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The odd sibling: features of β$_3$-adrenoceptor pharmacology[*]

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ABSTRACT

β_3-Adrenoceptor agonists have recently been introduced for the treatment of the overactive

urinary bladder syndrome. Their target, the β_3-adrenoceptor, was discovered much later than

β_1- and β_2-adrenoceptors and exhibits unique properties which make extrapolation of findings

from the other two subtypes difficult and the β_3-adrenoceptor a less understood subtype. This

article discusses three aspects of β_3-adrenoceptor pharmacology. Firstly, the ligand-

recognition profile of β_3-adrenoceptors differs considerably from that of the other two

subtypes, i.e. many antagonists considered as non-selective actually are β_3-sparing including

propranolol or nadolol. Many agonists and antagonists classically considered as being β_3-

selective actually are not, including BRL 37,344 or SR 59,230. Moreover, the binding pocket

apparently differs between the human and rodent β_3-adrenoceptor, yielding considerable

species differences in potency. Second, the expression pattern of β_3-adrenoceptors is more

restricted than that of other subtypes, particularly in humans; while this makes extrapolation

of rodent findings to the human situation difficult, it may result in a smaller potential for side

effects. The role of β_3-adrenoceptor gene polymorphisms has insufficiently been explored and

may differ even between primate species. Third, β_3-adrenoceptors lack the phosphorylation

sites involved in agonist-induced desensitization of the other two subtypes. Thus, they exhibit

down-regulation and/or desensitization in only some but not other cell types and tissues.

When desensitization occurs, it most often is at the level of mRNA or signaling molecule

expression. All three of these factors have implications for future studies to better understand

the β_3-adrenoceptor as a novel pharmacological target.

INTRODUCTION

Muscarinic receptor antagonists are the mainstay of symptomatic treatment of the overactive bladder syndrome, but due to limited efficacy and tolerability often are used for short periods only. β_3-Adrenoceptor agonists are an emerging alternative treatment option. In 2012 the first clinical proof of concept study for a member of this new drug class, solabegron, has been reported (Ohlstein et al., 2012); in 2013 the first member of this class, mirabegron (Chapple et al., 2014), has received marketing authorization in the US and Europe. This strengthened interest in β_3-adrenoceptors and their pharmacology.

Soon after the subdivision of β-adrenoceptors into the subtypes β_1 and β_2, it became clear that the pharmacological profile of some apparently β-adrenoceptor-mediated responses did not fit either of these two subtypes (Nergardh et al., 1977). However, the existence of a third subtype, the β_3-adrenoceptor, was not universally accepted until it was first cloned in 1989 (Emorine et al., 1989). In its 1994 adrenoceptor classification IUPHAR recognized the presence of three adrenoceptor subfamilies, the α_1-, α_2- and β-adrenoceptors, with β_1-, β_2- and β_3-adrenoceptors as members of the latter (Bylund et al., 1994).

Why did it take more than 30 and almost 25 years from discovery and cloning of the β_3-adrenoceptor, respectively, to the launch of its first ligand for clinical use? This manuscript discusses three areas which have historically limited studies on β_3-adrenoceptors but can also be seen as opportunities to develop highly targeted treatments, i.e. a unique and species-specific ligand recognition profile, a restricted expression, and a unique regulation pattern as compared to β_1- and β_2-adrenoceptors. Therapeutic opportunities resulting from this unique profile are discussed.

UNIQUE LIGAND RECOGNITION PROFILE

The canonical signaling of β_3-adrenoceptors, similar to the other subtypes, occurs via the G_s-cAMP pathway but coupling to G_i-proteins leading to activation of p38 protein kinase has also been reported (Sato et al., 2007;Sato et al., 2012). β_3-Adrenoceptors have similar affinity for norepinephrine as the other two subtypes but lower affinity for epinephrine (Table 1). Hence, all subtypes of innervated β-adrenoceptors are likely to similarly respond to neuronally released norepinephrine, particularly given its high concentrations in the synaptic cleft. On the other hand, circulating epinephrine may only poorly activate extrasynaptic β_3-adrenoceptors, potentially leading to some functional selectivity.

