Naunyn-Schmiedeberg's Arch Pharmacol (2007) 374:385–398
DOI 10.1007/s00210-006-0127-5

REVIEW

Tools to study β_3-adrenoceptors

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Received: 7 September 2006 / Accepted: 22 November 2006 / Published online: 9 January 2007

Abstract β_3-adrenoceptors mediate some of the effects of catecholamines on tissues such as blood vessels or the urinary bladder and are putative targets for the treatment of diseases such as the overactive bladder syndrome. Progress in the understanding of the presence, function, and regulation of β_3-adrenoceptors has been hampered by a lack of highly specific tools. "Classical" β_3-adrenoceptor agonists such as BRL 37,344 [(R*, R*)-(±)-4[2-[(3-chlorophenyl)-2-hydroxyethyl) amino] propyl] phenoxyacetic acid] and CGP 12,177 [(±)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one] are only partial agonists in many settings, have limited selectivity over other β-adrenoceptor subtypes, and may additionally act on receptors other than β-adrenoceptors. More efficacious and more selective agonists have been reported and, in some cases, are in clinical development but are not widely available for experimental studies. The widely used antagonist SR 59,230 [3-(2-ethylphenoxy)-1-[(1,S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propanoloxalate] is not selective for β_3-adrenoceptors, at least in humans, and may actually be a partial agonist. Radioligands, which are suitable either for the selective labeling of β_3-adrenoceptors or for the nonselective labeling of all β-adrenoceptor subtypes, are also missing. β_3- and β_1/β_2 double knockout mice have been reported, but their usefulness for extrapolations in humans is questionable based upon major differences between humans and rodents with regard to the ligand recognition and expression profiles of β_3-adrenoceptors. While the common availability of more selective agonists and antagonists at the β_3-adrenoceptor is urgently awaited, the limitations of the currently available tools need to be considered in studies of β_3-adrenoceptor for the time being.

Keywords β_3-adrenoceptor · BRL 37,344 · CGP 12,177 · SR 59,230 · Radioligand · Knock-out mice

The authors wish to dedicate this manuscript to Prof. Karl H. Jakobs on the occasion of his retirement.

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Introduction

β-adrenoceptors mediate many effects of the endogenous catecholamines adrenaline and noradrenaline. Following the initial subdivision of β-adrenoceptors into the β_1- and β_2-adrenoceptors (Lands et al. 1967), it became clear that not all β-adrenoceptor effects could be explained by these two subtypes. A detailed description of the early history of research into β-adrenoceptors other than β_1 and β_2 has been published elsewhere (Arch and Kaumann 1993). Meanwhile, not only β_1- and β_2-adrenoceptors but also a β_3-adrenoceptor has been cloned in humans (Emorine et al. 1989; Lelias et al. 1993), rats (Granneman et al. 1991), and other species (Nahmias et al. 1991), and the β_3-adrenoceptor has been incorporated into the official adrenoceptor subtype classification (Bylund et al. 1994).

The human β_3-adrenoceptor gene is located on chromosome 8p11.1-8p12 (Nahmias et al. 1991). In contrast to the genes of β_1- and β_2-adrenoceptor, the β_3-adrenoceptor gene contains one or more introns (Pietri-Rouxel and Strosberg 1995). While the species homologues of the β-

