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L-750355, a human ß3-adrenoceptor agonist; in vitro pharmacology and profile of activity in vivo in the rhesus monkey

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Abstract

The profile of in vitro and in vivo biology of a human ß3-adrenoceptor agonist, (*S*)-*N*-[4-[2-[[3[(2-amino-5-pyridinyl)oxy]-2-hydroxypropyl]amino]-ethyl]-phenyl]-4-isopropylbenzenesulfonamide, L-750355, is described. Using cloned human and rhesus ß1-, ß2- and ß3-adrenoceptors, expressed in Chinese hamster ovary (CHO) cells, L-750355 was shown to be a potent, albeit partial, agonist for the human (EC_{50} = 10 nM; % maximal receptor activation = 49%) and rhesus (EC_{50} = 28 nM; % maximal receptor activation = 34%) ß3-adrenoceptors. Furthermore, L-750355 stimulates lipolysis in rhesus adipocytes in vitro. L-750355 is a weak partial agonist (EC_{50} = 3.2 μM; % maximal receptor activation = 33%) for the human ß1-adrenoceptor but exhibits no agonist activity for rhesus ß1- or ß2-adrenoceptors of either human or rhesus origin. Administration of L-750355 to anesthetized rhesus monkeys, as a series of rising dose intravenous infusions, evokes dose-dependent glycerolemia and tachycardia with no change in mean arterial blood pressure or plasma potassium. The dose–response curve for L-750355-induced glycerolemia lies to the left of that for tachycardia. Propranolol, at a dose (0.3 mg/kg, i.v.) that attenuates isoproterenol-induced changes in heart rate and glycerolemia, abolished L-750355-induced tachycardia but had no effect on L-750355-induced glycerolemia.

Keywords: Obesity; ß3-adrenoceptor agonist; Lipolysis; Tachycardia; Rhesus

1. Introduction

Obesity affects approximately 30% of the adult population in the Western world (Kuczmarski et al., 1997), and is closely associated with the development of Type II diabetes, coronary artery disease, hypertension, some types of cancer and an increased mortality risk (Calle et al., 1999). In many cases, the morbidities associated with these diseases are partially reversed by weight loss. Obesity may be considered to arise from an imbalance between energy intake and energy expenditure. Current therapeutic approaches are focused on decreasing caloric intake, either by appetite suppression or decreased nutrient absorption. However, recent studies have shown that decreases in body weight in humans result in compensatory decreases in metabolic rate, with the result that weight loss is difficult to maintain by caloric restriction alone (Leibel et al., 1995). Therefore, an optimal therapeutic approach to obesity would include treatment to enhance energy expenditure by increasing metabolic rate. ß3-adrenoceptor agonists are proposed to act by such a mechanism.

In mammals, there are two phenotypically distinct types of adipose tissue that have common and unique features.

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White adipose tissue serves to store energy in the form of triglycerides. In contrast, brown adipose tissue functions to dissipate energy in the form of heat, through the action of mitochondrial uncoupling protein-1, a proton transporter that is unique to brown adipose tissue. Heat production by brown adipocytes results from a controlled uncoupling of oxidative phosphorylation by an uncoupling protein-1 mediated proton conductance pathway in the inner mitochondrial membrane. Sympathetic stimulation of both brown and white adipocytes activates ß-adrenoceptors on the cell surface, leading to increases in intracellular cAMP and stimulation of lipolysis, resulting in the breakdown of triglycerides into glycerol and free fatty acids. In brown adipose tissue, the free fatty acids resulting from the lipolytic reaction serve to activate uncoupling protein-1 by binding to an allosteric site on the protein. In addition, the increases in cAMP upon ß-adrenoceptor stimulation serve to up-regulate transcription of the uncoupling protein-1 gene, by activation of a cAMP response element in the promoter region. The result of uncoupling protein-1 activation is a net increase in energy utilization (Himms-Hagen, 1992).

