β_3 Receptors: role in cardiometabolic disorders

Ther Adv Endocrinol Metab
(2011) 2(2) 65–79
DOI: 10.1177/2042018810390259

Shraddha V. Bhadada, Bhoomika M. Patel, Anita A. Mehta and Ramesh K. Goyal

***Abstract*:** Pharmacological and molecular approaches have shown that an atypical β-adrenoceptor (AR), called β_3-AR, that is distinct from β_1-ARs and β_2-ARs, exists in some tissues in heterogeneous populations such as β_{3a}-ARs and β_{3b}-ARs. β_3-ARs belong to a superfamily of receptors linked to guanine nucleotide binding proteins (G proteins). The β_3-AR gene contains two introns whereas the β_1-AR and β_2-AR genes are intronless, leading to splice variants. β_3-ARs can couple to G_i and G_s and they are reported to be present in brown adipose tissue, vasculature, the heart, among other tissues. β_3-ARs cause vasodilation of microvessels in the islets of Langerhans and may participate in the pathogenesis of cardiac failure, during which modification of β_1-AR and β_2-AR expression occurs. The development of β_3-AR agonists has led to the elaboration of promising new drugs, including antiobesity and antidiabetic drugs. This article reviews the various pharmacological actions of β_3-ARs and their clinical implications for diabetes and cardiovascular diseases.

***Keywords*:** β_3-adrenoceptors, antidiabetic, vascular smooth muscles

Introduction

The pressor effect of adrenal extracts was first shown by Oliver and Schafer in 1895. The active principle was named epinephrine by Abel in 1899. The existence of more than one adrenergic receptor was first proposed by Ahlquist in 1948. He proposed the terms α and β for receptors on smooth muscle where catecholamines produce excitatory and inhibitory responses respectively. Almost 50 years after Ahlquist first discovered evidence of the heterogeneity of adrenergic receptors [Ahlquist, 1948], the number of receptor subtypes is still unclear. β-Adrenoceptors (β-ARs) were later subdivided into β_1 and β_2, which are present in the myocardium and smooth muscle respectively. Pindolol, a nonselective β-AR antagonist with significant agonist activity, was found to cause relaxation of canine-isolated perfused mesenteric vessels [Clark and Bertholet, 1983] and rat aorta precontracted with potassium chloride [Doggrell, 1990]. In both instances, the vasorelaxant effect of pindolol was not significantly antagonized by propranolol, suggesting the presence of a β-AR subtype different from the conventional β_1-ARs and β_2-ARs. The effect of isoprenaline was ascribed not only to activation of β_1-ARs and β_2-ARs, but also to that of an additional adrenoceptor [Doggrell, 1990; Clark and Bertholet, 1983]. Later on, the existence of a third β-AR came into light and Gauthier *et al.* [1996] found that stimulation of β_3-AR in human cardiac muscle, in contrast with β_1- and β_2-AR stimulation, resulted in a profound dose-dependent negative inotropic effect and hence suggested the participation of β_3-AR in the pathogenesis of cardiac failure. Moreover, various *in vivo* studies have also demonstrated that positive β_3-AR-related chronotropic effects were prevented by β_1- or β_2-AR antagonists and are likely due to baroreflex activation in response to β_3-adrenoceptor agonist- induced vasodilation [Wheeldon *et al.* 1994; Takayama *et al.* 1993; Wheeldon *et al.* 1993; Tavernier *et al* 1992].

Studies using molecular and biochemical techniques are likely to provide additional new and unexpected insights into the role of AR subtypes in both normal physiologic functions and diseases. Initially the presence of β_3-ARs was demonstrated in vasculature and heart, but later they were shown in adipocytes. β_3-ARs mediate lipolysis in white adipose tissues and thermogenesis in brown adipose tissues [Lönnqvist *et al.* 1993; Langin

Correspondence to:
Dr Ramesh K. Goyal, MSc(Medical), PhD(Pharmacy), F.I.C., FAMS, FICN, FIPS, FIACS, FNASc
Vice Chancellor, MS University of Baroda, Vadodara, Gujarat, India
goyalrkf@rediffmail.com

Shraddha V. Bhadada, MPharm
Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Bhoomika M. Patel, MPharm, PhD, D.P.M.M., D.P.Q.C.Q.A.M.
Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Anita A. Mehta, MPharm, PhD, MAMS
Department of Pharmacology, LM College of Pharmacy, Ahmedabad, Gujarat, India

et al. 1991; Zaagsma and Nahorski, 1990]. β_3-ARs represent a heterogeneous population, such as β_{3a}-ARs and β_{3b}-ARs, as suggested by the studies in Chinese hamster ovary cells. Furthermore, the activation and signal transduction of such a β_{3a}-AR and β_{3b}-AR complex may prevent the full potency of the β_3-AR agonist [Hutchinson *et al.* 2002]. The regulation of adrenergic receptors by receptor-specific agonists and antagonists has been actively studied for many years and is important clinically because alterations in these receptors have been suspected in many pathological states. The development of β_3-AR agonists has led to the elaboration of promising new drugs and they are a target for antiobesity and antidiabetic drugs [Lowell and Flier, 1995; Pietri-Rouxel and Strosberg, 1995]. Although considerable information is available on β_3-AR physiology in fat, there are many other areas in which β_3-ARs are involved. The existence of an atypical β-AR, called β_3-AR, distinct from β_1-ARs and β_2-ARs, has been demonstrated in various tissues by pharmacological [Berlan *et al.* 1993; Holloway *et al.* 1992; Tavernier *et al.* 1992; Langin *et al.* 1991; Mc Laughlin and MacDonald, 1990; Hollenga and Zaagsma, 1989; Bojanic *et al.* 1985] and molecular approaches [Granneman *et al.* 1991; Muzzin *et al.* 1991; Tate *et al.* 1991; Emorine *et al.* 1989]. The overview of location of β_3 receptors is given in Table 1. This review aims to provide an overview of the presence of β_3-ARs in various tissues, with special emphasis on the clinical implications for diabetes and cardiovascular diseases.

