β3-Adrenoceptor detection and signal transduction: focus on antibody validation and urinary bladder
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CHAPTER 2

The odd sibling features of $\beta_3$-adrenoceptor pharmacology

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The odd sibling features of 3-adrenoceptor pharmacology
ABSTRACT

\(\beta_3\)-Adrenoceptor agonists have recently been introduced for the treatment of the overactive urinary bladder syndrome. Their target, the \(\beta_3\)-adrenoceptor, was discovered much later than \(\beta_1\) and \(\beta_2\)-adrenoceptors and exhibits unique properties which make extrapolation of findings from the other two subtypes difficult and the \(\beta_3\)-adrenoceptor a less understood subtype. This article discusses three aspects of \(\beta_3\)-adrenoceptor pharmacology. Firstly, the ligand recognition profile of \(\beta_3\)-adrenoceptors differs considerably from that of the other two subtypes, i.e. many antagonists considered as non-selective actually are \(\beta_3\)-sparing including propranolol or nadolol. Many agonists and antagonists classically considered as being \(\beta_3\)-selective actually are not, including BRL 37,344 or SR 59,230. Moreover, the binding pocket apparently differs between the human and rodent \(\beta_3\)-adrenoceptor, yielding considerable species differences in potency. Second, the expression pattern of \(\beta_3\)-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 \(\beta_3\)-adrenoceptor gene polymorphisms has insufficiently been explored and may differ even between primate species. Third, \(\beta_3\)-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 \(\beta_3\)-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. \(\beta_3\)-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 \(\beta_3\)-adrenoceptors and their pharmacology.

Soon after the subdivision of \(\beta\)-adrenoceptors into the subtypes \(\beta_1\) and \(\beta_2\), it became clear that the pharmacological profile of some apparently \(\beta\)-adrenoceptor-mediated responses did not fit either of these two subtypes (Nergardh et al., 1977). However, the existence of a third subtype, the \(\beta_3\)-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 \(\alpha_1\)-, \(\alpha_2\)- and \(\beta\)-adrenoceptors, with \(\beta_1\), \(\beta_2\) and \(\beta_3\)-adrenoceptors as members of the latter (Bjeldstedt et al., 1994).

Why did it take more than 30 and almost 25 years from discovery and cloning of the \(\beta_3\)-adrenoceptor, respectively, to the launch of its first ligand for clinical use? This manuscript discusses three areas which have historically limited studies on \(\beta_3\)-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
The odd sibling features of $\beta_3$-adrenoceptor pharmacology compared to $\beta_1$- and $\beta_2$-adrenoceptors. Therapeutic opportunities resulting from this unique profile are discussed.

**Unique Ligand Recognition Profile**

The canonical signaling of $\beta_3$-adrenoceptors, similar to the other subtypes, occurs via the $G_{\text{s}}$-cAMP pathway but coupling to $G_{\text{q}}$-proteins leading to activation of p38 protein kinase has also been reported (Sato et al., 2007; Sato et al., 2012). $\beta_3$-Adrenoceptors have similar affinity for norepinephrine as the other two subtypes but lower affinity for epinephrine (Table 1). Hence, all subtypes of innervated $\beta$-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 $\beta_3$-adrenoceptors, potentially leading to some functional selectivity. A key roadblock to the acceptance of $\beta_3$-adrenoceptors was its unique ligand recognition profile. For example, sensitivity to the classic antagonist propranolol has long been viewed as a defining feature of $\beta$-adrenoceptors, but the affinity of $\beta_3$-adrenoceptors for this antagonist is about two log units lower than that of $\beta_1$- or $\beta_2$-adrenoceptors (Table 1). Many other classic antagonists, including the clinically used atenolol, bisoprolol and metoprolol, also display considerably lower affinity for $\beta_3$- as compared to $\beta_1$- and/or $\beta_2$-adrenoceptors (Table 1). The term “non-selective” $\beta$-adrenoceptor antagonist should describe drugs with similar affinity for all three subtypes; however, often but wrongly it refers to similar affinity for $\beta_1$- and $\beta_2$-adrenoceptors only. We propose that ligands with similar affinity for $\beta_1$- and $\beta_2$- but much lower affinity for $\beta_3$-adrenoceptors should be referred to as “$\beta_3$-sparing” in the future.

