β3-Adrenoceptor detection and signal transduction: focus on antibody validation and urinary bladder
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CHAPTER 5

Desirable properties of \( \beta_3 \)-adrenoceptor agonists: Implications for the selection of drug development candidates

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Desirable properties of 3-adrenoceptor agonists
ABSTRACT

β3-Adrenoceptor agonists are currently in clinical development for the treatment of overactive bladder and considered for several other indications. This Perspective discusses desirable properties of such drugs mainly based on the example of overactive bladder, but at least partly should also be applicable to other indications of β3-adrenoceptor agonists or other drug classes and therapeutic areas. These include degree of selectivity for the molecular target in terms of affinity, intrinsic efficacy and ligand-directed signalling. The ability to cause agonist-induced desensitization and the potential impact of gene polymorphisms also need to be considered. Depending on intended indication, specific pharmacokinetic considerations may also apply. These findings challenge the usefulness of high-throughput screening assays based upon a single molecular response in an artificial system and emphasize the need for early use of in vivo testing in species considered to be predictive for the human situation.

Keywords: β3-adrenoceptor, overactive bladder, ligand-directed signalling, selectivity

1. INTRODUCTION

β1- and β2-adrenoceptor agonists and/or antagonist have long been a cornerstone of cardiovascular and pulmonary medicine. More recently β3-adrenoceptors have emerged as a therapeutic target. β3-Adrenoceptor agonist have been proposed as treatments for several indications. Obesity and type 2 diabetes mellitus were one of the earliest candidates, but proof-of-concept studies have failed for lead compounds from several companies, probably because the expression pattern of β3-adrenoceptors in most animal species differs considerably from that in man, particularly in adipose tissue (Arch, 2008). Based upon animal studies and/or in vitro studies with human tissues, congestive heart failure (Rasmussen et al., 2009), retinopathy (Mori et al., 2010), depression and anxiety (Stemmelin et al., 2008) and tocolysis (Bardou et al., 2007) have been proposed as possible indications, but none of these proposals has undergone clinical proof-of-concept testing. On the other hand, a considerable body of evidence supports the concept of using β3-adrenoceptor agonist as a treatment for the overactive bladder symptom complex (OAB) (Michel and Vrydag, 2006). Several compounds are currently in clinical development for this indication, and at least for one of them, mirabegron (formerly known as YM 178) clinical proof-of-concept data have been presented (Chapple et al., 2010; Chapple et al., 2008). Against this background we will discuss desirable properties of β3-adrenoceptor agonists as therapeutics.

2. RISK FOR OFF-TARGET EFFECTS

β1- and even more so β2-adrenoceptors are widely expressed in human tissues. In contrast, β3-adrenoceptors show a much more restricted mRNA expression pattern with high expression e.g. in the urinary bladder and the gall bladder (Krief et al., 1993; Berkowitz et al., 1995). The expression of functional β3-adrenoceptor protein is much less established, largely due to limited suitability of available pharmacological tools (Michel et al., 2010). Within the urinary bladder, β3-adrenoceptor agonists could have beneficial effects by directly acting on smooth muscle but also by affecting mediator release from the urothelium (Masunaga et al., 2010;
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Otsuka et al., 2008) and/or afferent nerve activity (Aizawa et al., 2010; Leon et al., 2008). The limited expression pattern at the mRNA level suggests that highly selective $\beta_3$-adrenoceptor agonists, at least when used in the treatment of OAB, will affect few other tissues, but this remains to be explored in greater detail.

A second source of off-target effects does not relate to $\beta_3$-adrenoceptors in other tissues but rather to activation of $\beta_1$- and/or $\beta_2$-adrenoceptors. Due to the wide-spread expression of the latter, such activation is possible if the chosen $\beta_3$-adrenoceptor agonist has limited selectivity for its cognate receptor. Given that some $\beta_1$- and/or $\beta_2$-adrenoceptor functions exhibit major receptor reserve (Brown et al., 1992), even a minor cross-reactivity with these other subtypes may cause detectable off-target effects.