Molecular structure and signal transduction mechanism of β receptors

ARs are members of a large superfamily of receptors linked to guanine nucleotide binding proteins (G proteins). All G-protein coupled receptors share structural features, such as extracellular amino terminals with sites for *N*-linked glycosylation, seven α-helical domains that span the plasma membrane, and intracellular carboxy terminals containing amino acid sequences that indicate probable sites of phosphorylation by one or more protein kinases. The G proteins, linked to adrenergic receptors, are heterotrimeric proteins with α, β, and γ subunits. Each subunit is part of a family consisting of multiple members [Simon *et al.* 1991]: approximately 20 α subunits that have been divided into four subfamilies – α_s, α_i, α_q and α_{12}; at least five β subunits (β_{1-5}); and at least six γ subunits (γ_{1-6}). Although several hundred different subunit combinations (heterotrimers) are theoretically possible, the repertoire of G proteins used by a particular receptor system is limited [Hescheler and Schultz, 1994]. Each type of G protein can be used for signaling by more than one type of receptor. β_1, β_2 and β_3 share approximately 60% amino acid sequence identity within the presumed membrane spanning domains. The β_3-AR gene contains two introns [Lelias *et al.* 1993; Granneman *et al.* 1992] in contrast to β_1-AR and β_2-AR genes, which are intronless. This structure leads to splice variants. The B and C isoforms contain 12 and six additional amino acids, respectively, at their C terminus in comparison with the A isoform [Lelias *et al.* 1993; Granneman *et al.* 1992]. In rat adipocytes, a unique isoform is expressed that is close to the B isoform, whereas in human brown adipocytes, the C isoform is predominant [Lelias *et al.* 1993; Van Spronsen *et al.* 1993]. It was hypothesized that the physiological response to β_3-AR stimulation differs depending on the isoform expressed in a given species [Levasseur *et al.* 1995]. The human β_3-AR exists in at least two different agonist conformations with a similar high-affinity and low-affinity pharmacology analogous to β_1-AR. Both conformations are present in living cells and can be distinguished by their pharmacological characteristics [Baker, 2005].

All β-AR subtypes signal by coupling to the stimulatory G protein $G_{\alpha s}$, leading to activation of adenyl cyclase and accumulation of the second messenger cAMP [Emorine *et al.* 1989; Frielle *et al.* 1987; Dixon *et al.* 1986]. G_s can directly enhance the activation of voltage-sensitive Ca^{2+} channels in the plasma membrane of skeletal and cardiac muscle. However, some recent studies indicate that, under certain circumstances, β_3-AR can couple to G_i as well as to G_s [Gauthier *et al.* 1996; Xiao *et al.* 1995; Chaudry *et al.* 1994]. Multiple mechanisms control the signaling and density of G-protein-coupled receptors. Catecholamines, which are hydrophilic, do not bind to the highly charged extracellular domains of the receptors, as might be expected, but bind in the more hydrophobic membrane-spanning domains [Caron and Lefkowitz, 1993; Jasper and Insel, 1992].

On the basis of many pharmacological and molecular studies, the existence of a fourth β-AR subtype was postulated [Brodde and Michel, 1999; Galitzky *et al.* 1998; Strosberg *et al.* 1998; Kaumann, 1997; Strosberg, 1997;

Summers *et al.* 1997; Strosberg and Pietri-Rouxel, 1996; Barnes, 1995; Arch and Kaumann, 1993]. To date, at least nine subtypes of adrenergic receptors (three subtypes each of α_1-ARs, α_2-ARs, and β-ARs) have been identified. The precise function of all these receptors has not yet been defined, in part because of a dearth of highly specific agonists and antagonists. An alternative way to examine receptor function is to use molecular genetic techniques to overexpress or to knock out the expression of particular subtypes in laboratory animals [Milano *et al.* 1994a, 1994b; Bertin *et al.* 1993].

Pharmacological actions

Adipose tissue and diabetes

β_3-ARs mediate lipolysis in white adipose tissues and thermogenesis in brown adipose tissues [Lönnqvist *et al.* 1993; Langin *et al.* 1991; Zaagsma and Nahorski, 1990]. The presence of the Arg64 allele in the first intracellular loop of the β_3-AR gene may predispose patients to abdominal obesity, which may in turn predispose them to insulin resistance and the earlier onset of type 2 diabetes mellitus (T2DM) [Widén *et al.* 1995]. A naturally occurring variation, $Trp^{64}Arg$ β_3-AR mutation, found in about 8% of Europeans and North Americans, actually restores the arginine residue in humans, which is found present in animals [Strosberg, 1997]. This variation was found to be associated with the following:

1. An increased capacity of obese French patients to gain weight [Clément *et al.* 1995].
2. An early onset of T2DM in obese Pima Indians by altering the balance of energy metabolism in visceral adipose tissue and tend to have a lower resting metabolic rate [Walston *et al.* 1995]. Similar observations were also reported in Japanese participants [Fujisawa *et al.* 1996].
3. An early onset of T2DM and clinical features of the insulin resistance syndrome in Finns [Widén *et al.* 1995].