Table 1 Sequence comparison of β_3-adrenoceptors in various species

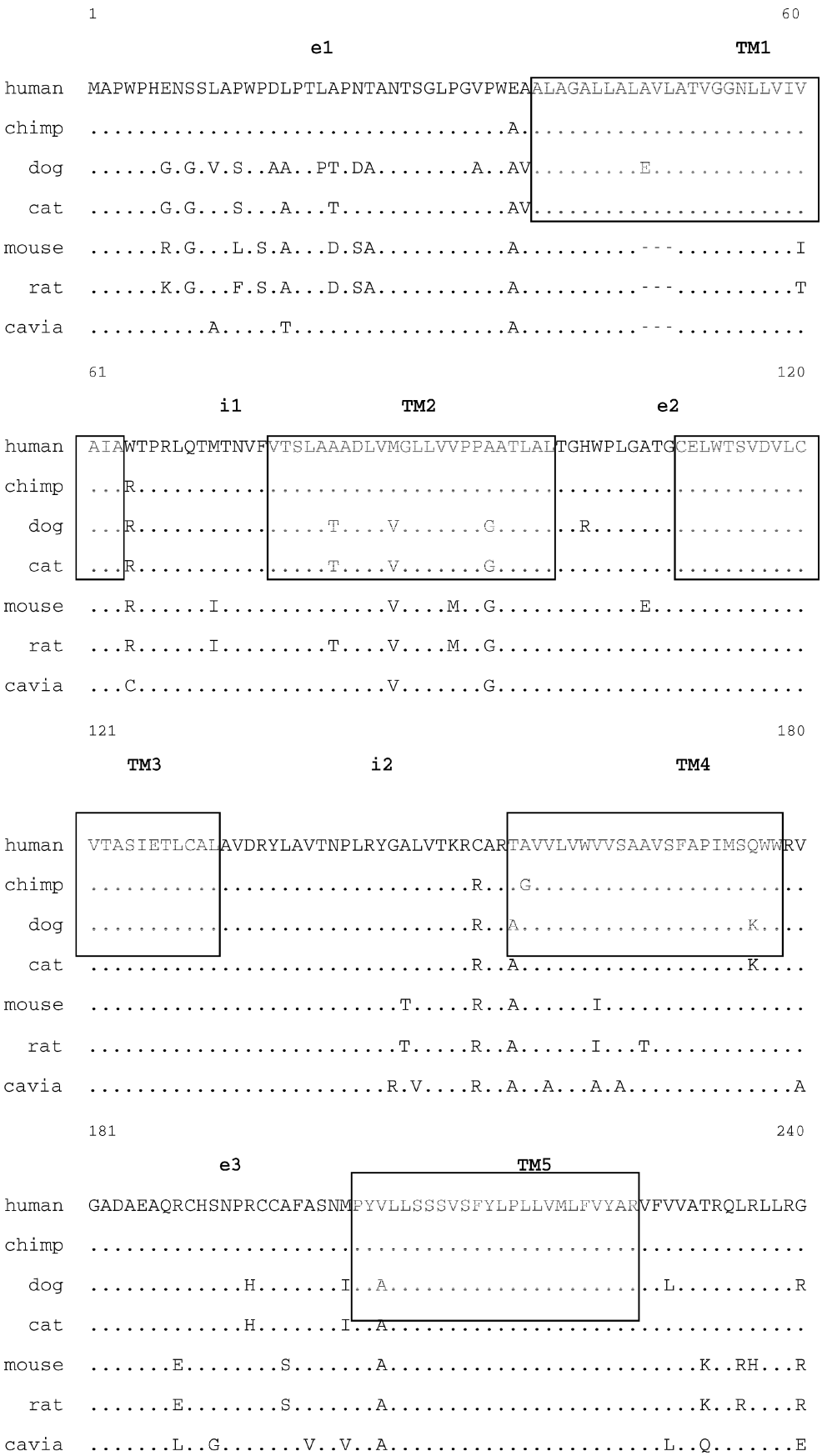
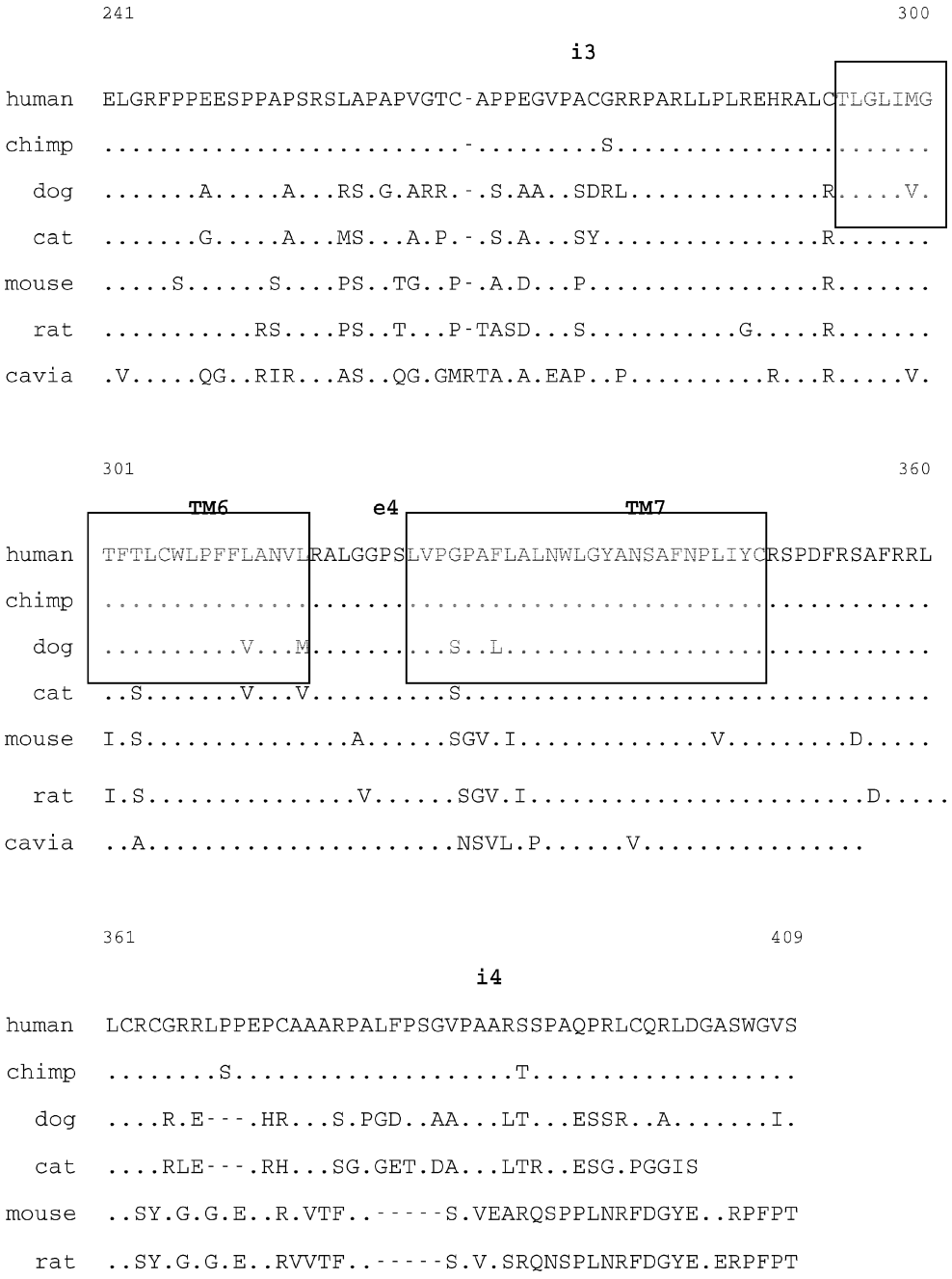


Table 1 (continued)



adrenoceptor subtypes have great amino acid homology (Table 1), minor differences in amino acid sequence between species homologues of β_3-adrenoceptors can translate into noticeable differences in ligand recognition patterns (Arch 2002; Cohen et al. 1999; Granneman et al. 1991; Hutchinson et al. 2006; Liggett 1992; Molenaar et al. 1997; Tate et al. 1991). An important structural difference between the β_3- and the β_1- and β_2-adrenoceptors is the absence of phosphorylation sites for cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) or G-protein-coupled receptor kinase in the short C terminus of the β_3-adrenoceptor, which is thought to be the basis for its relative resistance to agonist-induced down-regulation (Pietri-Rouxel and Strosberg 1995). Moreover, the organization of the β_3-adrenoceptor gene differs between species, with, e.g., the human gene having only two exons (the second one comprising only the final six amino acids), whereas the rodent gene has three exons (although the third does not code for any amino acid residues) (Pietri-Rouxel and Strosberg 1995). Finally, the murine receptor has splice variants, which are differentially expressed in tissues (Evans et al. 1999) and couple to

distinct signaling pathways (Hutchinson et al. 2002; Sato et al. 2005).