Pharmacologically distinct ß-adrenoceptors (Arch et al., 1984) termed ß3-adrenoceptors, have been characterized on adipocytes. Subsequently, the pertinent genes were cloned from rat (Granneman et al., 1992) and human libraries (Emorine et al., 1989). Although ß3-adrenoceptors have been identified in many tissues, by far the most abundant expression of the ß3-adrenoceptor is in adipocytes (Lowell and Flier, 1997) where activation results in increased lipolysis. Historically, several selective ß3-adrenoceptor agonists have been developed on the basis of their ability to stimulate lipolysis in rat adipocytes in the absence of ß1- or ß2-adrenoceptor effects. In rodent and canine models of obesity, these compounds (e.g. *R,R*-(±)-methyl-4-[2-[(2-hydroxy-2-phenylethyl)amino]-propyl]-benzoate,(*E*)-2-butenedioate, BRL 26830A; *R,R*-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl) ethylamino]-propyl]-phenoxyacetate hydrobromide, BRL 35135; and disodium (*R,R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxy-ethyl-amino]propyl]-1,3-benzodioxazole-2,2-dicarboxylate, CL 316,243) caused an increase in metabolic rate, weight loss, and improved glucose tolerance (Arch and Ainsworth, 1983; Cawthorne et al., 1992; Himms-Hagen et al., 1994). The weight loss resulted entirely from a decrease in body lipid content with no decrease in muscle mass. Similar weight loss studies with these agents in humans were inconclusive and were complicated by tremors (a ß2-adrenoceptor effect) and, in some cases, tachycardia(a ß1-adrenoceptor effect) (Cawthorne et al., 1992). However, subsequent analysis in our laboratories (Naylor et al., 1998) using cloned human ß1-, ß2- and ß3-adrenoceptors showed that these compounds are only weak partial agonists of the human ß3-adrenoceptor. Significantly, none of the compounds was particularly selective for the human ß3-adrenoceptor subtype: ß1- and/or ß2-adrenoceptor agonist activity was observed for all compounds, consistent with the side-effect profile reported in human subjects. To develop ß3-adrenoceptor agonists suitable for treatment of human obesity, we have used cloned human receptors as screening tools.

Differences in the binding affinity, to the ß3-adrenoceptor from different species, have been observed for various classes of ß3-adrenocpetor agonists (Liggett, 1992; Arch and Wilson, 1996). As our objective is to identify ß3-adrenoceptor agonists selective for the human ß3-adrenoceptor, we sought to identify test species wherein the pharmacological profile of ß3-adrenoceptor agonists more readily mimicked that of the human ß3-adrenoceptor. In vitro experiments with clonedrhesus receptors suggested that the profile of activity, efficacy and selectivity ofthe benzenesulfonamide class of ß3-adrenoceptor agonists for binding and activation of rhesus ß-adrenoceptors was similar to that of human ß-adrenoceptors (Fisher et al., 1998). Furthermore, the profile of activity, efficacy and selectivity of these ß3-adrenoceptor agonists for stimulation of lipolysis in rhesus adipocytes mimics the profile observed for the human ß3-adrenoceptor (Fisher et al., 1998). Accordingly, we have utilized changes in the level of circulating serum glycerol in the rhesus monkey as an index of ß3-adrenoceptor activity in vivo. In order to determine that, changes in serum glycerol in rhesus monkeys are mediated viaactivation of ß3-adrenoceptors, a series of experiments were performed to determine the effects of adrenergic agonists and antagonists on indices of ß1-, ß2- and ß3-adrenoceptor mediated responses. The ß3-adrenoceptor agonist used in these studies was a benzenesulfonamide derivative, (*S*)-*N*-[4-[2-[[3[(2-amino-5-pyridinyl)oxy]-2-hydroxy-propyl]amino]-ethyl]-phenyl]-4-isopropylbenzenesulfonamide, L-750355 (Weber et al., 1998). Changes in heart rate were used to monitor ß1-adrenoceptor activation and changes in serum potassium were used to monitor ß2-adrenoceptor activation.