The $Trp^{64}Arg$ β_3-AR genotype is associated with mild gestational diabetes and this polymorphism is associated with increased weight gain during pregnancy [Festa *et al.* 1999]. The increased amount of adipose tissue after menopause is considered to elevate estradiol production, which in turn increases the risk for breast cancer. Thus, genetic traits that are related to obesity may influence the risk of postmenopausal breast cancer in an indirect manner [Huang *et al.* 2001]. A missense mutation in codon 64 of the β_3-AR gene that results in substitution of tryptophan by arginine [$Trp^{64}\rightarrow$Arg] in the first intracellular loop of the receptor protein has been reported in various ethnic groups, including the Japanese [Kadowaki *et al.* 1995]. A review [Arner and Hoffstedt, 1999] identified a link between obesity and the Trp64→Arg polymorphism in 13 studies. Also a polymorphism in codon 27 of the ADRβ_2 gene that features a replacement of glutamine by glutamic acid [Gln27→Glu] is linked with obesity [Large *et al.* 1997]. Thus, β_3-ARs may constitute a target for antiobesity and antidiabetic drugs [Lowell and Flier, 1995; Pietri-Rouxel and Strosberg, 1995].

BRL 26830 (see Table 2), a selective β_3-AR agonist, caused a marked increase in blood flow to brown adipose tissue in the anesthetized rat [Takahashi *et al.* 1992]. The increase in blood flow may well be secondary to an augmented metabolic process [Shen and Claus, 1993], since BRL 37344 (see Table 2) causes marked increases in the plasma levels of free fatty acids and insulin. *In vitro* studies have demonstrated that in rat [Granneman, 1992] and dog [Galitzky *et al.* 1993] fat cells, catecholamines stimulate β_3-ARs at higher concentrations than those required to activate β_1-ARs or β_2-ARs. Similar results were demonstrated in dog *in vivo* studies [Pelat *et al.* 2003]. Also, BRL 26830A causes stimulation of insulin secretion in pancreas β cells [Yoshida *et al.* 1991].

In diabetic ZDF rats, CL 316243, a β_3 selective agonist (see Table 2) did not reduce hyperglycemia when given under acute conditions (single intravenous injections or subcutaneous infusions for a few days). However, long-term treatment of CL 316243 progressively normalized glycemia, reduced insulinemia and decreased the levels of circulating free fatty acids in obese diabetic ZDF rats. This treatment also markedly improved their glucose and insulin responses during an intravenous glucose tolerance test [Liu *et al.* 1998]. Hyperinsulinemic-euglycemic clamps combined with the [2-^{3}H]deoxyglucose method revealed that chronic CL 316243 treatment markedly increased insulin responsiveness in obese rats and that it increases glucose uptake in brown adipose tissue, white adipose tissue, the diaphragm, and skeletal muscles, but not in the heart. The maximal capacity of various tissues for glucose

Table 1. Location of β_3-adrenoceptors.

Organ	Species	Reference
Heart		
Atria	Human	Krief *et al.* (1993); Berkowitz *et al.* (1995)
Ventricle	Human	Gauthier *et al.* (1999)
Vascular smooth muscles		
Veins		Viard *et al.* (2000)
Cutaneous vascular smooth muscles	Canine	Berlan *et al.* (1994)
Vasculature		Tavernier *et al.* (1992); Shen *et al.* (1994); Rohrer *et al.* (1999)
Thoracic aorta	Rat	Trochu *et al.* (1999)
Internal mammary artery	Human	Rozec *et al.* (2005)
Non-vascular smooth muscles		
Gastrointestinal tract, brain, and prostate		Granneman *et al.* (1991); Emorine *et al.* (1989); Bensaid *et al.* (1993); Rodriguez *et al.* (1995)
Ileum	Rat	Roberts *et al.* (1995); Roberts *et al.* (1999)
Rectum and IAS membranes	Western blot studies	Rathi *et al.* (2003)
Urinary tract		Tomiyama *et al.* (1998)
Near-term myometrium	Human	Bardou *et al.* (2000)
Brown adipose tissues		Emorine *et al.* (1994)

Table 2. Agonists and antagonists of β_3-adrenoceptors (ARs).