3. LIGAND CHARACTERISTICS

While the selectivity of an antagonist is largely determined by its affinity for the target vs. other receptors, that of an agonist is more complex and can theoretically be achieved by a combination of two properties of the drug, subtype-selectivity for target recognition and for intrinsic activity at the target, two parameters which are not necessarily related (Baker, 2010). Given the problems with receptor reserve as well as the potential for an uneven drug distribution across body compartments/tissues, the desirable binding selectivity of an agonist exceeds what is considered adequate in most antagonist cases. While many antagonists considered being “selective” for $\beta_1$- over $\beta_2$-adrenoceptors have only 10-100 fold selectivity (Hoffmann et al., 2004; Baker, 2005), the desired selectivity margin for agonists should probably exceed this and may easily require to be 1000-fold. One possibility to enhance functional selectivity would be a much higher intrinsic activity at $\beta_3$- as compared to $\beta_1$- and/or $\beta_2$-adrenoceptors. While this seems to be the case with some of the compounds currently in clinical development such as mirabegron (Takasu et al., 2007), a low intrinsic activity at $\beta_1$- and/or $\beta_2$-adrenoceptors implies that a drug could act as an antagonist at these receptors. This could have considerable safety implications, e.g. in patients with obstructive airway disease, if sufficient occupancy of these subtypes is achieved by therapeutic doses. Therefore, a strong component of selective bimolecular interaction between agonist and receptor, as determined e.g. in radioligand binding studies (Hoffmann et al., 2004; Baker, 2010), is desirable to be a main cause of functional selectivity.

Historically, the interaction between an agonist and its receptor has been assumed to be very straightforward. I.e. even if there are multiple signalling responses to activation of that receptor, it was assumed that they are the same for each agonist at this receptor. We meanwhile know that this is not the case and that each ligand can induce a specific receptor confirmation which prefers a distinct signalling response (Michel and Alewijnse, 2007). This phenomenon has been designated “ligand-directed signalling” or “biased agonism” and has specifically also been shown to occur with $\beta_3$-adrenoceptors (Evans et al., 2010). As a result, a $\beta_3$-ligand, such as SR59230A, may display agonistic properties for activation of a specific signal transduction pathway, but might act as an antagonist for others, often depending on the receptor properties, such as the level of its expression (Sato et al., 2007). Such ligand-directed signalling could be a third source of generating functional selectivity for a desired response as compared to off-target effects. Interestingly, the signalling pathway underlying urinary bladder
smooth muscle relaxation apparently is largely independent of cAMP but has not been fully identified (Frazier et al., 2008). Possible alternative signalling pathways in the urinary bladder could involve the activation of BKca channels in smooth muscle (Frazier et al., 2008) and the activation of NO formation in the urothelium (Birder et al., 2002). Hence, it remains unclear whether cAMP assays provide a reliable indicator of efficacy in the urinary bladder or other tissues which are potential sources of off-target effects. This lack of reliability requires that development candidates are screened functionally for their selectivity for the target response. Specifically for β3-adrenoceptor agonists targeted for the treatment of bladder dysfunction, the question remains what the cellular target is. While it has long been assumed that detrusor smooth muscle is the primary target, more recent findings raise the possibility that receptors expressed in the urothelium (Masunaga et al., 2010; Otsuka et al., 2008) and/or in afferent nerves (Aizawa et al., 2010; Leon et al., 2008) should also be considered. In the absence of sufficient data allowing definitive conclusions about the cellular target within the bladder, the concept of ligand-directed signalling may even require using whole animals in the validation of novel compounds in their selectivity for the target. Species differences in the expression of a given receptor may further complicate this issue (Michel and Vrydag, 2006).