β_3-adrenoceptor messenger ribonucleic acid (mRNA) is abundantly expressed in human urinary bladder, gall bladder, and small and large intestine but less so or even absent in the central nervous system, pancreas, cardiac ventricle, kidney, and lung (Berkowitz et al. 1995; Nomiya and Yamaguchi 2003; Roberts et al. 1997). To what extent β_3-adrenoceptor mRNA is expressed in human adipose tissue has remained controversial (Berkowitz et al. 1995; Deng et al. 1996). In rats, β_3-adrenoceptor mRNA has mainly been found in adipocytes, various parts of the gastrointestinal tract (Evans et al. 1996; Granneman et al. 1991), and also in the urinary bladder (Seguchi et al. 1998). The strong expression in adipocytes may relate to the presence of an enhancer element in an intron of the β_3-adrenoceptor gene, which drives expression in this tissue (Granneman et al. 1992). The expression profile of the two murine splice variants differs (Evans et al. 1999).

Much initial attention focussed on a role of β_3-adrenoceptors in the regulation of adipocyte function, but later studies showed that its important role in rodents is not paralleled by a major role in human adipocytes (Barbe et al. 1996; Rosenbaum et al. 1993). Meanwhile, a role for β_3-adrenoceptors has been established in blood vessels (Guimaraes and Moura 2001; Rozec and Gauthier 2006) and the urinary bladder (Michel and Vrydag 2006). Based upon such information, a systematic search for β_3-adrenoceptor agonists and antagonists has been initiated, and some of these agonists have entered clinical drug development. Originally, β_3-adrenoceptor agonists were investigated for the treatment of obesity and diabetes mellitus type 2 but met with only limited success (Arch 2002). Currently, β_3-adrenoceptor agonists are in clinical development for treatment of depression [e.g., SR 58,611; ((RS)-N-[(2S)-7-ethyoxycarbonylmethoxy-1,2,3,4-tetrahydronapth-2-yl]-(2R)-2-(3-cholophenyl)-2-hydroxyethanamine hydrochloride)] (Anonymous 2003) or voiding disorders [e.g., solabegron, ritobegron (formerly known as KUC 7483) and YM 178]. Nevertheless, knowledge about characteristics, presence, function, and regulation of β_3-adrenoceptors is lagging far behind that for β_1- and β_2-adrenoceptors. To further such understanding, suitable tools are required. These may not only further our physiological knowledge but could potentially also become therapeutically useful drugs.

Cell lines

While many laboratories have created cell lines stably expressing β_3-adrenoceptors, only few cell lines have been reported that natively express this subtype. This includes SK-N-MC neuroblastoma cells, but their use is complicated by the concomitant expression of β_1-adrenoceptor (Chaudhry and Granneman 1994; Curran and Fishman 1996; Esbenshade et al. 1992). Others have reported β_3-adrenoceptors in 3T3-F4424 cells (Feve et al. 1995, 1992) and other adipocyte-derived cell lines (Thomas et al. 1992).

β_3-adrenoceptor antagonists

Many β_3-adrenoceptor ligands have been described and characterized based upon their properties in tissues and cell lines natively expressing receptors distinct from β_1- and β_2-adrenoceptors (Arch and Kaumann 1993). While such studies were instrumental to get the field started, the use of such data for definition of the properties of these ligands can be misleading for several reasons. Firstly, circular reasoning applies if ligands are used to define which receptor is natively expressed at the protein level, and then the same tissue or cell line is used to define the properties of such ligands. Secondly, there is some heterogeneity in reported ligand properties across tissues and cell lines; it remains unclear whether this heterogeneity reflects random scatter in measured values or, rather, heterogeneity in the pharmacological properties of the expressed receptors. For this reason, this manuscript largely relies on heterologous expression of cloned β-adrenoceptor subtypes to define the properties of the various ligands.

"Classical" antagonists

A key reason that β_3-adrenoceptors were identified so much later than β_1- and β_2-adrenoceptors is that many classical β-adrenoceptor antagonists have much lower affinity for them (Baker 2005; Hoffmann et al. 2004). For example, the prototypical antagonist propranolol was reported to have K_i values at β_1-, β_2,- and β_3-adrenoceptors of 1.8, 0.8, and 186 nM, respectively (Table 2). Similarly, much lower β_3- than β_1- and β_2-adrenoceptor affinity was also reported for the clinically used drugs sotalol, alprenolol, carvedilol, metoprolol, atenolol, and bisoprolol in most studies (Baker 2005; Hoffmann et al. 2004), although one study reported high β_3-adrenoceptor affinity of carvedilol (Candelore et al. 1999). Among frequently used experimental antagonists, the selectivity of ICI 118,551 for β_2-adrenoceptors relative to β_3-adrenoceptors is comparable to that relative to β_1-adrenoceptors (Table 2) (Baker 2005; Hoffmann et al. 2004). Similarly, the selectivity of CGP 20,712A for β_1-adrenoceptors relative to β_3-adrenoceptors is comparable to that relative

to β_2-adrenoceptors (Table 2) (Baker 2005; Hoffmann et al. 2004). Thus, most well-established β-adrenoceptor antagonists are unsuitable to also block β_3-adrenoceptors.

β_3-adrenoceptor antagonists

SR 59,230 [3-(2-ethylphenoxy)-1-[(1,S)-1,2,3,4-tetrahydronapth-1-ylamino]-2S-2-propanoloxalate] was originally described as a β_3-adrenoceptor-selective antagonist (Manara et al. 1996; Nisoli et al. 1996). This was, e.g., based on studies in which it antagonized cAMP formation in rat brown adipose tissue but not in rat brain frontal cortex. Subsequently, it has been used in numerous studies in which inhibition of a functional response by this compound has been taken as evidence for mediation of this response by a β_3-adrenoceptor (Sarma et al. 2003; Yamanishi et al. 2002b, 2003). However, more recent radioligand binding studies with cloned human β-adrenoceptor subtypes have reported that SR 59,230 is not selective for β_3-adrenoceptors and, if anything, has slightly lower affinity for this subtype than for β_1- and β_2-adrenoceptors (Table 2) (Candelore et al. 1999; Hoffmann et al. 2004; Niclauß et al. 2006). Moreover, SR 59,230 can exhibit agonist rather than antagonist properties and, in some systems, even behave as a full agonist (Horinouchi and Koike 2001; Hutchinson et al. 2005). Even more importantly, SR 59,230 appears to inhibit not only other β-adrenoceptor subtypes but also α_1-adrenoceptors and may have rather similar affinity for the two adrenoceptor subfamilies (Brahmadevara et al. 2004; Briones et al. 2005; Leblais et al. 2005).