β_3-Agonist	Chemical name
BRL 26830A	methyl 4-[2-[[2-hydroxy-2-phenethyl]amino]propyl]benzoate-2-butanedioate
BRL 35135	methyl 4-[2-[2-hydroxy-2-[3-chlorophenyl]ethylamino] propyl] phenoxyacetate
BRL 37344	4-[-[[2-hydroxy-[3-chlorophenyl] ethyl]- amino] propyl] phenoxyacetate
CGP 12177	4-[3-t-butylamino-2-hydroxypropoxy]benzimidazol-2-one
CGP 20712A	2-hydroxy-5-[2-[{2-hydroxy-3-[4-[1-methyl-4-trifluoromethyl-2-imidazolyl]phenoxy]propyl}amino]ethoxy] benzamide
CL 316243	disodium[*R*,*R*]-5-[2[[2-[3-chlorophenyl]-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate
FR-149175	ethyl-[[S]-8-[[R]-2-[3-chlorophenyl]-2-hydroxyethylamino]-6,7,8,9-tetrahydro-5H-benzocyclohepten-2-yloxy]acetate monohydrochloride monohydrate
ICI 198157	methyl [4-[2-[[2-hydroxy-3-phenoxypropyl]amino]ethoxy]phenoxy]acetate
ICI D7114	[S]-4-[2-hydroxy-3-phenoxypropylaminoethoxy]-N-[2-methoxyethyl]phenoxyacetamide
L 742,791	[S]-N-[4-[2-{[3-[4-hydroxyphenoxy]-2-hydroxypropyl]amino}ethyl]phenyl]-4-iodobenzenesulfonamide
L 750355	3-pyridyloxypropanoloamine derivative
L755,507	4-[[(Hexylamino)carbonyl]amino]-*N*-[4-[2-[[(2*S*)-2-hydroxy-3-(4-hydroxyphenoxy) propyl] amino]ethyl]phenyl]-benzenesulfonamide
L 770,644	[*R*]-4-[4-[3-cyclopentylpropyl]-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl]-*N*-[4-[2-[[2-hydroxy-2-[3-pyridinyl]ethyl]amino] ethyl]phenyl]- benzenesulfonamide
L 796568	[R]-N -[4-[2-[[2-hydroxy-2-[3-pyridinyl]ethyl]amino]ethyl]-phenyl]-4-[4-[4-[trifluoromethyl]phenyl]thiazol-2-yl]-benzenesulfonamide, dihydrochloride
LY 79771	[R-[R*,S*]] alpha-[[[3-[4-hydroxyphenyl]-1-methylpropyl]amino]methyl]benzenemethanol
RO363	[-]-1-[3,4-dimethoxyphenethylamino]-3-[3,4-dihdroxyphenoxy]-2-propanol] oxalate
SB 251023	[4-[1-{2-[S]-hydroxy-3-[4-hydroxyphenoxy]-propylamino} cyclopentyl methyl]phenoxymethyl]phenylphosphonic acid lithium salt
SR 58611	[RS]-*N*-[[25]-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronapth-2-yl]-[2R]-2-[3-chlorophenyl]-2-hydroxyethanamine hydrochloride
SR 58611A	[N2S]-7-carbethoxymethoxy-1,2,3,4-tetrahydronaphth-2-yl-[2R]-2-hydroxy-2-chlorophenyl ethanamine hydrochloride
SR 59104A	N-[[6-hydroxy-1,2,3,4-tetrahydronaphthalen-[2R]-2-yl]methyl]-[2R]-2-hydroxy-2-[3-chlorophenyl]ethanamine hydrochloride
SR 59119A	N-[[7-methoxy-1,2,3,4-tetrahydronaphthalen-[2R]-2-yl]methyl]-[2R]-2-hydroxy-2-[3-chlorophenyl]ethanamine hydrochloride
ZD2079	4-[2-[[(2*R*)-2-Hydroxy-2-phenylethyl] amino]ethoxy]-benzeneacetic acid hydrochloride
ZD 7114	[*S*]-4-[2-hydroxy-3-phenoxypropylaminoethoxy]-*N*-[2-methoxyethyl]phenoxyacetamide
ZM 215001	[S]-4-[2-hydroxy-3-phenoxy-propylamino-ethoxy] phenoxyacetic acid [*R*]-*N*-[4-[2-[[2-hydroxy-2-[3-pyridinyl]ethyl]amino]ethyl]phenyl]-1-[4-octylthiazol-2-yl]-5-indolinesulfonamide
β_3-Antagonist	Chemical name
L 748328	[S]-N-[4-[2-{[3-[3-{aminosulfonyl}phenoxy]-2-hydroxypropyl]-amino}ethyl] phenyl]benzenesulfonamide
SR 59230A	3-[2-ethylphenoxy]-1[1*S*]-1,2,3,4-tetrahydronaphth-1-ylaminol-[2*S*]2-propanol oxalate

uptake in CL 316243-treated animals varied in the following order: brown adipose tissue > heart > diaphragm > skeletal muscles > white adipose tissue [Liu *et al.* 1998]. This sequence of potencies agrees with previous observations for normal rats treated with insulin [Vallerand *et al.* 1987] or norepinephrine [Liu *et al.* 1994] as well as with cold-exposed animals [Vallerand *et al.* 1990]. Acute treatment of obese rodents with CL 316243 causes a number of diverse metabolic effects, including an increase in oxygen consumption and insulin levels, and a decrease in food intake. The mechanism by which CL 316243 increases insulin secretion in rodents is not known. Nevertheless, the stimulus for insulin secretion is extremely potent as it causes a 50 to 100-fold increase in insulin levels, which remain elevated (24-fold increase) despite the presence of hypoglycemia. This effect cannot be mediated by the direct effects of CL 316243 on pancreatic β cells because pancreatic islets appear not to express detectable levels of β_3-AR mRNA and because insulin secretion is not stimulated following addition of CL 316243 to cultured pancreatic islets [Grujic *et al.* 1997].

In another study by Fu and colleagues [Fu *et al.* 2007], CL 316243 and BRL 37344 downregulated adiponectin, but upregulated adiponectin receptor 2 (not receptor 1) in epididymal and/or subcutaneous white adipose tissue and in brown adipose tissue. Tumor necrosis factor-α (TNF-α) expression was upregulated only in epididymal adipose tissue, which suggests that upregulation of TNF-α and downregulation of adiponectin by β-AR activation may contribute to the pathogenesis of catecholamine-induced insulin resistance, and that upregulation of adiponectin receptor 2 may be a feedback result of reduced adiponectin [Fu *et al.* 2007]. In addition, the effects of CL 316243 were investigated in obese diabetic KKAy mice by Fu and colleagues [Fu *et al.* 2008]. Two weeks' subcutaneous administration of CL 316243 reduced serum levels of glucose, insulin, triglyceride, free fatty acid and TNF-α, and increased adiponectin. CL 316243 recovered the mRNA expressions of adiponectin, adiponectin receptors and β_3-ARs, which were reduced in epididymal white adipose tissue in KKAy mice. Meanwhile, CL 316243 suppressed the overexpressed mRNA level of TNF-α in both epididymal white adipose tissue and brown adipose tissue. These data suggest that the normalization of adiponectin, adiponectin receptors and TNF-α may result in the amelioration of obesity-induced insulin resistance [Fu *et al.* 2008].