A key roadblock to the acceptance of β_3-adrenoceptors was its unique ligand recognition profile. For example, sensitivity to the classic antagonist propranolol has long been viewed as a defining feature of β-adrenoceptors, but the affinity of β_3-adrenoceptors for this antagonist is about two log units lower than that of β_1- or β_2-adrenoceptors (Table 1). Many other classic antagonists, including the clinically used atenolol, bisoprolol and metoprolol, also display considerably lower affinity for β_3- as compared to β_1- and/or β_2-adrenoceptors (Table 1). The term "non-selective" β-adrenoceptor antagonist should describe drugs with similar affinity for all three subtypes; however, often but wrongly it refers to similar affinity for β_1- and β_2-adrenoceptors only. We propose that ligands with similar affinity for β_1- and β_2- but much lower affinity for β_3-adrenoceptors should be referred to as "β_3-sparing" in the future.

Three antagonists which have often been used to block apparent β_3-adrenoceptor responses deserve special consideration. Bupranolol, while not being selective for the β_3-subtype, was found to inhibit some responses which were resistant to other antagonists such as nadolol or propranolol (Atef et al., 1996;Igawa et al., 1998;Takeda et al., 2002). Accordingly, bupranolol

has been used to explore β_3-adrenoceptor involvement in *in vivo* responses (Reverte et al.,

1993;Atef et al., 1996), particularly under conditions where the same response was not

blocked by antagonists such as nadolol. However, in studies with cloned human subtypes,

bupranolol also exhibited lower affinity for β_3- as compared to β_1- and particularly β_2-

adrenoceptors (Table 1).

Therefore, investigators have turned to antagonists which are supposedly β_3-selective. The

most frequently used one is SR 59,230, but it does not fulfill the criteria for a useful β_3-

selective antagonist in two ways. First, SR 59,230 has consistently failed to display β_3-

selectivity in studies with cloned human subtypes; if anything its affinity was somewhat less

than for the other two subtypes (Table 1). Second, SR 59,230 is a biased agonist at β_3-

adrenoceptors with poor efficacy for cAMP formation and greater efficacy for p38 activation

(Hutchinson et al., 2005;Sato et al., 2007). It is a partial agonist for smooth muscle relaxation

with an efficacy of up to 80% (Horinouchi and Koike, 2001;Frazier et al., 2011). These

findings limit the use of SR 59,230 as an antagonist to identify β_3-adrenoceptor responses.

Until recently, it was thought that L 748,337 may be a better alternative to selectively block

β_3-adrenoceptors. Indeed in radioligand binding studies with cloned human subtypes it

exhibited much higher affinity for β_3- than β_1- or β_2-adrenoceptors (K_i 4 vs. 390 and 204 nM,

respectively) (Candelore et al., 1999). Accordingly, a tritiated version of L 748,337 has been

proposed to be a β_3-selective radioligand in studies with human receptors (van Wieringen et

al., 2013). However, the latter studies also identified two possible problems in using L

748,337. Firstly, its affinity for rodent β_3-adrenoceptors apparently is considerably lower than

for the human subtype. Secondly, L 748,337 binds to a low affinity site which is distinct from

the catecholamine binding site of the β_3-adrenoceptor, an observation in line with those in

other studies (Baker, 2010); however, this site has not specifically been mapped and its

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relevance for function of the receptor remains unclear. Moreover, similar to SR 59,230, L 748,337 is a biased agonist with low efficacy for cAMP formation and much greater for p38 activation, the latter apparently involving a pertussis toxin-sensitive G-protein (Sato et al., 2008). Nonetheless L 748,337 remains the most suitable among the poor β_3-adrenoceptor antagonists. While there is no anticipated clinical use for a selective, non-biased, high-affinity β_3-adrenoceptor antagonist, such a compound would considerably support future research in this area. The molecular basis for the unique ligand recognition profile of β_3-adrenoceptors has not been established, as modelling studies have not been reported after the crystal structure of β_1- and β_2-adrenoceptors was revealed.

