1 agonists such as isoproterenol, as the latter is rapid in onset and is maximal within 60 s of agonist addition. Evaluation of the dose dependence of these responses indicated that significant increases in metabolic rate and lipolysis were evident at a dose of L-755,507

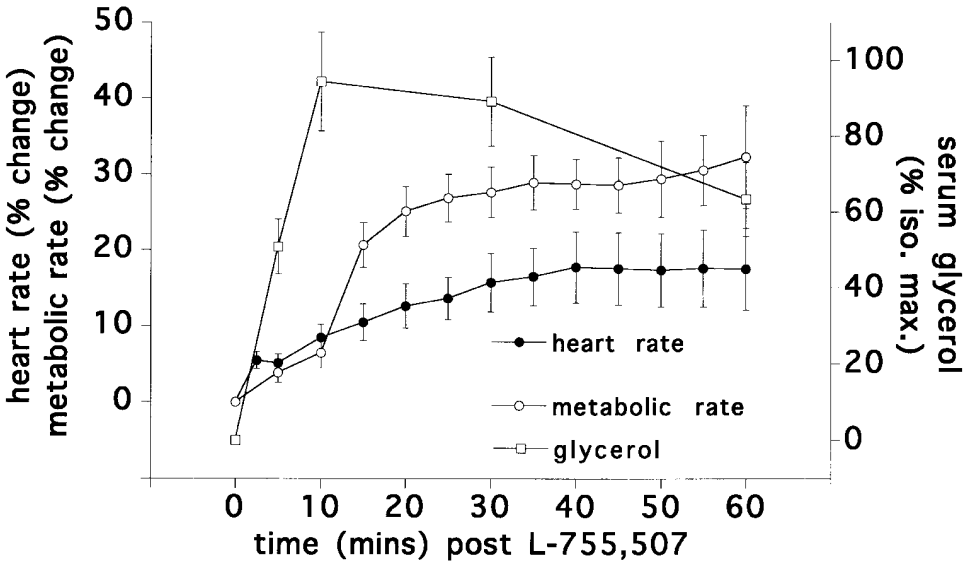


Figure 3. The effects of L-755,507 (0.1 mg/kg, i.v.) on metabolic rate (*open circles*), serum glycerol (*open squares*), and heart rate (*solid circles*) were determined in the anesthetized rhesus. Metabolic rate and heart rate are expressed as the percent change from baseline values, and serum glycerol is expressed as a percentage of the response evoked by a maximally effective dose of isoproterenol in the same animals. No changes in any of the parameters were evident after vehicle administration in place of L-755,507. Each point represents the mean of determinations in three to four animals and the vertical bars the standard error of the mean.

($\leq$ 0.01 mg/kg) at which no significant tachycardia was evident (Fig. 4).

Chronic exposure of rhesus monkeys to L-755,507. Female rhesus monkeys were administered vehicle (25% ethanol, 25% polyethylene glycol 400, 50% saline, 1 ml/kg, i.v., twice daily) or L-755,507 (3 mg/kg, i.v., twice daily) daily for up to 28 d. Samples of axillary brown adipose tissue, so defined because of the presence of UCP1, multilocular lipid droplets, and a significant cytoplasm: lipid ratio (vide infra), were obtained before and at 2 and 4 wk after initiation of treatment, and the morphology of, and UCP1 levels in axillary BAT were determined.

As shown in Fig. 5, UCP1 levels were increased by 62% (P = 0.04) and 132% (P = 0.05) after 2 and 4 wk of administration of L-755,507, respectively. No changes in UCP2 expression were noted. Histological examination of samples of BAT obtained 4 wk after administration of vehicle or L-755,507 revealed changes at both the light and electron microscopic levels (Fig. 6). Light micrographs (Fig. 6, *A* and *B*) demonstrate that, in comparison to vehicle treated control animals, treatment with L-755,507 results in a decrease in the size of intracellular lipid droplets coupled with an increase in the number of droplets per cell. Moreover, the cytoplasm:lipid ratio is increased in the tissue from animals treated with the β_3 agonist. A further structural effect of chronic exposure to L-755,507, which is evident when specimens are observed by electron microscopy (Fig. 6, *C* and *D*), is that there are many more cristae within the mitochondria of tissue from L-755,507–treated animals. In tissue from β_3 agonist–treated animals, the cristae are usually found as tightly packed arrays of parallel membranes