β_3-Agonists appear to be of significance not only in obesity but also in terms of the risks of cardiovascular disorders because visceral obesity is the most dangerous form of regional fat accumulation, the form of obesity that is more directly linked to β_3-AR activity [Arner, 1995].

Vascular smooth muscles

β_3-ARs produce sustained peripheral vasodilation that is predominant in skin and fat [Shen *et al.* 1994; Berlan *et al.* 1993]. A study showed that the relaxation of rat thoracic aorta was caused by selective β_3-AR agonists like CGP 12177 [Mohell and Dicker, 1989], cyanopindolol [Engel *et al.* 1981], ZD 2079 [Grant *et al.* 1994], ZM 215001 [Tesfamariam and Allen, 1994], and SR 58611 [Trochu *et al.* 1999] (see Table 2), further supporting the presence of β_3-ARs [Brawley *et al.* 2000a, 2000b]. A β_3-AR-mediated vasorelaxation was also observed in the canine pulmonary artery, an effect that was exerted through a cAMP-dependent pathway [Tagaya *et al.* 1999]. In the rat carotid artery, the selective β_3-AR agonist BRL 37344 and the selective β_2-AR agonist, salbutamol, were not antagonized by propranolol (100 nM), and pretreatment of the artery segments with BRL 37344 did not desensitize the tissue to the relaxant effect of isoprenaline and salbutamol [Oriowo, 1994]. In the same tissue, MacDonald and colleagues [MacDonald *et al.* 1999] confirmed the presence of β_3-AR by the relaxant effects of two selective β_3-AR agonists, BRL 37344 and ZD 2079. In normal dogs, the infusion of BRL 37344 or CL 316243 or CGP 12177 induced an increase in heart rate and cutaneous blood flow (evaluated in the internal part of the ear) [Berlan *et al.* 1994].

The $Trp^{64}Arg$ mutation of β_3-AR has been suggested to confer susceptibility to essential hypertension [Morris *et al.* 1994] and this was confirmed by Tonolo and colleagues [Tonolo *et al.* 1999]. These authors concluded that the $Trp^{64}Arg$ polymorphism of the β_3-AR gene is associated more often with high blood pressure than with normal blood pressure. Isoproterenol, BRL 37344 and CGP 12177 are reported to produce a reduction in arterial blood pressure. In sinoaortic denervated animals, isoproterenol infusion provoked tachycardia and hypotension [Tavernier *et al.* 1992]. It was demonstrated that a higher dose of isoproterenol is required

to obtain *in vivo* β_3-mediated vasodilation than that necessary for β_1- or β_2-mediated vasodilation. The reason for this may be that β_3-AR is a 'back-up' receptor activated during extreme or stressful conditions [Pelat *et al.* 2003].

Significant increases in systolic blood pressure and Doppler stroke distance occurred with BRL 37344 and salbutamol, which were unaffected by pretreatment with bisoprolol and completely blocked by nadolol, in keeping with the β_2-mediated effects. BRL 37344 and salbutamol produced significant chronotropic effects, which were unaffected by β_1-AR blockade [Wheeldon *et al.* 1994]. In a clinical study, isoprenaline produced an increase in systolic blood pressure and left ventricular stroke distance that was not attenuated by a dose of nadolol, which produced complete blunting of β_1- and β_2-mediated responses but not of β_3-mediated effects [Wheeldon *et al.* 1993].

Experimental *in vivo* studies have demonstrated that positive β_3-AR-related chronotropic effects were prevented by β_1-AR or β_2-AR antagonists and are likely due to baroreflex activation in response to β_3-AR agonist-induced vasodilation [Tavernier *et al.* 1992; Takayama *et al.* 1993]. Apart from this, positive chronotropic effects were not observed in denervated animals and thus it was concluded that tachycardia resulted from a baroreceptor-mediated reflex in response to a drop in blood pressure caused by the vasodilating action of β_3-AR agonists [Berlan *et al.* 1994; Shen *et al.* 1994]. In normal dogs, infusion of isoproterenol, BRL 37344 or CGP 12177 increased heart rate with the following order of potency: BRL 37344 > isoproterenol >> CGP 12177. Isoproterenol stimulated adenylate cyclase activity in heart membranes from normal dogs, whereas CGP 12177 and BRL 37344 were without any stimulating action [Tavernier *et al.* 1992]. *In vitro* studies are more suitable in analyzing the cardiac effects of β_3-ARs. A typical example of the masking effects of baroreflex activation lies with 1,4-dihydropyridines, which induce a negative inotropic effect *in vitro*, but a positive chronotropic and inotropic effect *in vivo* as a consequence of vasodilation [Piepho, 1991].