Similar problems have long existed for β_3-adrenoceptor agonists. Historically, BRL 37,344 has been used most often but is a poor choice (Vrydag and Michel, 2007). At least for human receptors it exhibits poor subtype-selectivity (Table 1), and accordingly in both rats (Mori et al., 2010) and humans (Pott et al., 2003) exerts many of its effects via β_1- or β_2-adrenoceptors. Moreover, at least when acting on β_2-adrenoceptors, it is a biased agonist favoring the G_s-cAMP pathway (Ngala et al., 2013) and, in high concentrations, can additionally exhibit muscarinic receptor antagonism (Vrydag and Michel, 2007). L 755,507 apparently recognizes two sites on the β_3-adrenoceptor, of which only that with higher affinity displays relevant selectivity towards the other subtypes (Baker, 2010). CL 316,243 has low potency at the human β_3-adrenoceptor; while it exhibits selectivity towards β_1-adrenoceptors, that towards β_2-adrenoceptors is only poor (Baker, 2005). More hope comes from β_3-adrenoceptor agonists which have entered clinical development such as mirabegron, ritobegron and solabegron; for experimental use of these compounds it should be considered that mirabegron and ritobegron may preferentially act on the human and rat orthologue, respectively (Igawa et al., 2012;Igawa and Michel, 2013).

Finally, many β₃-adrenoceptor ligands exhibit relevant species differences in affinity. For instance, the agonist BRL 37,344 (Nahmias et al., 1991;Liggett, 1992), the weak partial agonist CGP 12,177 (Liggett, 1992) and the antagonist/biased agonist L 748,337 (Candelore et al., 1999;van Wieringen et al., 2013) exhibit affinity differences of 10-fold or more between rat and human or rat and rhesus monkey β₃-adrenoceptors, indicating important species differences in the ligand binding pocket; a better understanding of such difference awaits resolving the crystal structure of β₃-adrenoceptors. Based on such species differences, several investigators have turned to monkeys to explore properties of novel β₃-adrenoceptor agonists (Maruyama et al., 2012;Hatanaka et al., 2013).

RECEPTOR POLYMORPHISMS AND EXPRESSION PATTERN

Soon after the cloning of the human β₃-adrenoceptor it became clear that the corresponding gene is polymorphic (Clement et al., 1995;Walton et al., 1995). The most frequently studied polymorphism is an exchange of tryptophan in position 64 for an arginine (Trp64Arg). The frequency of this polymorphism has been compared in cross-sectional studies for various conditions including obesity (Engelhardt and Ahles, 2014). While some of these studies have reported associations with disease, particularly with states compatible with hypofunctional β₃-adrenoceptors, many other studies have not confirmed such findings. While this could at least partly be linked to a reporting bias, the overall available data are in favor of the Trp64Arg polymorphism being associated with at least some pathophysiological conditions; however, such associations may be too weak to be robustly detected. Accordingly, the β₃-adrenoceptor gene locus has not shown up in genome-wide association studies for any common disease (Engelhardt and Ahles, 2014).

Recreation of the Trp64Arg polymorphism by site-directed mutagenesis studies has also

yielded inconsistent results (Vrydag et al., 2009;Engelhardt and Ahles, 2014). On the other

hand, sequencing studies showed that the Trp64Arg polymorphism forms a haploblock with

several polymorphisms in the non-coding part of the β_3-adrenoceptor gene, including single

nucleotide and TG dinucleotide length polymorphisms (Table 2). This raises the possibility

that Trp64Arg itself may not modify expression or function of the receptor but rather may be

an indicator for the presence of other polymorphisms; however, the functional role of these

non-coding polymorphisms has not been defined. Based on the use of non-human primates in

β_3-adrenoceptor research (Maruyama et al., 2012;Hatanaka et al., 2013), it is interesting that

the haploblock consistently identified in the human β_3-adrenoceptor gene is not mirrored in

non-human primates including chimpanzees (Table 2), indicating that it has emerged late in

phylogenesis.