2. Materials and methods

2.1. ß-adrenoceptor mediated cAMP generation

The human ß3-adrenoceptor was obtained from Dr. J. Grannemann (Wayne State University, Detroit, MI) and other receptors were cloned as described previously (Frielle et al., 1987; Kobilka et al., 1987). Human and rhesus monkey ß1-, ß2- and ß3-receptors were expressed in mammalian cell lines for the primary screening assays. Chinese hamster ovary (CHO) cells, stably transfected with the cloned ß-adrenoceptors were harvested in enzyme-free dissociation media 3 days after plating. Cells were counted and distributed in the assay tubes, after being resuspended in 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 isobutylmethylxanthine). 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.

2.2. Binding of ß-adrenoceptor agonists to cloned ß-adrenoceptors

CHO cells expressing the cloned human and rhesus ß-adrenoceptors were grown in selective media for 3 days and membranes prepared by hypotonic lysis in 1mM 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).

2.3. Lipolysis assays

Lipolysis assays were performed on adipose tissue obtained from rhesus monkeys following surgical biopsy of subcutaneous adipose depots. Tissue samples were promptly transported to the laboratory in Krebs–Ringer solution containing 4% fatty acid-free bovine serum albumin at 37°C. Adipose cells were harvested from the tissue after digestion with collagenase D as described by Rodbell (1964). Briefly, the tissues were incubated with shaking at 37°C under a CO_2 atmosphere with collagenase D in 4% fatty acid-free bovine serum albumin, 0.2 mM 2-hydroxy-2,4,6 cycloheptatrienone, in Krebs–Ringers buffer for 20–45 min. The cells were segregated from undigested tissue by passing through nylon mesh. Fat cells were collected by allowing them to float to the surface while fibroblasts and stromal cells were discarded with the pellet. Adipocytes were washed free of the collagenase. The lipolysis assay was initiated by the addition of 50–100 μl of packed cells to wells containing the test compound in lipolysis buffer (Krebs–Ringers, 2% fatty acid-free bovine serum albumin, 1 mM ascorbic acid). The incubation mixture was shaken gently in a 37°C incubator under 5% CO_2 atmosphere for 2 h. The assay was terminated by low speed centrifugation to float the cells, and the infranatant collected for glycerol determination. The glycerol content of the samples was determined utilizing Sigma kit 337A according to manufacturer's instructions.

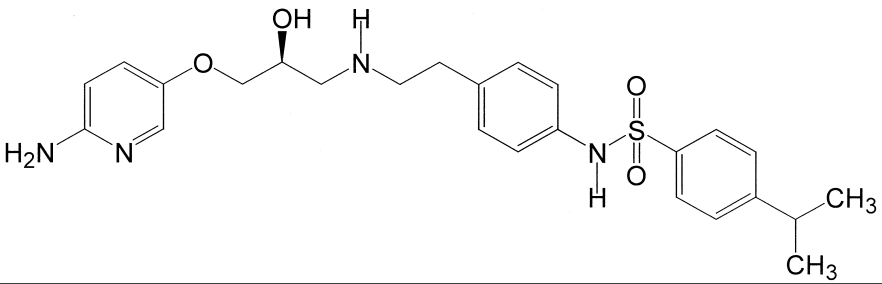
2.4. Measurement of compound potency and efficacy in vivo

All animal procedures were reviewed and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Male rhesus monkeys (4–7 kg, body weight) were fasted for 16–24 h and were lightly anesthetized with ketamine (10 mg/kg, i.m.). A 22-gauge angiocatheter was placed in a saphenous vein for the administration of test compounds after which the animals were administered Nembutal (25 mg/kg, i.v.). A 20-gauge angiocatheter was introduced into a femoral artery and subsequently connected to a Statham pressure transducer for monitoring arterial blood pressure. Blood samples were also taken from the femoral artery. Electrocardiogram (ECG) leads were connected for the continuous measurement of heart rate, which together with blood pressure were monitored for approximately 30 min until stable baseline values were obtained, at which time animals were administered either propranolol (0.3 mg/kg, i.v.) or an equivalent volume of saline. After a new stable baseline had been achieved (approximately 10 min) animals were administered a series of rising dose infusions (0.1 ml/min) of agonists (isoproterenol, salbutamol, L-750355) or an equivalent volume of vehicle (ethanol/polyethylene glycol 400/saline, 20:60:20, v/v/v) over a 15-min period. Infusion periods were separated by approximately 1 min. Blood samples (2 ml) were collected from the femoral artery into heparin 1 min prior to propranolol or saline administration, 1 min prior to the initiation of compound or vehicle infusions and 14 min into each infusion period.