The presence of β_3-ARs has also been reported in veins. In the rat portal vein, activation of β_3-ARs stimulates L-type Ca^{2+} channels through a $G\alpha_s$-induced stimulation of the cyclic AMP/protein kinase, a pathway and the subsequent phosphorylation of the channels [Viard *et al.* 2000]. In rats, the selective β-AR agonist CL 316243 induced marked increases in islet blood flow and plasma insulin level, and these increases were stopped by bupranolol, a β_1-AR, β_2-AR and β_3-AR antagonist, but not by nadolol, a β_1-AR and β_2-AR antagonist, indicating that β_3-ARs caused a vasodilation of microvessels in the islets of Langerhans [Atef *et al.* 1996].

Cardiac effects

β_3-AR stimulation of the human cardiac muscle, in contrast with β_1-AR and β_2-AR stimulation, resulted in a profound dose-dependent negative inotropic effect. This unexpected finding suggests that β_3-ARs may participate in the pathogenesis of cardiac failure, during which modification of β_1-AR and β_2-AR expression occurs [Brodde, 1993]. Functional β_3-ARs stimulation, which occurs in the normal left ventricle, causes direct inhibition on $(Ca^{2+})_{iT}$ and $I_{Ca,L}$(L-type Ca channels) and produces a negative inotropic action [Cheng *et al.* 2001]. In another study, it was found that β_3-AR activation inhibits the L-type Ca^{2+} channel in both normal and heart failure rat myocytes. In heart failure, β_3-AR stimulation-induced inhibition of Ca^{2+} channels is enhanced, which is responsible for reduced inotropic response [Zhang *et al.* 2005]. β_3-AR agonists induce negative inotropic effects with the following order of potency: BRL 37344 > SR 58611 = CL 316243 > CGP 12177, similar to that observed in Chinese hamster ovary cells transfected with human β_3-ARs [Pietri-Rouxel and Strosberg, 1995; Dolan *et al.* 1994]. In another study, the mechanical effects of BRL 37344 were not modified by pretreatment with metoprolol (β_1-AR antagonist) or nadolol, indicating that this effect was not mediated by β_1-ARs or β_2-ARs. By contrast, bupranolol, which possesses β_3-AR antagonist properties [Pietri-Rouxel and Strosberg, 1995; Galitzky *et al.* 1993; Sugasawa *et al.* 1992], antagonized the negative inotropic effects of BRL 37344 with a pA_2 value similar to that determined in adipocytes [Galitzky *et al.* 1998; Pietri-Rouxel and Strosberg, 1995].

In heart failure, increased activity of the sympathetic nervous system leads to downregulation of cardiac β_1-ARs and β_2-ARs [Brodde, 1993] resulting from their phosphorylation by cAMP-dependent protein kinase or β-AR kinase. Reduced β_1-ARs and β_2-ARs lead to a decrease in the contractile response to β-AR agonists

[Strosberg, 1993]. Contrary to β_1-ARs and β_2-ARs, the abundance of the negatively inotropic β_3-ARs increases in the failing myocardium [Moniotte *et al.* 2001]. β_3-ARs lack phosphorylation sites for cAMP-dependent protein kinase or β-AR kinase [Strosberg, 1993], and thus may not be downregulated in heart failure. According to this hypothesis, the high adrenoceptor tone during heart failure may alter the cardiac contractile activity as a result of unmasked β_3-AR stimulation in the presence of reduced β_1-ARs and β_2-ARs [Gauthier *et al.* 1996]. Overstimulation of the relatively desensitization-resistant β_3-AR [Liggett *et al.* 1993] after increased sympathetic tone and norepinephrine release in the setting of heart failure in humans may further decrease cardiac inotropy [Moniotte *et al.* 2001]. The levels of β_3-AR mRNA and proteins show an increase in the failing heart compared with the nonfailing heart. The β_3-AR agonist BRL 37344 was found to markedly aggravate the cardiac function and stimulate cardiac myocytes apoptosis in the failing heart. If the levels of β_3-AR are too high, they might contribute to the loss of cardiac function and be the foundation of the functional degradation of heart failure [Kong *et al.* 2004]. Moreover, another study in isoproterenol-induced chronic heart failure rats suggests that the myocardial upregulation of β_3-AR in heart failure is associated with increased oxidative stress [Kong *et al.* 2010]. These studies open the perspective for correcting the disordered adrenergic regulation of the failing heart with specific antagonists of the human cardiac β_3-AR. By contrast, Rasmussen *et al.* (2009) reported that as increased intracellular myocyte Na+ levels represent a key adverse pathophysiological feature of heart failure, and the β_3-AR mediates the stimulation of the only export route for Na+ – the Na+–K+ pump – the upregulation of this receptor may also represent a useful compensatory mechanism. Data from animal studies and circumstantial observations from clinical trials suggest that β_3-AR activation is beneficial in severe heart failure, and that β_3-AR agonists are a promising therapeutic option for the treatment of this disease (Rasmussen *et al.* 2009). In transgenic mice with cardiac-specific overexpression of protein of the human β_3-AR (TGβ_3 mice), the human β_3-AR is quiescent until stimulated with a selective agonist L 755,507, at which point there is a marked augmentation in left ventricular contractility. In addition, because β3-AR is relatively insensitive to catecholamines, it would be minimally activated by endogenous catecholamines. This approach could have important therapeutic potential in patients with heart failure, in which delivery of the human β3-AR by gene therapy could provide a functionally inactive signaling protein that becomes activated only when a highly selective agonist is exogenously administered [Kohout *et al.* 2001].