Three antagonists which have often been used to block apparent $\beta_3$-adrenoceptor responses deserve special consideration. Bupronolol, while not being selective for the $\beta_3$-subtype, was found to inhibit some responses which were resistant to other antagonists such as nadolol or propranolol (Atif et al., 1996; Igawa et al., 1998; Takeda et al., 2002). Accordingly, bupronolol has been used to explore $\beta_3$-adrenoceptor involvement in *in vivo* responses (Reverte et al., 1993; Atif 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, bupronolol also exhibited lower affinity for $\beta_3$- as compared to $\beta_1$- and particularly $\beta_2$-adrenoceptors (Table 1). Therefore, investigators have turned to antagonists which are supposedly $\beta_3$-selective. The most frequently used one is SR 59,230, but it does not fulfill the criteria for a useful $\beta_3$-selective antagonist in two ways. First, SR 59,230 has consistently failed to display $\beta_3$-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 $\beta_3$-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 $\beta_3$-adrenoceptor responses.
Table 1: Affinity comparison of commonly used ligands at human β-adrenoceptor subtypes. Values represent ratios of reported affinity estimates (Ki values β3/β1 and β3/β2) 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 β3-adrenoceptors, whereas those >1 represent selectivity for the other subtypes; data are range of 2-4 reports. Ki value ratio β3/β1, Ki value ratio β3/β2.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Kᵢ value ratio β3/β1</th>
<th>Kᵢ value ratio β3/β2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>1.6-2.1</td>
<td>0.16-0.77</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>2.8-32</td>
<td>27-171</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>3.5-7.0</td>
<td>3.4-13</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>2.1-22</td>
<td>25-107</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>0.25-4.5</td>
<td>292-1259</td>
</tr>
<tr>
<td>BRL 37,344</td>
<td>0.01-0.05</td>
<td>0.05-1.2</td>
</tr>
<tr>
<td>Atenolol</td>
<td>168-355</td>
<td>76-80</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>145-405</td>
<td>7.9-11</td>
</tr>
<tr>
<td>Bupranolol</td>
<td>29-29</td>
<td>125-650</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>126-215</td>
<td>3.4-54</td>
</tr>
<tr>
<td>Propranolol</td>
<td>17-103</td>
<td>141-233</td>
</tr>
<tr>
<td>CGP 12,177</td>
<td>17-166</td>
<td>18-251</td>
</tr>
<tr>
<td>CGP 20,712</td>
<td>502-4169</td>
<td>0.58-8.3</td>
</tr>
<tr>
<td>CL 316,243</td>
<td>0.008</td>
<td>0.10</td>
</tr>
<tr>
<td>ICI 118,551</td>
<td>1.2-12</td>
<td>661-873</td>
</tr>
<tr>
<td>L 748,337</td>
<td>0.003-0.01</td>
<td>0.02-0.03</td>
</tr>
<tr>
<td>SR 59,230</td>
<td>1.1-7.4</td>
<td>2.0-12</td>
</tr>
</tbody>
</table>

Until recently, it was thought that L 748,337 may be a better alternative to selectively block β3-adrenoceptors. Indeed in radioligand binding studies with cloned human subtypes it exhibited much higher affinity for β3- than β1- or β2-adrenoceptors (Ki 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 β3-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 β3-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 β3-adrenoceptor, an observation in line with those in other studies (Baker, 2010); however, this site has not specifically been mapped and its 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 β3-adrenoceptor antagonists. While there is no anticipated clinical use for a selective, non-biased, high-affinity β3-adrenoceptor antagonist, such a compound would considerably support future research in this area. The molecular basis for the unique ligand recognition profile of β3-adrenoceptors has not been established, as modelling studies have not been reported after the crystal structure of β1- and β2-
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adrenoceptors was revealed. Similar problems have long existed for \( \beta_3 \)-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 \( \beta_1 \)- or \( \beta_2 \)-adrenoceptors. Moreover, at least when acting on \( \beta_2 \)-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 \( \beta_3 \)-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 \( \beta_3 \)-adrenoceptor; while it exhibits selectivity towards \( \beta_1 \)-adrenoceptors, that towards \( \beta_2 \)-adrenoceptors is only poor (Baker, 2005). More hope comes from \( \beta_3 \)-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 \( \beta_3 \)-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 (Candello 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 \( \beta_3 \)-adrenoceptors, indicating important species differences in the ligand binding pocket; a better understanding of such difference awaits resolving the crystal structure of \( \beta_3 \)-adrenoceptors. Based on such species differences, several investigators have turned to monkeys to explore properties of novel \( \beta_3 \)-adrenoceptor agonists (Maruyama et al., 2012; Hatanaka et al., 2013).