Table 2 Affinity and efficacy of drugs at β-adrenoceptors

	β_1-adrenoceptor		β_2-adrenoceptor		β_3-adrenoceptor	
	pK_i	Efficacy	pK_i	Efficacy	pK_i	Efficacy
Isoprenaline	−6.61	100	−6.34	100	−5.80	100
Adrenaline	−5.40	133	−6.13	110	−3.90	106
Noradrenaline	−5.45	123	−4.58	103	−5.37	122
Propranolol	−8.75	−35	−9.10	−35	−6.73	−1
Pindolol	−8.59	−25	−8.32	−34	−7.36	13
CGP 12,177	−8.35	−19	−8.37	−32	−7.11	36
BRL 37,344	−4.42	−5	−5.04	−7	−6.37	28
SR 59,230	−7.79	−19	−7.21		−6.91	5
CGP 20,712A	−8.33	−25	−5.39	−30	−5.63	−20
ICI 118,551	−7.31	−22	−9.15	−32	−6.21	−30

Data are taken from Hoffmann et al. (2004) and based upon Chinese hamster ovary (CHO) cells transfected with the indicated human β-adrenoceptor subtype at similar density. Protein kinase inhibitor (pK_i) are given in molar concentrations and based on radioligand binding, whereas efficacy is given in % of the isoprenaline response in adenylyl cyclase stimulation studies. Negative values for efficacy indicate inverse agonism

Bupranolol is a clinically used β-adrenoceptor antagonist, which also has been used to demonstrate the involvement of β_3-adrenoceptor in functional responses (Horinouchi and Koike 2001; Igawa et al. 1998; Kaumann 1996; Takeda et al. 2002a). However, in competition binding studies with human β-adrenoceptor subtypes, bupranolol has lower affinity for β_3-adrenoceptors than for β_1- and β_2-adrenoceptors (Baker 2005; Candelore et al. 1999). Moreover, bupranolol, which is chemically related to SR 59,230, shares its α_1-adrenoceptor antagonist effects (Brahmadevara et al. 2004; Leblais et al. 2005).

Pindolol has similar affinity at human β_1- and β_2-adrenoceptors and approximately ten-fold lower affinity at β_3-adrenoceptors (Table 2) (Hoffmann et al. 2004). It has long been known to be a weak partial agonist (Jasper et al. 1988) with an intrinsic activity selectively acting on β_2-adrenoceptors in human tissues in vivo (Michel et al. 1988). Pindolol also is an agonist for the propranolol-resistant site of the β_1-adrenoceptor (Joseph et al. 2003). Studies with cloned human subtypes expressed in Chinese hamster ovary (CHO) cells found pindolol to have intrinsic activity only at β_3-adrenoceptors but not at β_1- and β_2-adrenoceptors (Hoffmann et al. 2004). Its partial agonism at β_3-adrenoceptors was also seen with rat and human receptor expressed in CHO cells, where it had slightly greater potency and efficacy with the human receptor than its rat homologue (Granneman et al. 1991; Liggett 1992). Nevertheless, its congener cyanopindolol has been used as an antagonist to demonstrate involvement of a β_3-adrenoceptor in certain functional responses (de Groot et al. 2003). Finally, it should be considered that pindolol can also act on some subtypes of serotonin receptors (Guan et al. 1992) and may even have some α_1-adrenoceptor effects (Brahmadevara et al. 2004).

L-748,337 [(S)-N-[4-[2-[[3-[3(acetamidomethyl)phenoxy]-2-hydroxypropyl]amino]ethyl]phenylbenzenesulfonamide] was reported to have an affinity of 4 nM at the cloned human β_3-adrenoceptor compared with 390 and 204 nM at the β_1- and β_2-adrenoceptors, respectively, and to be practically devoid of agonist activity at all three subtypes (Candelore et al. 1999). It is about 100-fold less potent at the rat β_3-adrenoceptor (Candelore et al. 1999). Nevertheless, it was reported to abolish some nadolol-insensitive β-adrenoceptor responses in rat heart (Zhang et al. 2005) or aorta (Mallem et al. 2004) and also to inhibit propranolol-resistant responses in the opossum esophagus (Sarma et al. 2003) or anal sphincter (Banwait and Rattan 2003). L-748,337 distinguishes the β_3-adrenoceptor from the propranolol-resistant site of the β_1-adrenoceptor (Joseph et al. 2003). Therefore, at least in humans, L-748,337 probably is one of the very few antagonists with selectivity for the β_3-adrenoceptor, but unfortunately, it is not widely available.

β_3-adrenoceptor agonists

General considerations

The properties of agonists at β-adrenoceptor subtypes are more difficult to describe than those of antagonists, and this also applies to studies of β_3-adrenoceptors (Arch 2002). Their effects are characterized not only by potency but also by efficacy. Agonist potency and efficacy depend on both intrinsic properties of the agonist (affinity, intrinsic efficacy) as well as those of the cell/tissue in which the response is measured. Moreover, G-protein-coupled receptors can exist in guanosine triphosphate (GTP)-dependent high- and low-affinity states for agonists, which makes even the assessment of affinity, e.g., in a radioligand binding assay, difficult (Brown et al. 1992). Studies with β_3-adrenoceptors expressed in CHO cells at three different densities illustrate these points (Wilson et al. 1996): With increasing receptor density, the potency of all agonists increased. Concomitantly, the efficacy relative to that of the full agonist isoprenaline increased for partial agonists such as BRL 37,344 or CGP 12,177 (see below), an observation confirmed by reports for all three subtypes (Hoffmann et al. 2004). Furthermore, different potencies were found in these studies when adenylyl cyclase activation in cell membranes was compared with cAMP accumulation in intact cells, the latter exhibiting a much greater potency, particularly for the full agonists. When a down-stream response such as activation of PKA was compared with an early signaling response, such as cAMP accumulation, all agonists exhibited a greater potency for the more distal response, and the partial agonists additionally exhibited an increased efficacy for the down-stream response. Finally, all of these factors may additionally differ between species (Arch 2002). Taken together, these factors considerably complicate studies into the selectivity of β_3-adrenoceptor agonists. Definitive evaluations of such selectivity may require comparisons at all three subtypes using the same cell type, receptor density, and cellular response. Therefore, we will largely refer to two such systematic studies (Baker 2005; Hoffmann et al. 2004). However, even the results obtained under such carefully controlled conditions may not be fully representative for all other cell types, tissues, and responses. These limitations need to be kept in mind in the interpretation of the data discussed below.