2.5. Measurement of plasma glycerol and potassium

Plasma glycerol was measured using an enzymatic colorimetric assay and potassium was determined by flame photometry via a Boehringer Mannheim Hitachi 911 clinical chemistry analyzer. Glycerol levels were determined using a Triglyceride (GPO-TRINDER) kit obtained from Sigma Diagnostics (St Louis, MO). Plasma samples or standards (20 μl) were placed in polystyrene cuvettes. One milliliter of Triglyceride Reagent A which contained ATP (0.375 mmol/l), magnesium salt (3.75 mmol/l), 4 aminoantipyrine (0.188 mmol/l), sodium-*N*-ethyl-*N*-(3-sulfopropyl)-*m*-anisidine (2.11 mmol/l), glycerol kinase (1250U/l), glycerol phosphate oxidase (2500 U/l), peroxidase (horseradish, 2500 U/l), and sodium azide (0.05%) added as a preservative, was added to the cuvette. After 10 min at room temperature, the cuvettes were read at 540 nm in a Beckman spectrophotometer. A standard curve was generated using glycerol (Boehringer Mannheim Biochem-

Table 1
Potency and selectivity of L-750355 at human and rhesus adrenoceptors



Species		β3			β1			β2		
		EC_{50}[a]	% act[b]	IC_{50}	EC_{50}	% act	IC_{50}	EC_{50}	% act	IC_{50}
Human	Mean	10	49	49	3175	33	3180	> 10,000	3.7	526
	S.E.M.	1.7	5.4	2.4	680	2.6	783		0.5	29.7
	n	5	5	5	4	4	5	3	3	5
Rhesus	Mean	28	34	69	> 10,000	13	820	> 10,000	0	508
	S.E.M.	6.1	4.6	18.2			41			130
	n	3	3	3	1	1	3		1	4

[a] EC_{50} and IC_{50} values are in nM.
[b] Adenyl cyclase activity expressed as a percentage relative to maximal activation achieved with isoproterenol.

icals). The data was generated by linear regression analysis using the protocol in Microsoft Excel.

3. Results

3.1. Effects of L-750355 on binding and activation of adrenergic receptors in vitro

In CHO cells transfected with the human or rhesus ß3 adrenergic receptors, L-750355 is a moderately potent (EC_{50} = 10 and 28 nM, respectively) partial agonist (% maximal receptor activation = 49% and 34%, respectively) for stimulation of adenylyl cyclase (Table 1). The inhibition by L-750355, for binding of [^{125}I]-cyanopindolol to CHO cells is observed in the micromolar range for cells transfected with human or rhesus ß1-adrenoceptors (3.2 and 0.82 μM, respectively) or for cells transfected with human or rhesus ß2-adrenoceptors (0.53 and 0.51 μM, respectively). L-750355 exhibited no agonist activity at human or rhesus ß1- or ß2-adrenoceptors aside from weak (EC_{50} = 3.2 μM) partial agonist activity (% maximal re-