**Receptor Polymorphisms and Expression Pattern**

Soon after the cloning of the human \( \beta_3 \)-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 \( \beta_3 \)-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 \( \beta_3 \)-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 \( \beta_3 \)-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 β3-adrenoceptor research (Maruyama et al., 2012; Hatanaka et al., 2013), it is interesting that the haploblock consistently identified in the human β3-adrenoceptor gene is not mirrored in non-human primates including chimpanzees (Table 2), indicating that it has emerged late in phylogenesis.

Table 2: Genotypes of human, chimpanzee and macaque β3-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).

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Position</th>
<th>Wild-type</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>T190C</td>
<td>Coding</td>
<td>T</td>
</tr>
<tr>
<td>g1219t</td>
<td>Intron</td>
<td>g</td>
</tr>
<tr>
<td>a2135g</td>
<td>Intron</td>
<td>a</td>
</tr>
<tr>
<td>g25002c</td>
<td>3'-UTR</td>
<td>g</td>
</tr>
<tr>
<td>a3558t</td>
<td>3'-UTR</td>
<td>a</td>
</tr>
<tr>
<td>Δtg3273/3274</td>
<td>3'-UTR</td>
<td>-</td>
</tr>
</tbody>
</table>

The expression of β3-adrenoceptor mRNA is more restricted than that of β1- and β2-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 β3-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 β3-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 β3-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 β3-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 β3-
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adrenoceptor desensitization is considered undesirable in this indication to maintain therapeutic efficacy. An interesting molecular feature of the β3-adrenoceptor is the lack of phosphorylation sites implied in agonist-induced desensitization and down-regulation of β1- and β2-adrenoceptors (Liggett et al., 1993; Nantel et al., 1993). Accordingly, studies with β3-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 Gs expression (Chambers et al., 1994). On the other hand, β3-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 β3-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 β1- and β2-adrenoceptors observed in almost every cell type ever studied.

Interestingly, in cases where β3-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 β3-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 β2- and β3-adrenoceptors (Michel and Vrydag, 2006), have explored this question (Michel, 2014). The β2-component of relaxation exhibited desensitization upon a 6 h exposure to agonists such as fenoterol or isoprenaline. In contrast, the β3-component exhibited much less if any desensitization, and was detectable for the experimental agonist CI 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 β3-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 $\beta_3$-adrenoceptor agonists is the symptomatic treatment of the overactive bladder syndrome. Human bladder relaxation is mediated predominantly if not exclusively by the $\beta_3$-adrenoceptor subtype (Igawa et al., 2012), and $\beta$-adrenoceptor agonists are effective in every experimental model of bladder dysfunction ever investigated (Michel and Barendrecht, 2008). Accordingly, two $\beta_3$-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 $\beta_3$-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 $\beta$-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 M3 subtype (Schneider et al., 2004), but in pathological settings additional mediators such as ATP or bradykinin may contribute.

Table 3: Potency (pEC50) and efficacy ($E_{max}$, expressed as % of 10 $\mu$M forskolin-induced relaxation) of the $\beta_3$-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.

<table>
<thead>
<tr>
<th></th>
<th>Time control</th>
<th>KUC-7322</th>
<th>Isoprenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_{max}$</td>
<td>pEC50</td>
<td>$E_{max}$</td>
</tr>
<tr>
<td>Passive tension</td>
<td>21 ± 4</td>
<td>7.29 ± 0.09</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>KCl</td>
<td>23 ± 2</td>
<td>7.09 ± 0.07</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>Carbachol</td>
<td>13 ± 2</td>
<td>6.10 ± 0.67</td>
<td>71 ± 14</td>
</tr>
<tr>
<td>Serotonin</td>
<td>15 ± 2</td>
<td>7.36 ± 0.11*</td>
<td>95 ± 3*</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>16 ± 3</td>
<td>7.03 ± 0.08</td>
<td>82 ± 2</td>
</tr>
</tbody>
</table>

Therefore, it was interesting to observe that $\beta$-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 $\beta_3$-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 $\beta_2$- and $\beta_3$-adrenoceptors (Michel and Vrydag, 2006). These findings have two implications with clinical relevance: First, $\beta_3$-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 $\beta_3$-adrenoceptor agonists have been proposed including tocolysis (Bardou et al., 2007), congestive
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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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