Isoprenaline

In radioligand binding studies in CHO cells stably transfected with the human subtypes, isoprenaline exhibited an order of potency of $\beta_1 > \beta_2 > \beta_3$ (Table 2), but the differences between the subtypes were small, i.e, affinities at β_1- and β_3-adrenoceptors differed by less than ten-fold, and isoprenaline was a full agonist for all three subtypes (Hoffmann et al. 2004). Thus, isoprenaline may be the most suitable full agonist to yield a balanced stimulation of all three subtypes, and indeed, most studies have used isoprenaline as the reference agonist to describe the relative efficacy of other agonists.

Adrenaline and noradrenaline

In radioligand binding studies, adrenaline showed an order of potency of $\beta_2 > \beta_1 > \beta_3$ in CHO cells stably transfected with human subtypes, whereas noradrenaline exhibited a rank order of $\beta_3 \approx \beta_1 > \beta_2$ (Table 2) (Hoffmann et al. 2004). Adenylyl cyclase stimulation studies based upon the same cell lines found both adrenaline and noradrenaline to be full agonists at all three subtypes relative to isoprenaline (Hoffmann et al. 2004). Using a similar approach with independently created CHO cells, other investigators reported an order of potency for cAMP accumulation in intact cells of $\beta_2 \geq \beta_1 > \beta_3$ for adrenaline and of $\beta_1 > \beta_3 > \beta_2$ for noradrenaline (Tate et al. 1991). Thus, the endogenous catecholamines are not suitable to specifically stimulate certain subtypes, but in cells or tissues coexpressing multiple subtypes, a given concentration of adrenaline or noradrenaline may preferentially stimulate one of them. Moreover, they can be used for selective subtype stimulation when applied in the presence of suitable antagonists of other subtypes.

BRL 37,344

BRL 37,344 [(R*, R*)-(±)-4[2-[(3-chlorophenyl)-2-hydroxyethyl) amino] propyl] phenoxyacetic acid] is probably the most often used prototypical β_3-adrenoceptor-selective agonist. Radioligand binding studies with human β_3-adrenoceptors have typically reported an affinity in the high nanomolar range (pK_i 5.8–6.8) (Blin et al. 1994; Hoffmann et al. 2004; Liggett 1992). Similar values were reported for the murine receptor (Blin et al. 1994), but the affinity of BRL 37,344 at the rat and bovine receptor may be 20-fold and 100-fold higher, respectively, compared with its human orthologue (Liggett 1992; Pietri-Rouxel and Strosberg 1995).

A direct comparative study of all three human subtypes found a rank order of potency of $\beta_3 > \beta_2 > \beta_1$ with an approximately 20- and 100-fold selectivity, respectively, for the former vs. the two latter subtypes (Table 2) (Hoffmann et al. 2004). Moreover, the same study reported BRL 37,344 to have an efficacy for adenylyl cyclase stimulation of 28% of isoprenaline, whereas it lacked efficacy at the other two subtypes. Another study measuring cAMP accumulation in intact cells confirmed the rank order of potency of $\beta_3 > \beta_2 > \beta_1$, but BRL 37,344 was an agonist for

all three human subtypes, and the difference in potency between the subtypes was less than ten-fold (Tate et al. 1991). Studies including only β_3-adrenoceptors reported BRL 37,344 to be a full agonist for cAMP accumulation in intact CHO cells at human and mouse but a partial agonist at rat β_3-adrenoceptors (Blin et al. 1994; Liggett 1992). BRL 37,344 has similar affinity for both splice variants of the murine β_3-adrenoceptor, but its effects, similar to that of many other agonists, are somewhat lower at the β_{3b}- compared with the β_{3a}-adrenoceptor, as the former couples to both G_s and G_i proteins (Hutchinson et al. 2002). Given that the selectivity of BRL 37,344 for β_3- relative to other β-adrenoceptor subtypes is only moderate in binding studies, the question as to whether it has intrinsic efficacy at β_1- and/or β_2-adrenoceptors becomes of major importance to determine its usefulness in functional studies. In this regard, studies in neonatal rat liver (Fraeyman et al. 1992) and in guinea pig heart (Kozlovski et al. 2003) support the idea of at least some intrinsic activity at subtypes other than the β_3-adrenoceptor. However, we are not aware of other studies that have directly compared its efficacy at the β-adrenoceptor subtypes of any species.

Several studies have reported on the potency and efficacy of BRL 37,344 for a variety of functional responses in tissues of rats and other species, including humans. While a comprehensive listing of reported values is beyond the scope of this manuscript, we will give a few examples. Relaxation of rat urinary bladder strips was characterized by partial agonism and pEC_{50} values of 6.6–8.0 (Frazier et al. 2006; Longhurst and Levendusky 1999; Oshita et al. 1997); when bladder strips had been precontracted with a muscarinic agonist, BRL 37,344 behaved as a full agonist for relaxation but with rather shallow concentration–response curves and a low overall potency ($pEC_{50}<6$), possibly reflecting that it may act as a direct muscarinic receptor antagonist (Kubota et al. 2002; Matsubara et al. 2002). The potency of BRL 37,344 for production of free fatty acids in adipocytes was much higher in rats than in humans (pEC_{50} 8.3 vs. 5.5) (Hollenga et al. 1991), possibly reflecting not only species differences in binding to the β_3-adrenoceptor but also stronger involvement of this subtype in rodents than in humans (Rosenbaum et al. 1993). The tissue effects of BRL 37,344 in rats typically are not inhibited by low propranolol concentrations, i.e., those sufficient to block β_1- and β_2-adrenoceptors, but, rather, by CGP 12,177 (see below) or SR 59,230 (Longhurst and Levendusky 1999), supporting that it acts via β_3-adrenoceptors despite its limited selectivity for this subtype. However, it has also been found to act via β_1- and/or β_2-adrenoceptors, e.g., in the human heart (Pott et al. 2003).