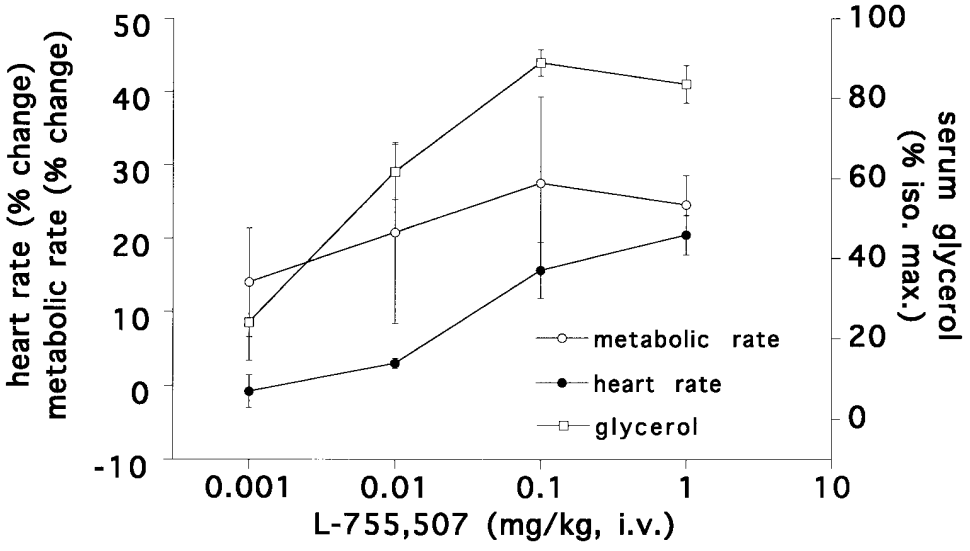


Figure 4. The dose dependence of the effects of L-755,507 (0.001–1 mg/kg, i.v.) on metabolic rate (*open circles*), serum glycerol (*open squares*), and heart rate (*solid circles*) were determined in the anesthetized rhesus, and the data reported are those obtained 30 min after compound administration. Metabolic rate and heart rate are expressed as the percent change from baseline values, and serum glycerol is expressed as a percentage of the response evoked by a maximally effective dose of isoproterenol in the same animals. No changes in any of the parameters were evident after vehicle administration in place of L-755,507. Each point represents the mean of determinations in three to four animals and the vertical bars the standard error of the mean.

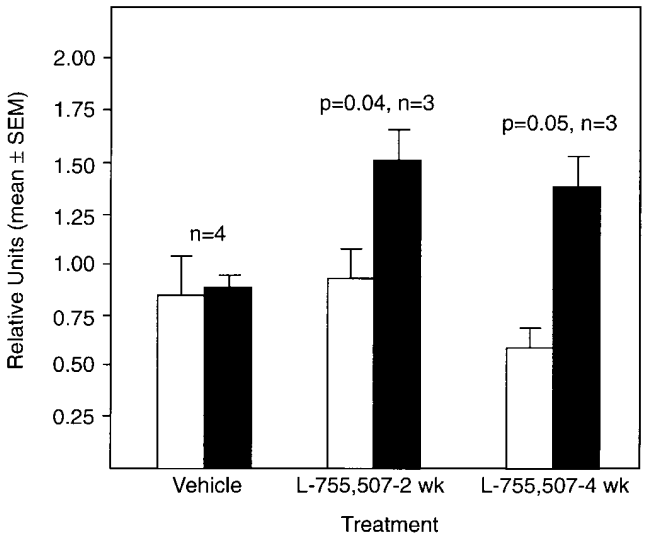


Figure 5. Effects of chronic administration of L-755,507 on mitochondrial UCP levels in axillary brown adipose tissue. UCP levels were determined by Western analysis of membrane fractions from BAT obtained by surgical biopsy before (*open bars*) and after (*closed bars*) 2 or 4 wk of administration of vehicle or L-755,507 (3 mg/kg, i.v., twice daily). Analysis was performed three times for each tissue sample, and data for samples on each gel were normalized to one posttreatment vehicle sample before averaging the three values for each sample. Data shown are the mean±SEM for the normalized data for each animal within a treatment group (four vehicle-treated animals, three L-755,507–treated animals for 2 or 4 wk). Statistical analysis was performed using a 1-tailed paired *t* test.

whereas in tissue from vehicle-treated animals there are fewer cristae per mitochondria and they generally fill only a portion of the mitochondrial volume.