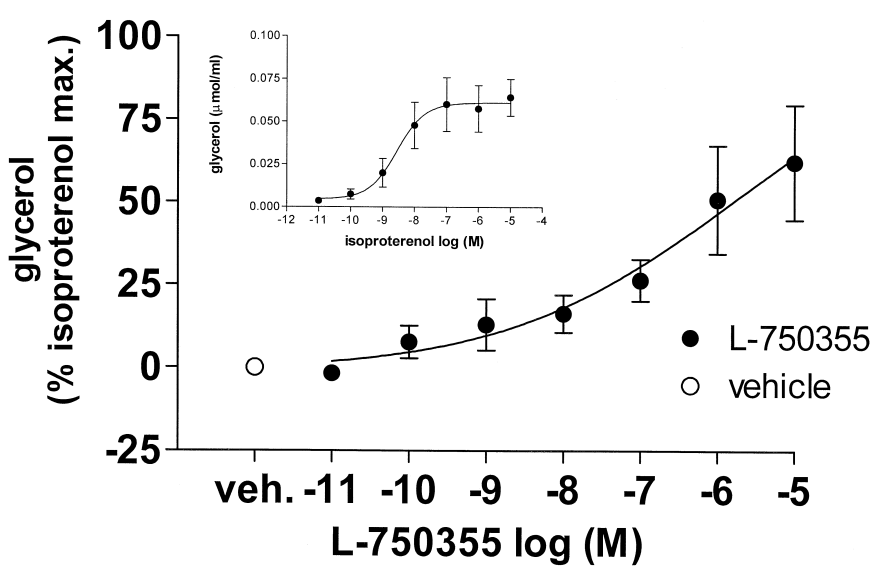


Fig. 1. Effects of L-750355 and isoproterenol (inset) on glycerol release from rhesus adipocytes in vitro. Adipose tissue was obtained from anesthetized rhesus monkeys by surgical biopsy. Adipocytes were isolated by collagenase digestion and incubated with buffer, isoproterenol or L-750355 at the concentrations shown for 2 h at 37°C. Glycerol levels were determined by an enzymatic colorimetric assay. Each data point represents the mean of triplicate determinations and vertical lines the standard error of the mean.

ceptor activation = 33%) at the cloned human ß1-adrenoceptor. With respect to lipolysis, incubation of L-750355 with rhesus adipocytes evoked a concentration dependent production of glycerol (Fig. 1) with an estimated EC_{50} for lipolysis of approximately 100 nM. The EC_{50} for lipolysis is similar to the EC_{50} determined for stimulation of cAMP production in ß3-adrenoceptor transfected CHO cells (28 nM, Table 1). Furthermore, in agreement with partial agonist activity observed in CHO cells, the maximal stimulation of lipolysis by L-750355 was approximately 60% of that obtained with a maximal concentration of isoproterenol (100 nM).

3.2. Effects of L-750355 on lipolysis, cardiovascular function and plasma potassium levels in rhesus monkeys

The intravenous infusion of isoproterenol (5 ng/kg–15 μg/kg) or the ß3-adrenoceptor agonist L-750355 (30 μg/kg–30 mg/kg) produced dose-dependent increases in plasma glycerol and heart rate (Fig. 2) with no significant effect on mean arterial pressure (data not shown). The extent of the increases in plasma glycerol and heart rate produced by L-750355 were less, approximately 80% and 30%, respectively, than that evoked by a maximal dose of isoproterenol, suggesting partial agonist effects. Partial agonist activity induced by L-750355 was also observed at human ß1 and ß3 receptors in in vitro assays (Table 1). The dose–response curve for isoproterenol-induced tachycardia (ß1-adrenoceptor-mediated effect) lies to the left of that for stimulation of lipolysis (ß3-adrenoceptor-mediated effect). In contrast, the dose–response curve for L-750355-induced tachycardia lies to the right of that for stimulation of lipolysis. These data are consistent with the greater potency of isoproterenol for activation of ß1-adren-