The expression of β_3-adrenoceptor mRNA is more restricted than that of β_1- and β_2-

adrenoceptors and in humans largely limited to brown adipose tissue, gall bladder and ileum

and, at a lower level, in white adipose tissue and the urinary bladder (Thomas and Liggett,

1993;Berkowitz et al., 1995). Notably, only little β_3-adrenoceptor expression has been found

in human brain, heart, arteries, veins, liver, lung or skeletal muscle. The expression pattern in

rats (Muzzin et al., 1991) and mice (Regard et al., 2008) is qualitatively similar; while direct

comparative studies are largely lacking, it appears that expression in rodents in most tissues is

greater than in humans, for instance in brain, white and brown adipose tissue, stomach and

colon. Expression studies at the protein level have long been hampered by a lack of target

selectivity of many β_3-adrenoceptor antibodies (Cernecka et al., 2012). Suitable radioligands

have also been missing until recently (van Wieringen et al., 2013). Therefore, most tissue

mapping has relied on functional studies. While this has provided robust evidence for

dominant role of the β_3-subtype in human bladder smooth muscle relaxation (Michel and

Vrydag, 2006), the evidence for involvement of this subtype in the regulation of other tissues is less convincing due to a limited number of studies and poor selectivity of many of the tools used in those studies (Michel et al., 2010).

UNIQUE REGULATION PROFILE

The overactive bladder syndrome is the only validated therapeutic use of β_3-adrenoceptor agonists. In this indication they provide symptomatic relief but no cure of the condition (Chapple et al., 2014), necessitating long-term treatment. Therefore, agonist-induced β_3-adrenoceptor desensitization is considered undesirable in this indication to maintain therapeutic efficacy.

An interesting molecular feature of the β_3-adrenoceptor is the lack of phosphorylation sites implied in agonist-induced desensitization and down-regulation of β_1-and β_2-adrenoceptors (Liggett et al., 1993;Nantel et al., 1993). Accordingly, studies with β_3-adrenoceptors transfected into Chinese hamster ovary cells reported a lack of desensitization with agonist exposure of up to 1 h (Chaudhry and Granneman, 1994), whereas longer exposure (6-24 h) resulted in desensitization (Chambers et al., 1994;Candelore et al., 1996); however, the latter occurred in the absence of receptor down-regulation and was rather explained by a reduced G_s expression (Chambers et al., 1994). On the other hand, β_3-adrenoceptor desensitization and down-regulation can occur when transfected into other cell types such as human embryonic kidney cells (Chaudhry and Granneman, 1994;Michel-Reher and Michel, 2013). Studies with natively expressed β_3-adrenoceptor have yielded both negative (Carpene et al., 1993;Curran and Fishman, 1996) and positive findings (Granneman and Lahners, 1992;Bengtsson et al., 1996;Scarpace et al., 1999;Hutchinson et al., 2000), which is in sharp contrast to the desensitization of β_1-and β_2-adrenoceptors observed in almost every cell type ever studied.

Interestingly, in cases where β_3-adrenoceptor desensitization was observed it often occurred at

the level of receptor mRNA expression (Granneman and Lahners, 1992;Bengtsson et al.,

1996;Scarpace et al., 1999) or that of signaling molecules activated by the receptor (Chambers

et al., 1994;Michel-Reher and Michel, 2013), whereas down-regulation of the receptor itself

at the protein level has rarely been reported (Michel-Reher and Michel, 2013). The Trp64Arg

polymorphism of the receptor apparently affects neither the lack of down-regulation in

Chinese hamster ovary cells (Candelore et al., 1996) nor the desensitization in human

embryonic kidney cells (Vrydag et al., 2009). Taken together, agonist-induced β_3-

adrenoceptor desensitization can occur in some but not other cell types and tissues but, in line

with the lack of phosphorylation sites, when occurring largely involves down-regulation of

corresponding mRNA and/or an altered expression of signaling molecules.