Discussion

Using cloned human receptors for screening, a novel β_3 adrenergic receptor agonist, L-755,507, was discovered. This compound is a potent β_3 agonist at both the human and rhesus receptors, and is $>$ 1,000-fold selective for activation of the β_3 receptor versus activation of the β_1 receptor in both species. Selectivity is $>$ 400-fold in both species when quantified with respect to activation of the β_3 receptor and activation of or binding to β_1 and β_2 receptors.

We and others (20–22) have shown that it is only possible to detect β_3 adrenergic receptor mRNA in omental white adipose tissue (WAT) and in BAT from humans and rhesus monkeys by reverse transcription PCR. However, β_1 and β_2 adrenergic receptor mRNA can be detected by Northern blots. Thus, in contrast to rodent adipocytes, the β_3 adrenergic receptor is a minor component of the complement of β adrenergic receptors expressed on primate adipocytes. However, CGP12177 stimulates triglyceride breakdown in human abdominal WAT consistent with its activation of β_3 receptors (15). In addition, we have evaluated the effects of L-755,507 and 14 other selective β_3 agonists on lipolysis in human abdominal WAT and demonstrated that lipolytic activity correlates well with their affinity for the human β_3 adrenergic receptor ($r^2 = 0.34$, slope = 0.38 [$P = 0.02$]). In contrast, there is no correlation between their lipolytic potential and their affinity

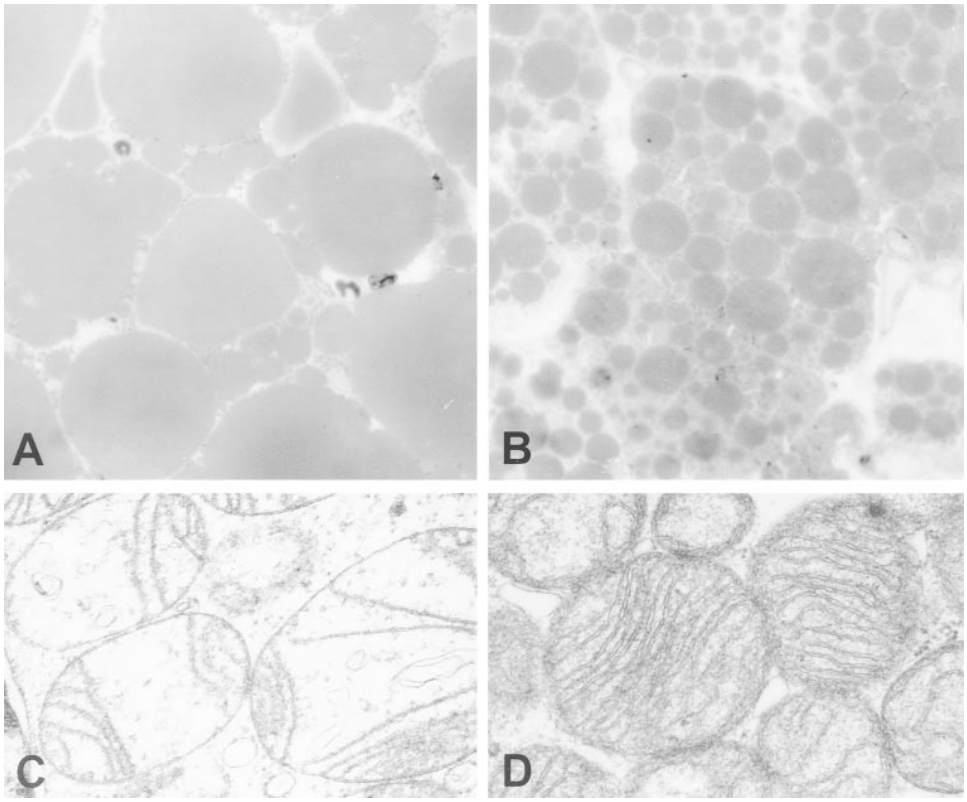


Figure 6. Structural observation of axillary brown adipose tissue collected from rhesus which had been treated for 28 d with L-755,507 (3 mg/kg, i.v., twice daily) (B and D) or vehicle (A and C). Magnification, *A* and *B* = 900, *C* and *D* = 70,000.