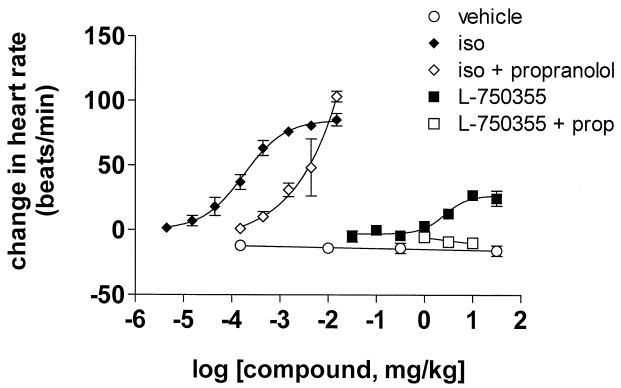


Fig. 3. Effects of propranolol on isoproterenol- and L-750355-induced heart rate changes in the anesthetized rhesus. Anesthetized rhesus monkeys were administered either propranolol (0.3 mg/kg, i.v.) or saline prior to rising dose intravenous infusions (0.1 ml/min over a 15-min period) of isoproterenol (5 ng/kg–15 μg/kg), the human ß3-adrenoceptor agonist L-750355 (30 μg/kg–30 mg/kg) or vehicle (ethanol/polyethylene glycol 400/saline, 20:60:20, v/v/v). Heart rate was continuously monitored via ECG tracings. Each point represents the mean of determinations in two to five animals and the vertical bars the standard error of the mean.

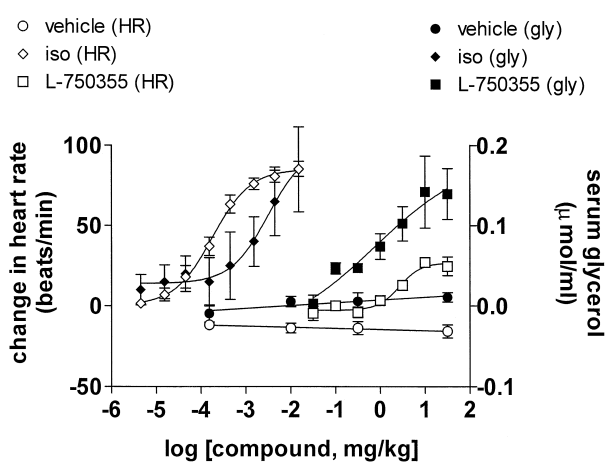


Fig. 2. Effects of isoproterenol and L-750355 on heart rate and lipolysis in anesthetized rhesus monkeys. Isoproterenol (5 ng/kg–15 μg/kg), the human ß3-adrenoceptor agonist L-750355 (30 μg/kg–30 mg/kg) or vehicle (ethanol/polyethylene glycol 400/saline, 20:60:20, v/v/v) were administered as a series of rising dose intravenous infusions each of 15 min duration (vehicle was administered as a series of four infusions of 15 min duration each). Heart rate was monitored continuously from ECG leads and glycerol was measured by an enzymatic colorimetric assay on an arterial blood sample collected 1 min prior to the conclusion of each infusion. Each point represents the mean of determinations in two to five animals and the vertical bars the standard error of the mean.

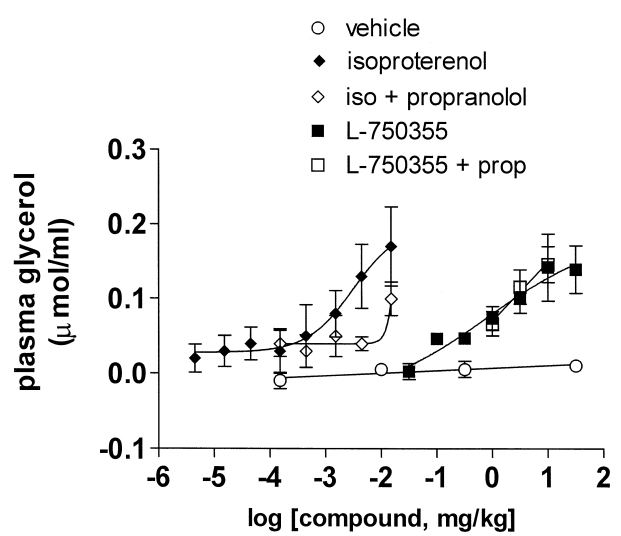


Fig. 4. Effects of propranolol on isoproterenol- and L-750355-induced increases in plasma glycerol in the anesthetized rhesus. Anesthetized rhesus monkeys were administered either propranolol (0.3 mg/kg, i.v.) or saline prior to rising dose intravenous infusions (0.1 ml/min over a 15-min period) of isoproterenol (5 ng/kg–15 μg/kg). the human ß3-adrenoceptor agonist L-750355 (30 μg/kg–30 mg/kg) or vehicle (ethanol/polyethylene glycol 400/saline, 20:60:20, v/v/v). Blood samples for the determination of plasma glycerol were obtained from the femoral vein 14 min after the start of each infusion period. Each point represents the mean of determinations in two to five animals and the vertical bars the standard error of the mean.