In congestive heart failure (CHF), β_3-AR expression is increased. This augmentation is proposed to exacerbate the dysfunctional $[Ca^{2+}]_i$ regulation, enhance inhibition of cardiac contraction and relaxation, and lead to worsening of cardiac failure [Cheng *et al.* 2001]. In CHF, when marked increases in sympathetic tone and cardiac norepinephrine release have rendered the positive inotropic β_1-AR system relatively unresponsive, the upregulated β_3-AR pathways would continue to exhibit a negative inotropic effect. This altered balance between opposing inotropic influences of β_1-ARs and β_3-ARs in CHF may contribute to progressive cardiac dysfunction in CHF. The enhanced response to β_3-AR stimulation in CHF may also be related to increased numbers of β_3-ARs or an altered signal transduction [Cheng *et al.* 2001]. As shown in Figure 1, in CHF, NO-cGMP signaling may be altered [Mohan *et al.* 1996], thereby altering CHF myocyte response to β_3-AR stimulation [Cheng *et al.* 2001]. The enhanced contractile response to β_3-AR stimulation in CHF myocytes of dogs may be coupled to G_i through both NO-dependent and NO-independent mechanisms [Cheng *et al.* 2001]. The activation of G_i also has the potential to couple β_3-ARs to other important signaling pathways such as mitogen-activated protein kinase [Soeder *et al.* 1999]. The increase in other neurohormonal activation, such as TNF-α, endothelin 1, and angiotensin II, may also differentially modulate β_3-AR expression and function. These studies indicate that using β_3-AR agonists for the treatment of obesity and diabetes [Arch *et al.* 1984] may have cardiac side effects, especially in patients with CHF [Cheng *et al.* 2001]. Also, these studies suggest several novel therapeutic strategies for the treatment of CHF, such as the use of β_3-AR antagonists or G_i inhibitors. Gan and colleagues [Gan *et al.* 2007a] reported that a β_3-AR antagonist SR 59230A can block the β_3-AR–nitric oxide synthase (NOS)–cyclic GMP pathway and improve cardiac function in heart failure in rats if administered long term. SR 59230A can also attenuate cardiac remodeling by inhibition of interstitial

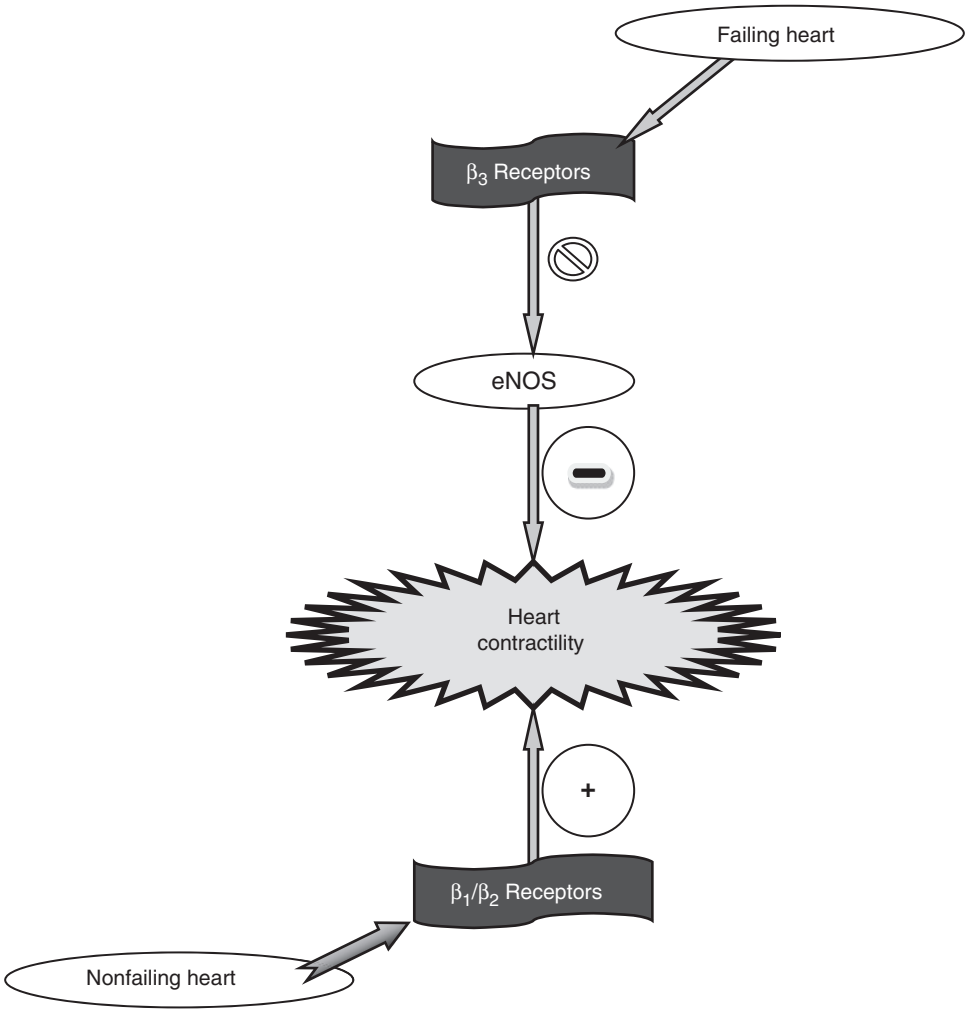

Figure 1. Postulated changes in β-adrenoceptor signaling in cardiomyocytes from nonfailing to failing myocardium.
eNOS, endothelial nitric oxide synthase.

fibrosis to a certain degree, which may help to improve cardiac function in heart failure [Gan *et al.* 2007b]

β_3-ARs are involved in the vasomotor control of the internal mammary artery and thus open new fields of investigation in coronary bypass graft management, heart failure, and hypertension [Rozec *et al.* 2005]. In the hearts of long-term diabetic rats, the expression of β_1-ARs decreases, whereas that of β_3-ARs increases. This may suggest that a decrease in β_1-AR together with an increase in β_3-AR expression might be involved in the development of diabetes-induced cardiac dysfunction [Dinçer *et al.* 2001].