Based on the above it cannot be extrapolated from other tissues whether desensitization

occurs in target tissue for agonist treatment, the urinary bladder. While such studies are

missing in human bladder, recent experiments in rat bladder, where relaxation involves both

β_2- and β_3-adrenoceptors (Michel and Vrydag, 2006), have explored this question (Michel,

2014). The β_2-component of relaxation exhibited desensitization upon a 6 h exposure to

agonists such as fenoterol or isoprenaline. In contrast, the β_3-component exhibited much less

if any desensitization, and was detectable for the experimental agonist CL 316,243 but not for

the clinically used agonist mirabegron. In line with these observations therapeutic effects

appeared stable over a one-year treatment period (Chapple et al., 2014).

THERAPEUTIC OPPORTUNITIES

Originally it had been assumed that β_3-adrenoceptor agonists may be useful in the treatment

of obesity and type 2 diabetes, but negative clinical proof-of-concept studies with multiple

compounds have invalidated this concept; the most likely reason for this is the differential

expression pattern between rodents and humans, particularly with regard to adipose tissue

(Muzzin et al., 1991;Thomas and Liggett, 1993;Berkowitz et al., 1995;Regard et al., 2008).

Thus, the only validated therapeutic use of β_3-adrenoceptor agonists is the symptomatic

treatment of the overactive bladder syndrome. Human bladder relaxation is mediated

predominantly if not exclusively by the β_3-adrenoceptor subtype (Igawa et al., 2012), and β-

adrenoceptor agonists are effective in every experimental model of bladder dysfunction ever

investigated (Michel and Barendrecht, 2008). Accordingly, two β_3-adrenoceptor agonists have

shown efficacy in placebo-controlled studies, i.e. solabegron (Ohlstein et al., 2012) and

mirabegron (Chapple et al., 2014), the latter recently having obtained regulatory approval. A

notable observation in these studies was a tolerability profile close to placebo, apparently

reflecting the limited expression of β_3-adrenoceptors outside the urinary bladder. The current

standard of care in overactive bladder treatment is muscarinic receptor antagonists.

Considerable experimental evidence supports the view that combinations of β-adrenoceptor

agonists and muscarinic antagonists exhibit at least additive effects on smooth muscle tone

(Dale et al., 2014). In the human bladder physiological contraction during voiding is

predominantly mediated by muscarinic receptors of the M_3 subtype (Schneider et al., 2004),

but in pathological settings additional mediators such as ATP or bradykinin may contribute.

Therefore, it was interesting to observe that β-adrenoceptor agonists produce weaker

inhibition of bladder contraction by a muscarinic agonist as compared to any other contractile

stimulus (Michel and Sand, 2009). In this regard a β_3-selective agonist showed less consistent

differences between contractile stimuli than isoprenaline (Table 3), probably reflecting that in

contrast to human bladder that of rat bladder involves a mixture of β_2- and β_3-adrenoceptors

(Michel and Vrydag, 2006). These findings have two implications with clinical relevance:

First, β_3-adrenoceptor agonists will preferentially inhibit pathological contractile stimuli over

physiological voiding. Second, to enable inhibition by an increased cholinergic tone in overactive bladder a combination with a muscarinic antagonist appears promising. Clinical data will be required to test this concept, and initial data are emerging (Abrams et al., 2014).

Based on animal studies and/or *ex vivo* studies with human tissue several other possible uses of β_3-adrenoceptor agonists have been proposed including tocolysis (Bardou et al., 2007), congestive heart failure (Rasmussen et al., 2009), retinal disease (Gericke et al., 2013) and anxiety and depression (Stemmelin et al., 2008). However, all of these will require validation studies in patients (Michel et al., 2010).