for the human β_1 or β_2 adrenergic receptors (r^2 = 0.02 and 0.08, slope = 0.08 [P = 0.6] and 0.24 [P = 0.3], respectively). The stimulation of lipolysis in human WAT by compounds selective for the human β_3 receptor suggests that the β_3 receptor is expressed at levels sufficient to be pharmacologically exploited to selectively activate adipose tissue.

L-755,507 is potent and efficacious at the rhesus β_3 receptor, and was thus chosen for more detailed studies. Preliminary pharmacokinetic analyses in various species indicated that this class of compounds was poorly orally bioavailable, with relatively short terminal half lives. Accordingly, the profile of activity of the compound in rhesus was evaluated after intravenous administration. When administered as sequential rising dose infusions over 15 min into catheterized and instrumented barbiturate anesthetized monkeys, L-755,507 and isoproterenol dose dependently stimulated lipolysis and tachycardia. For L-755,507, the dose–response curve for glycerolemia lies significantly to the left of that for tachycardia, whereas the converse is true for isoproterenol. Although L-755,507 is less potent than isoproterenol in stimulating lipolysis, the maximum extents of glycerolemia evoked by L-755,507 and isoproterenol are similar when measured in the same animal, indicating that the compound behaves as a full agonist with respect to lipolysis in vivo. These data indicate that the profile of activity of L-755,507 in vivo in the rhesus monkey differs markedly from that of the nonselective β agonist isoproterenol.

L-755,507 stimulates metabolic rate after acute bolus intravenous administration to rhesus monkeys. The maximum increase in metabolic rate ($\sim$ 30% above baseline) was evident at 0.1 mg/kg L-755,507, and was accompanied by lipolysis and slowly developing tachycardia. However, significant increases in metabolic rate and lipolysis were evident at a dose of L-755,507 ($\leq$ 0.01 mg/kg) at which no significant tachycardia was evident. In these lean animals baseline respiratory quotient (RQ) is $\sim$ 0.8, indicating predominance of fat over carbohydrate as substrates for oxidation in the fasted state. No reductions in RQ were evident after L-755,507 administration, presumably because of the low RQ before β_3 agonist administration. In support of this contention, we have shown that other β_3 agonists can reduce RQ in conscious fed animals, where baseline RQ is $\sim$ 0.9 (unpublished observations). Activation of lipolysis and metabolic rate elevation in nonhuman primates by L-755,507 at doses which fail to elicit significant tachycardia (a β_1 effect) or hypokalemia (a β_2 effect) suggest that human-selective β_3 receptor agonists will display an improved side-effect profile, in comparison to rodent-selective β_3 agonists, in humans.

As noted above, L-755,507 shows only very weak agonist activity at the rhesus β_1 receptor in vitro. This, together with the observation that the tachycardia evoked by bolus L-755,507 is much slower in onset than that evoked by isoproterenol, suggests that the tachycardia observed in rhesus monkeys after L-755,507 administration may not be a direct effect of activation of cardiac β_1 receptors by the β_3 agonist. Additional studies, to be described elsewhere, using selective human β_3 agonists in anesthetized and conscious rhesus monkeys established that the tachycardia evoked by β_3 agonists is reflexogenic in origin, consequent upon evoked increases in metabolic rate and direct β_3 receptor–mediated peripheral vasodilatation which occurs in the absence of changes in mean arterial pressure.