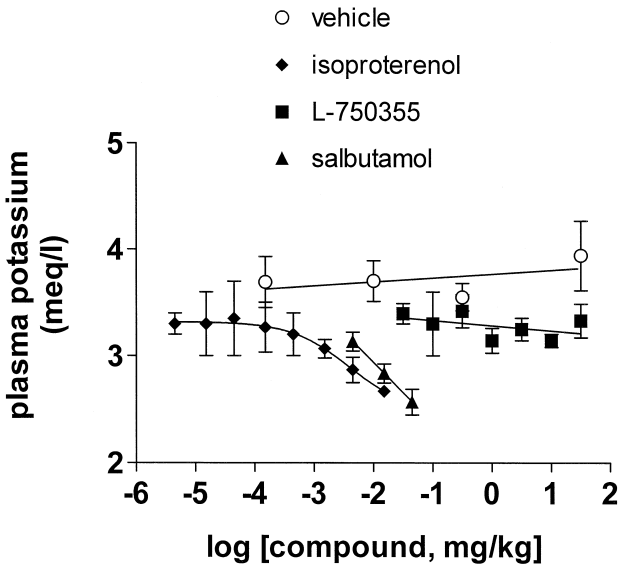


Fig. 5. Effects of ß-adrenoceptor agonists on changes in plasma potassium in the anesthetized rhesus. Anesthetized rhesus monkeys were administered rising dose intravenous infusions (0.1 ml/min over a 15-min period) of isoproterenol (5 ng/kg–15 μg/kg), salbutamol (4.5–45 μg/kg), the human ß3-adrenoceptor agonist L-750355 (30 μg/kg–30 mg/kg) or vehicle (ethanol/polyethylene glycol 400/saline, 20:60:20, v/v/v). Blood samples for the determination of plasma potassium, using flame photometry, were obtained from the femoral vein 14 min after the start of each infusion period. Each point represents the mean of determinations in two to five animals and the vertical bars the standard error of the mean.

oceptors versus ß3-adrenoceptors and of L-750355 for activation of ß3-adrenoceptors versus ß1-adrenoceptors receptors.

In the presence of propranolol (0.3 mg/kg, i.v.) the effects of isoproterenol were attenuated, and the dose–response curves for isoproterenol-induced tachycardia (Fig. 3) and glycerolemia (Fig. 4) were shifted to the right by approximately 1 log unit. L-750355-induced tachycardia was also abolished in the presence of propranolol. However, L-750355-induced glycerolemia was unchanged in the presence and absence of propranolol.

The intravenous infusion of isoproterenol (5 ng/kg–15 μg/kg) or salbutamol (4.5–45 μg/kg) produced dose-dependent hypokalemia (ß2 effect) whereas L-750355 (30 μg/kg–30 mg/kg) produced no significant change in plasma potassium (Fig. 5). Hypokalemia evoked by salbutamol was abolished in animals that received propranolol (0.3 mg/kg, data not shown).

4. Discussion

Using cloned human and rhesus ß-adrenoceptors expressed in CHO cells as screening tools, we identified L-750355 as a potent and selective ß3-adrenoceptor partial agonist that stimulates lipolysis in isolated human and rhesus adipocytes in vitro. Furthermore, following infusion to anesthetized rhesus monkeys, L-750355 evokes glycerolemia and at higher doses tachycardia with no effects on mean arterial pressure or plasma potassium levels. The studies described in anesthetized rhesus provide an effective and relatively simple method for the in vivo assessment of the potency, efficacy and selectivity of novel ß3-adrenoceptor agonists.