Another issue in the use of BRL 37,344 is its effects unrelated to any subtype of β-adrenoceptors, which may occur in a similar concentration range as those used for β-adrenoceptor activation. Thus, this compound is an antagonist of muscarinic acetylcholine receptors (Kubota et al. 2002), a feature that may cause problems in functional experiments with the urinary bladder where precontraction is frequently induced by a muscarinic receptor agonist (see above). Moreover, BRL 37,344 was also reported to be an α_1-adrenoceptor antagonist in low micromolar concentrations (Brahmadevara et al. 2003, 2004; Briones et al. 2005; Leblais et al. 2005). The poor subtype selectivity and β-adrenoceptor-independent effects of BRL 37,344 seriously limit its usefulness in pharmacological studies.

CGP 12,177

CGP 12,177 [(±)-4-(3-*t*-butylamino-2-hydroxypropoxy) benzimidazol-2-one] was primarily introduced as an antagonist of β_1- and β_2-adrenoceptors, and its hydrophilic properties made it useful for the specific detection of cell-surface receptors (Staehelin et al. 1983). However, it can exhibit agonistic effects in some cases. While some of these may relate to a second ligand recognition site on β_1-adrenoceptors (Joseph et al. 2004a,b), CGP 12,177 can also act on β_3-adrenoceptors (Table 2), albeit at least 20-fold higher concentrations than those required to inhibit the classical catecholamine recognition site on β_1- and β_2-adrenoceptors (Baker 2005; Hoffmann et al. 2004; Niclauß et al. 2006). Indeed, a tritiated form of CGP 12,177 has been used as a radioligand to label β_3-adrenoceptors (Feve et al. 1991), but its affinity is much lower than for the other subtypes (Baker 2005).

The efficacy of CGP 12,177, measured as the ability to stimulate adenylyl cyclase activity in membrane preparations, was 36% of that of isoprenaline at β_3-adrenoceptors at near physiological expression levels, whereas it was an inverse agonist at β_1- and β_2-adrenoceptors (Hoffmann et al. 2004). Partial agonism at the cloned human, rat, and murine β_3-adrenoceptor was also reported from other studies (Blin et al. 1994; Liggett 1992). Accordingly, CGP 12,177 was also only a partial agonist for relaxation of isolated rat bladder strips, an effect not antagonized by low propranolol concentrations (Frazier et al. 2006; Longhurst and Levendusky 1999). Moreover, CGP 12,177 was also reported to have agonist properties at nadolol-sensitive, i.e., β_1- and/or β_2-adrenoceptors in guinea pig heart (Kozlovski et al. 2003).

Apart from its weak partial agonism, two factors complicate the use of CGP 12,177 as a β_3-adrenoceptor agonist. Firstly, it can also activate β_1-adrenoceptors under some conditions by acting on a second ligand recognition site on this subtype (Joseph et al. 2004a,b). Secondly, similarly to BRL 37,344, direct antagonism of α_1-adrenoceptors has also been described for CGP 12,177 (Brahmadevara et al.

2004; Briones et al. 2005). Accordingly, effects of high concentrations of CGP 12,177 in guinea pig heart were not sensitive to any β-adrenoceptor antagonist, including the β_3-selective L-748,337 (Kozlovski et al. 2003). Thus, CGP 12,177 clearly is not very suitable to selectively stimulate β_3-adrenoceptors.

CL 316,243

CL 316,243 ((R,R)-5-(2-[{2-(3-chlorophenyl)-2-hydroxy-ethyl}-amino]propyl)-1,3-benzodioxole-2,2,dicarboxylate) has some selectivity for β_3- relative to β_1- and β_2-adrenoceptors (pK_i 5.1 vs. <3 and 4.1, respectively) (Baker 2005). CL 316,243 appears to be a rodent-selective agonist, as its potency to stimulate cAMP formation in transfected CHO cells was much higher at the mouse than at the human β_3-adrenoceptor (pEC_{50} 8.7 vs. 4.3) (Hutchinson et al. 2006). CL 316,243 has repeatedly been used in studies on the rat (Kaidoh et al. 2002; Takeda et al. 2000, 2002b, 2003; Woods et al. 2001) and human urinary bladder (Igawa et al. 1999, 2001) and also in the ureter (Murakami et al. 2000; Park et al. 2000), esophagus (Sarma et al. 2003), colon (Kaumann and Molenaar 1996) and blood vessels (Atef et al. 1996; Kuratani et al. 1994; Leblais et al. 2005). In line with the above differences in potency between mouse and human receptors, it also exhibited a consistently higher potency at rat compared with human tissues.

L-755,507

L-755,507 is a 4-acylaminobenzenesulfonamide derivative (Parmee et al. 1998) that activates cloned human and rhesus monkey β_3-adrenoceptors about 1,000-fold more potently than the other two subtypes (Fisher et al. 1998). It causes lipolysis in monkey (Fisher et al. 1998) and human adipose tissue (Umekawa et al. 1999) and relaxation of the human urinary bladder (Nomiya and Yamaguchi 2003) in sub-nanomolar concentrations. While L-755,507 lacks inotropic effects in wild-type mice, it increases cardiac contractility in transgenic mice with cardiac overexpression of the human β_3-adrenoceptor (Kohout et al. 2001). Numerous analogues of L-755,507 have been reported (Parmee et al. 1998), but unfortunately, none of them has become widely available.

Miscellaneous other agonists

A variety of other drugs have been reported to be agonists at β_3-adrenoceptors, including clenbuterol (Hutchinson et al. 2006) and zinterol (Hutchinson et al. 2006) and the experimental compounds ICI 215,001 (Baker 2005; Blin et al. 1994; Tesfamariam and Allen 1994), FK175 (Fujimura et al. 1999), FR 149175 (Hatakeyama et al. 2004), FR 165101 (Uchida et al. 2005), GS-332 (Morita et al. 2000), KUL-7211 (Tomiyama et al. 2003), and L-796,568 (van Baak et al. 2002). Some of these compounds may have much greater selectivity for β_3- compared with β_1- and β_2-adrenoceptors and may also lack the ancillary properties of BRL 37,344 or CGP 12,177, but overall, too little data have been published to allow a thorough evaluation. SR 58,611 (Blin et al. 1994; Longhurst and Levendusky 1999; Nisoli et al. 1995) is in development for the treatment of depression (Anonymous 2003), and solabegron, ritobegron, and YM 178 (chemical structure not disclosed) are in clinical development for the treatment of overactive bladder.