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Footnotes

Conflict of interest

HC is employed by a grant from Astellas to her institution. CS does not report a conflict of interest. MCM has received consultant honoraria and/or research support in the β_3-adrenoceptor field from AltheRX, Astellas and Boehringer Ingelheim; he currently is an employee of Boehringer Ingelheim.

<u>Table 1</u>: Affinity comparison of commonly used ligands at human β-adrenoceptor subtypes.

Values represent ratios of reported affinity estimates (K_i values β_3/β_1 and β_3/β_2) based on

(Blin et al., 1993;Candelore et al., 1999;Hoffmann et al., 2004;Baker, 2005;Niclauß et al.,

2006;Baker, 2010). Values <1 represent selectivity for β_3-adrenoceptors, whereas those >1

represent selectivity for the other subtypes; data are range of 2-4 reports.

	K_i value ratio β_3/β_1	K_i value ratio β_3/β_2
Norepinephrine	1.6-2.1	0.16-0.77
Epinephrine	2.8-32	27-171
Isoproterenol	3.5-7.0	3.4-13
Salbutamol	2.1-22	25-107
Salmeterol	0.25-4.5	292-1259
BRL 37,344	0.01-0.05	0.05-1.2
Atenolol	168-355	76-80
Bisoprolol	145-405	7.9-11
Bupranolol	29-29	125-650
Metoprolol	126-215	3.4-54
Propranolol	17-103	141-233
CGP 12,177	17-166	18-251
CGP 20,712	502-4169	0.58-8.3
CL 316,243	0.008	0.10
ICI 118,551	1.2-12	661-873
L 748,337	0.003-0.01	0.02-0.03
SR 59,230	1.1-7.4	2.0-12

Table 2: Genotypes of human, chimpanzee and macaque β_3-adrenoceptors. The mutation and position of the 5 SNPs are depicted in column 1 and 2, respectively. SNP T190C is the Trp64Arg mutation. Δtg3273/3274 refers to the repeat polymorphism, where the nine repeats in wild-type are reduced to eight in the polymorphic human gene. Based upon data from (Michel et al., 2008;Vrydag et al., 2009;Teitsma et al., 2013).

Polymorphism	Position	Wild-type		
		Human	Chimpanzee	Macaque
T190C	Coding	T	C	C
g1219t	Intron	g	g	g
a2135g	Intron	a	a	a
g25002c	3'-UTR	g	c	c
a3558t	3'-UTR	a	t	t
Δtg3273/32744	3'-UTR	-	-	-

Table 3: Potency (pEC$_{50}$) and efficacy (E$_{max}$, expressed as % of 10 µM forskolin-induced relaxation) of the β$_3$-adrenoceptor agonist KUC-7322 (Igawa et al., 2012) and isoprenaline against various pre-contraction stimuli in rat bladder. Isoprenaline data are adapted from (Michel and Sand, 2009), those for KUC-7322 were obtained within the same series of experiments. Data are expressed as means ± SEM of 6-8 experiments, *$p < 0.05$ versus carbachol-induced contraction in a one-way ANOVA followed by Dunnett's multiple comparison tests.

	Time control	KUC-7322		isoprenaline	
	E$_{max}$	pEC$_{50}$	E$_{max}$	pEC$_{50}$	E$_{max}$
Passive tension	21 ± 4	7.29 ± 0.09	90 ± 3	8.76 ± 0.08*	84 ± 2*
KCl	23 ± 2	7.09 ± 0.07	75 ± 2	8.00 ± 0.06*	80 ± 1*
Carbachol	13 ± 2	6.10 ± 0.67	71 ± 14	7.27 ± 0.15	57 ± 2
Serotonin	15 ± 2	7.36 ± 0.11*	95 ± 3*	8.54 ± 0.05*	91 ± 1*
Bradykinin	16 ± 3	7.03 ± 0.08	82 ± 2	8.66 ± 0.10*	79 ± 2*