Lipolysis and increased energy expenditure in adipocytes can be achieved via activation of ß1-, ß2- or ß3-adrenoceptors (Himms-Hagen, 1992). However, the clinical utility of ß-adrenoceptor agonists as therapeutic agents for the treatment of obesity via an increase in energy expenditure, would be compromised by unacceptable side-effects if compounds do not have appropriate selectivity for activation of ß3- versus ß1- or ß2-adrenoceptors. This contention was evident from previous studies with ß-adrenoceptor agonists in humans which produced tremors and tachycardia, presumably via stimulation of ß2- and ß1-adrenoceptors, respectively (Connacher et al., 1988; Wheeldon et al., 1994). Accordingly, our strategy for identification of ß3-adrenoceptor agonists has been to screen for compounds that activate cloned and expressed human ß3-adrenoceptors and have diminished activity for binding to and activation of human ß1- and ß2-adrenoceptors. Using this strategy, we have identified L-750355 as a potent and selective ß3-adrenoceptor agonist that preferentially stimulates lipolysis over tachycardia when administered to anesthetized rhesus monkeys.

Although a compound's profile of activity at individually cloned and expressed receptors is readily achieved in vitro, it is critical that a compound's profile of activity be defined in vivo under conditions where receptors are in their native state and expression level. This we have achieved in the anesthetized rhesus monkey using tachycardia as a read-out for ß1-adrenoceptor activation, hypokalemia as a read-out for ß2-adrenoceptor activation, and glycerolemia as a read-out for ß3-adrenoceptor activation. That ß3-adrenoceptor agonist-induced changes in plasma glycerol can be dissociated from ß1- or ß2-adrenoceptor-mediated effects in the rhesus in vivo is suggested by the following observations. (1) Isoproterenol evokes tachycardia (ß1-adrenoceptor mediated effect) at doses which do not evoke hyperglycerolemia whereas L-750355 evokes hyperglycerolemia (presumed ß3-adrenoceptor mediated effect) at doses which do not evoke tachycardia. (2) The ß1- and ß2-adrenoceptor selective antagonist propranolol (0.3 mg/kg, i.v.) produced a shift to the right of approximately 10-fold in the dose–response curves for isoproterenol-induced tachycardia and hyperglycerolemia and abolished salbutamol-induced hypokalemia. This dose of propranolol also abolished the L-750355-induced tachycardia but had no effect on the L-750355-induced glycerolemia. (3) At doses of L-750355 which produce glycerolemia, there is no indication of hypokalemia indicating an absence of ß2-adrenoceptor activation by L-750355.

Propranolol completely inhibited L-750355-induced tachycardia but did not reduce L-750355-induced lipolysis, suggesting that the increase in heart rate was mediated via activation of ß1-adrenoceptors. This is something of a paradox given that the dose, and hence presumed concentration, of L-750355 required to increase heart rate is approximately 10-fold greater than that required for stimulation of lipolysis, whereas in vitro, L-750355 is >350-fold selective for activation of rhesus ß3- versus ß1-adrenoceptors. Furthermore, studies in rhesus, with other human selective ß3-adrenoceptor agonists, determined that the kinetics of ß3-adrenoceptor agonist-induced tachycardia are slower than those for isoproterenol-induced tachycardia (Fisher et al., 1998). Together, these observations suggest that ß3-adrenoceptor agonist-induced tachycardia may not be mediated via direct activation of cardiac ß1-adrenoceptors. Additional studies, to be described elsewhere, using selective human ß3-adrenoceptor agonists in anesthetized rhesus monkeys established that tachycardia evoked by ß3-adrenoceptor agonists is reflexogenic in origin, consequent upon evoked increases in metabolic rate and direct ß3-adrenoceptor mediated peripheral vasodilatation.

In summary, these data in barbiturate anesthetized rhesus monkeys indicate that isoproterenol-evoked tachycardia and glycerolemia and L-750355 evoked tachycardia are mediated proximally via activation of ß1-adrenoceptors, whereas L-750355 evoked glycerolemia in the rhesus is mediated via activation of ß3-adrenoceptors. Furthermore, the anesthetized rhesus monkey provides a relatively simple assay system for reporting indices of potency, efficacy and receptor specificity of compounds in a species wherein the receptor pharmacology is similar to that of man.

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