Detection of β_3-adrenoceptors

In many types of investigations, it is not the primary aim to activate or inhibit β_3-adrenoceptor but, rather, to detect their presence and possible alterations of their expression, e.g., in disease states. This can be done both at the protein and the mRNA level. Although some β_3-adrenoceptor antibodies have been reported (Chamberlain et al. 1999) and are commercially available, to the best of our knowledge, none of them has been well validated and shown to display a useful selectivity for β_3-adrenoceptors relative to other β-adrenoceptor subtypes or other receptors in general. Therefore, as with most G-protein-coupled receptors, detection of β_3-adrenoceptors at the protein level is largely confined to radioligand binding studies. Radioligands have proven powerful to detect expression of receptors at the protein level with many receptor classes. When used in autoradiographic techniques, they can identify the location of receptors, and when used in binding assays with intact cells or homogenates of cells or tissues, they can be used to determine the expression density of a given receptor and its regulation by physiological and pathophysiological conditions as well as by drug treatment.

The identification of β-adrenoceptors at the protein level is typically based upon binding studies with radioligands such as [^{125}I]-iodocyanopindolol and its homologue [^{125}I]-iodopindolol, [^{3}H]-CGP 12,177 or [^{3}H]-dihydroalprenolol (Fig. 1). [^{125}I]-iodocyanopindolol and [^{3}H]-CGP 12,177 have much lower affinity for β_3- than for β_1 or β_2-adrenoceptors (Baker 2005; Hoffmann et al. 2004; Niclauß et al. 2006; Tate et al. 1991). While [^{3}H]-dihydroalprenolol has been proposed to label β_3-adrenoceptors in porcine lower urinary tract tissues (Yamanishi et al. 2002a,b), a systematic comparison of its binding to all three cloned human subtypes found only very poor labeling of β_3-adrenoceptors (Niclauß et al. 2006). This is

entirely consistent with the low β_3-adrenoceptor affinity of unlabeled alprenolol (Hoffmann et al. 2004). While high concentrations of [^{125}I]-iodocyanopindolol and [^{3}H]-CGP 12,177 have successfully been used to label β_3-adrenoceptors in transfected cells, the use of similarly high concentrations in tissues yields very high nonspecific binding and will saturate β_1- and β_2-adrenoceptors (Roberts et al. 1993). Moreover, at high concentrations, [^{125}I]-iodocyanopindolol may also bind to α_1-adrenoceptors (Brahmadevara et al. 2004), serotonin receptors (Hoyer et al. 1985), and other nonadrenergic sites (Sugasawa et al. 1997). Taken together, these problems make the detection of β_3-adrenoceptors in tissues expressing mixed β-adrenoceptor subtype populations almost impossible.

[^{3}H]-SB 206,606, a stereoisomer of BRL 37,344, has been introduced as a β_3-selective radioligand and found to label the cloned rat β_3-adrenoceptor or that natively expressed in rat brown adipose tissue (Klaus et al. 1995; Muzzin et al. 1994). However, its affinity for rat β_3-adrenoceptors is only low for a tritiated radioligand (K_d values of 200–500 nM), and, based upon data with nonradioactive BRL 37,344 (see above) may even be lower at the human receptor; this low affinity seriously limits its use in tissues. This may explain why no follow-up studies with this radioligand have been reported despite it being commercially available.

Based upon these difficulties in detecting β_3-adrenoceptor protein, studies into expression of the corresponding mRNA may be a suitable alternative. However, it should be noted that it has not been established whether alterations of β_3-adrenoceptor mRNA are predictive for those of functional receptor protein. While early studies into mRNA detection in rats (Muzzin et al. 1991) and humans (Berkowitz et al. 1995) have relied on techniques such as