In cardiac myocytes, repolarization of the action potential is produced by several potassium currents [Barry and Nerbonne, 1996] like very slow activating and deactivating delayed rectifier potassium current (IKs). This current represents the predominant repolarizing current during increased heart rate [Zeng *et al.* 1995; Jurkiewicz and Sanguinetti, 1993; Varnum *et al.* 1993]. The channel underlying IKs is formed by the assembly of two transmembrane proteins, the KvLQT1 and MinK protein [Barhanin *et al.* 1996; Sanguinetti *et al.* 1996]. The IKs current amplitude in the heart is increased by catecholamines, which are mediated by β-ARs [Lo and Numann, 1998; Sanguinetti *et al.* 1991; Duchatelle-Gourdon *et al.* 1989]. Catecholamines develop negative inotropic effects and shorten the human cardiac action potential through β_3-ARs [Gauthier *et al.* 1996]. The shortening of the human cardiac action potential under β_3-AR stimulation may be because they can couple functionally to the KvLQT1/MinK potassium channel in the

Xenopus oocyte expression system, which involves G proteins [Kathofer *et al.* 2000]. The shortening of cardiac action potentials is expected to affect the repolarization process, thereby potentially triggering arrhythmias. The coupling of the KvLQT1/MinK channel to the β_3-AR may have important implications for arrhythmogenesis in the heart and thus may open new perspectives for the prevention and treatment of cardiac arrhythmias [Kathofer *et al.* 2000]. The findings of Zhou and colleagues [Zhou *et al.* 2008] suggested that β-AR blocking agents with β_3-AR agonist properties might be useful for cardiac arrhythmia control after myocardial infarction, especially in treating ventricular tachycardia storms.

Endothelium

After L-NAME treatment or removal of endothelium, relaxant responses to isoprenaline were found to be unaffected by propranolol, suggesting that they were mediated by β_3-ARs and/or the low-affinity state of β_1-ARs, formerly proposed as putative β_4-ARs [Brawley *et al.* 1998].

In the rat thoracic aorta, β_3-ARs act in conjunction with β_1-ARs and β_2-ARs to mediate relaxation through activation of an NOS pathway and subsequent increase in cyclic GMP levels [Trochu *et al.* 1999]. In human vessels, β_3-AR relaxation was also found to be mediated partly through NO production. This was evidenced by its complete abrogation by NOS inhibition under circumstances when both prostanoids and endothelium-derived hyperpolarizing factors (EDHFs) are inoperative (that is, after cyclooxygenase inhibition and preconstriction with high potassium chloride respectively) [Dessy *et al.* 2004]. This may be caused by functional coupling of β_3-AR agonists to NO production in whole human ventricular muscle through $G_{\alpha i}$ proteins [Moniotte *et al.* 2001; Gauthier *et al.* 1998]. Endothelial cells produce a hyperpolarization leading to vascular muscle relaxation through activation of calcium-dependent K^+ channels [Busse *et al.* 2002]. Dessy and colleagues [Dessy *et al.* 2004] demonstrated vessel hyperpolarization in response to β_3-AR agonists and the abrogation of β_3-AR-mediated relaxation after vessel pretreatment with the K^+ channel inhibitors charybdotoxin and apamin, two signatures of an EDHF response. These results are also in agreement with the recent proposition of β_3-AR-mediated relaxation through K^+ channel activation in rat aorta [Rautureau *et al.* 2002]. Functional β_3-AR vasorelaxation mediated in part by EDHFs in human coronary resistance arteries may have a major bearing on our understanding of regulating coronary perfusion in circumstances such as dyslipidemia, diabetes and atherosclerosis, all associated with decreased NO production and/or bioavailability.

Conclusions

Almost 50 years after Ahlquist first uncovered evidence of the heterogeneity of adrenergic receptors, the number of receptor subtypes is still unclear, although nine subtypes are well documented (three subtypes each of α_1-ARs, α_2-ARs and β-ARs). Adrenergic receptors are members of a large superfamily of receptors linked to G proteins. The identification of new subtypes of receptors offers the promise of new therapeutic agents. β_3-ARs, which are found at unique sites such as in brown adipose tissue and the gallbladder, are potential targets for antiobesity drugs. Although considerable information is available on β_3-AR physiology in fat, there are many other areas in which β_3-ARs are involved. The presence of β_3-ARs in vasculature and heart provides new avenues for the development of innovative type-specific drugs. Since alterations in adrenergic receptors have a role in many clinical settings, the development of such agonists and antagonists may give therapeutic potential for the treatment of various disorders, including diabetes mellitus, hypertension, dyslipidemia, cardiac arrythmias, heart failure and diabetes-induced cardiac dysfunction . It is known that using β_3-AR agonists to treat obesity and diabetes may have cardiac side effects, especially in patients with CHF. However, with the knowledge that there are two types of β_3-ARs (β_{3a} and β_{3b}), it may be possible to develop subtype-specific drugs that are more effective and have fewer side effects than those currently available.

Conflict of interest statement

This article received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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