To evaluate the sequelae of chronic exposure to L-755,507, female rhesus monkeys were administered vehicle or L-755,507 (3 mg/kg, i.v., twice daily) daily for up to 28 d. Preliminary studies in conscious rhesus monkeys established that L-755,507 does not elicit significant direct β_1 receptor activation, as reported by rapidly developing tachycardia, but produces a peak increase ($\sim$ 20%) in metabolic rate, that is relatively transient and returns to the baseline value within 3 h. From the extent and duration of the L-755,507-induced increase in metabolic rate, as measured acutely over 3 h, we estimate the maximum increase in 24-h energy expenditure that could be achieved using this dosing regimen of L-755,507 is $\sim$ 5%. Accordingly, we chose not to attempt to measure 24-h energy expenditure in the chronically dosed animals using doubly labeled water, because in primates the sensitivity of the technique is sufficient to report increases in energy expenditure that are at least 10–15%. Consistent with the predicted small increases in 24-h energy expenditure, where a 5% increase (i.e., $\sim$ 24 kcal/d) deriving from increased fat metabolism (calorific value = 9.3 kcal/gram) would yield a weight loss of $<$ 3 grams/d in rhesus monkeys, there were no differences in weight loss between vehicle-treated and L-755,507–treated animals. To place these observations in rhesus monkeys in proper perspective, we estimate that a 10% increase in 24-h energy expenditure in humans will yield a clinically meaningful weight loss at the rate of $\sim$ 1 kg per month.

A significant increase in UCP1 levels was observed after 2 and 4 wk of administration of L-755,507. In addition, morphologic changes in the axillary fat of treated animals were consistent with BAT activation. Studies using other β_3 agonists in male rhesus indicate that the morphological effects of chronic β_3 agonist administration in the rhesus are not gender specific. Consistent with previous reports in rodents (23), chronic exposure of rhesus monkeys to this selective β_3 agonist did not alter expression of the newly discovered homolog UCP2 in axillary BAT.

Given the relative paucity (but not absence) of BAT in primates, including humans (24), it is likely that the acute lipolytic and metabolic rate effects of β_3 agonists such as L-755,507 are mediated predominantly, if not exclusively, via activation of β_3 receptors on white adipocytes. The evoked thermogenesis may derive in part from uncoupling of fatty acid β oxidation from ATP generation via the intermediation of UCP1, UCP2, and/or UCP3 (25) in BAT, or from UCP2 and/or UCP3 in WAT. This concept is consistent with the observations in transgenic mice where the acute effects of β_3 agonists on O_2 consumption are more marked in animals which express β_3 receptors exclusively in WAT than in BAT (26). As we have shown that chronic exposure to β_3 agonists upregulates BAT in rhesus, the presumption is that under these conditions BAT would make a greater contribution to the increase in metabolic rate. β_3 agonists also cause sustained metabolic effects, including up-regulation of nascent BAT and weight loss in other species (e.g., dogs) which, like humans and nonhuman primates, have relatively sparse BAT in adulthood (5, 24, 27). Moreover, hibernomata and pheochromocytoma in humans are associated with BAT expansion and up-regulation, respectively, and both are accompanied by marked weight loss (24). It remains to be shown whether chronic therapy with β_3 agonists in humans elicits upregulation of BAT with resulting negative energy balance and weight loss.

Conclusions. L-755,507 is the first β_3 agonist demonstrated to be potent and selective for the receptor in humans and

rhesus monkeys, and is the first selective compound to activate lipolysis in adipocytes isolated from these species. Thus, although the expression levels of β_3 receptors found in primate adipocytes are quite low, and reportedly variable among primate species (14, 28), activation of these receptors by selective agonists produces the desired pharmacological response in rhesus monkeys. In addition, our data demonstrate that pharmacological activation of β_3 receptors in primates acutely stimulates lipolysis in vivo and increases metabolic rate, suggesting that responses to appropriate β_3 agonists in primates mimic the responses previously seen in rodents. Finally, in a manner similar to that previously observed in rodents and dogs, chronic administration of these compounds activates and differentiates rhesus monkey BAT, as evidenced by increased UCP1 expression, which process is controlled by β_3 receptor activation (29). Thus, the data suggest that selective β_3 agonists with high affinity for the human receptor may be useful for increasing energy expenditure and inducing weight loss in humans.

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