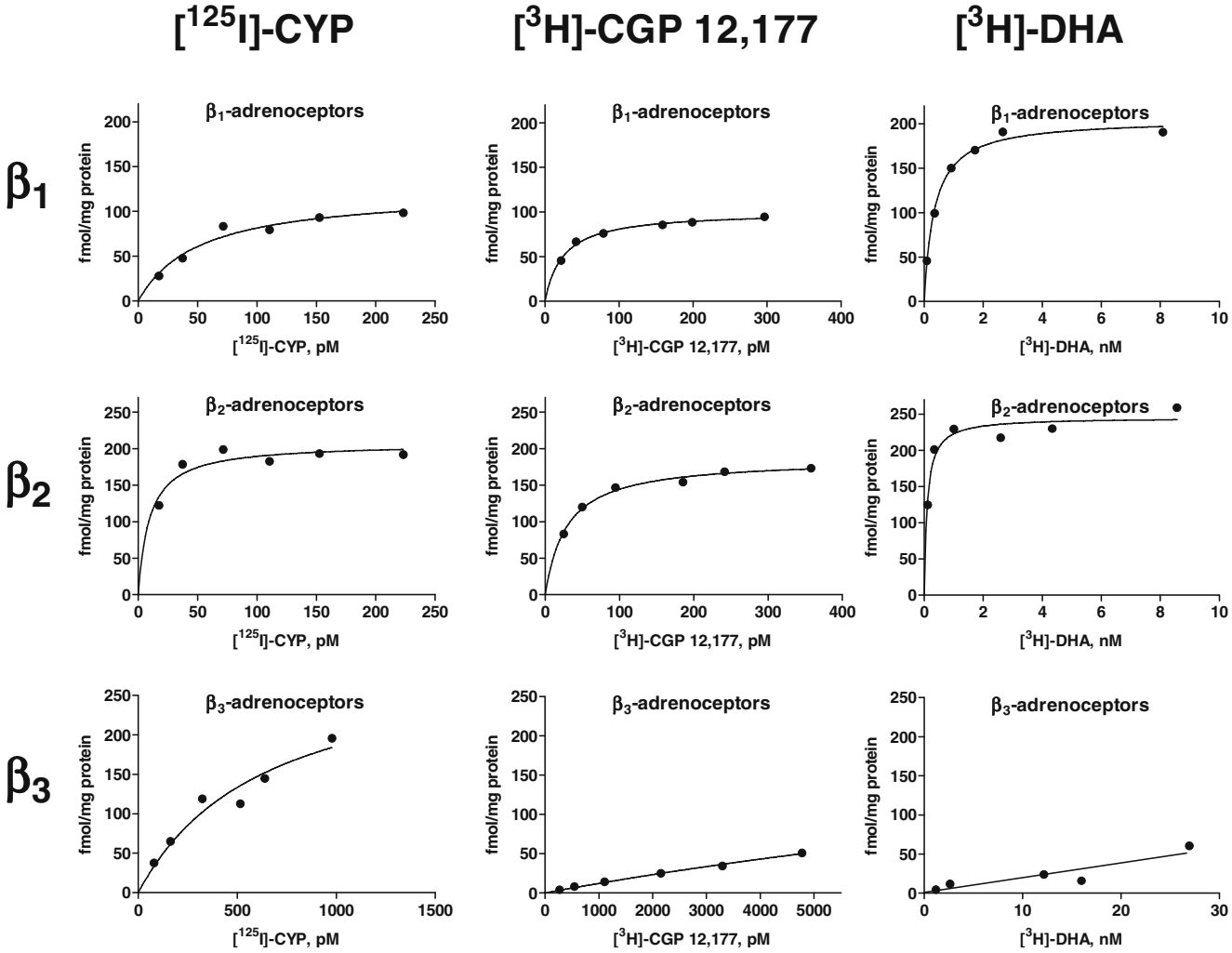


Fig. 1 Saturation binding isotherms for [^{125}I]-iodocyanopindolol ([^{125}I]-CYP), [^{3}H]-CGP 12,177 and [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) at human β-adrenoceptor subtypes. Taken from Niclauß et al. (2006)

Northern blotting or ribonuclease (RNAse) protection assays, this has meanwhile almost fully been superseded by real-time polymerase chain reaction (RT-PCR) techniques. The following studies give examples of primer pairs used for the measurement of β_3-adrenoceptor mRNA in mice (Lefrere et al. 2002; Monjo et al. 2005), rats (Alemzadeh and Tushaus 2004; Hatakeyama et al. 2004) and humans (Moniotte et al. 2001; Nomiya and Yamaguchi 2003). Based upon the sequence information of the β_3-adrenoceptor, it also appears possible to develop antisense or small interfering RNA (siRNA) approaches, but to the best of our knowledge, neither has been applied to this target until now.

In conclusion, a radioligand labeling all three β-adrenoceptor subtypes with similar affinity or a specific, high-affinity radioligand for β_3-adrenoceptors is still missing. This situation, along with the lack of validated specific β_3-adrenoceptor antibodies, seriously hampers further research into the presence and regulation of β_3-adrenoceptors in tissues. Whether measurements of corresponding mRNA can compensate for the lack of quantification at the protein level remains unknown.

Genetically modified animals

β_3-adrenoceptor knockout mice have been generated by two independent groups of investigators (Preitner et al. 1998; Susulic et al. 1995); while these two knockout strains display somewhat different phenotypes, the underlying reasons have not been fully elucidated. The lipolytic responses to CL 316,243 were abolished in such animals, but they exhibited only modestly increased fat stores, possibly due to a compensatory up-regulation of β_1- and, to a lesser extent, β_2-adrenoceptors (Susulic et al. 1995). On the other hand, adipocyte responses to CGP 12,177, similar to those to β_1- and β_2-adrenoceptor agonists, remained fully intact according to one study (Preitner et al. 1998), whereas another study reported that the high-affinity component remained intact and the low-affinity component disappeared (Konkar et al. 2000). Consequently, effects of CGP 12,177 in other tissues presumably representing the propranolol-resistant site of the β_1-adrenoceptor, such as heart (Kaumann et al. 1998) and colon, esophagus, and ureter (Oostendorp et al. 2000), also remained largely intact in β_3-adrenoceptor knockout mice. On the other hand, β_3-adrenoceptor knockout mice were also reported to lack the inhibitory effects of β_3-adrenoceptor agonists on gastrointestinal motility (Fletcher et al. 1998) or on ileum contraction (Hutchinson et al. 2001) as well the stimulatory effects on brain tryptophan content (Lenard et al. 2003). Transgenic mice with cardiac overexpression of the human β_3-adrenoceptor have been reported, but their physiological relevance remains unclear in light of the absence of cardiac β_3-adrenoceptor responses in wild-type mice (Kohout et al. 2001).

An alternative approach to the genetic analysis of the functional role of β_3-adrenoceptors is the use of β_1/β_2 double knockout mice. This approach is based upon the assumption that the three known β-adrenoceptor genes encode all functional β-adrenoceptors. Based on this assumption, any functional β-adrenoceptor response remaining in β_1/β_2 double knockout mice can be ascribed to β_3-adrenoceptors. This approach has been used and, e.g., allowed to determine that the proposed β_4-adrenoceptor represents a second site on the β_1-adrenoceptor rather than a distinct receptor (Devic et al. 2001; Kaumann et al. 2001; Zhou et al. 2000). However, it should be noted that compensatory β_3-adrenoceptor expression may occur in tissues of β_1/β_2 double knockout mice, which do not prominently express β_3-adrenoceptors in wild-type mice (Devic et al. 2001).

While knockout approaches can yield useful information on β_3-adrenoceptor in mice, any extrapolation of these findings to other species should be made with caution in light of the findings on species differences in ligand recognition profile and tissue expression pattern between rodent and nonrodent β_3-adrenoceptors.

Conclusions

At present, various molecular probes are available to study and modulate the expression of β_3-adrenoceptors. This is complemented by β_3-adrenoceptor knockout mice and β_1/β_2 double knockout mice. Considerable progress has also been made in the synthesis of β_3-adrenoceptor-selective agonists, although many of the most promising compounds are not yet available on a wide scale. A key bottleneck for research in this field is the lack of a widely available β_3-adrenoceptor-selective antagonist and, possibly derived thereof, a β_3-adrenoceptor-selective radioligand. Such antagonists are the only way to probe the functional role of β_3-adrenoceptors across multiple species, a need that is more pressing in this area than in many others due to the major differences between rodent and nonrodent receptors. Finally, highly selective β_3-adrenoceptor agonists that can be administered to humans will not only be helpful to better understand the role of this receptor subtype in humans but may also yield useful therapeutic agents. Clinical testing of some β_3-adrenoceptor agonists is currently under way.

Acknowledgement Work in the authors' lab has partly been funded by the Deutsche Forschungsgemeinschaft, Astellas and Boehringer Ingelheim.

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