4. ROLE OF DESENSITIZATION

An additional layer of complication in the use of agonists as drugs is their potential to cause sensitization which may limit their therapeutic effects. Based upon a lack of certain acceptor amino acids as phosphorylation sites believed to important in agonist-induced desensitization, it has long been believed that β3-adrenoceptors are relatively resistant to such desensitization (Nantel et al., 1993). Indeed experiments in transfected Chinese hamster ovary cells and some other cell types have supported this conclusion (Carpene et al., 1993; Chaudhry and Granneman, 1994). However, agonist-induced desensitization of β3-adrenoceptors has been found in other cell types such as human embryonic kidney cells (Chaudhry and Granneman, 1994; Vrydag et al., 2009). This raises the question whether and to which extent desensitization occurs in target and/or off-target tissues. Limited data in rat bladder indicate that desensitization may occur with some but not other β3-adrenoceptor agonists (Vrydag and Michel, 2009). Depending on the condition to be treated, the relative presence or absence of such desensitization may be another criterion to evaluate the suitability of β3-adrenoceptor agonists as drugs.

5. ROLE OF TARGET GENE POLYMORPHISMS

Similar to most other genes, that encoding the human β3-adrenoceptor is polymorphic. While the NCBI database lists many single nucleotide polymorphisms in the β3-adrenoceptor gene, the Trp64Arg polymorphism is the only one being present at more than 1% of the general population and having been explored in cross-sectional as well as mechanistic studies (Leineweber et al., 2004). Studies with polymorphisms of β1- and β2-adrenoceptors have shown that gene polymorphisms can affect agonist potency and/or efficacy and/or the susceptibility to agonist-induced desensitization (Leineweber et al., 2004). After several studies, largely based upon heterologous expression of polymorphisms generated by site-directed mutagenesis, the
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role of the Trp64Arg polymorphism for agonist responses remains unclear as increased, unchanged and decreased responses have been reported (Candelore et al., 1996; Pietri-Rouxel et al., 1997; Umekawa et al., 1999; Kimura et al., 2000; Isogaya et al., 2002; Vrydag et al., 2009). Given the prevalence of this polymorphism in the general population and potentially its even greater prevalence among OAB patients (Honda et al., 2006), it remains to be clarified whether similar effects on wild-type and mutated receptor are a desirable property of $\beta_3$-adrenoceptor agonists. Of note, the only studies exploring a possible impact of this polymorphism on desensitization have reported similar findings with wild-type and mutated receptor (Candelore et al., 1996; Vrydag et al., 2009).

6. PHARMACOKINETIC CONSIDERATIONS

Finally, there may be desirable pharmacokinetic properties in a $\beta_3$-adrenoceptor agonist. While a good penetration into the central nervous system is obviously required for the proposed anxiety and depression indication (Stemmelin et al., 2008), their use in OAB and other indications would probably benefit from a lack of penetration across the blood-brain-barrier to limit the potential of adverse effects.

Use of $\beta_3$-adrenoceptor agonists in OAB treatment raises an additional potentially desirable drug property that is renal excretion in active form (parent compound or active metabolite). Studies of muscarinic agonists used in OAB treatment have indicated that some are excreted in active form in urine, and that urine from treated subjects when instilled into the bladder of animal models of OAB has a beneficial effect (Chuang et al., 2008). If that property was also applicable to a $\beta_3$-adrenoceptor agonist for OAB treatment, it could provide yet another means to generate functional selectivity for target vs. off-target tissues, particularly if the cellular target resides in the urothelium rather than smooth muscle cells of the bladder.

7. CONCLUSIONS

While $\beta_3$-adrenoceptor agonists are a promising class of novel compounds for the treatment of OAB and perhaps other conditions, lack of basic knowledge on the cellular target of such drugs as well as relevant signalling mechanisms being involved complicate the selection of suitable drug development candidates. These data challenge reliance on high-throughput assays using artificial model systems and rather emphasize the need for using in vivo models predictive for the human situation early on in the selection of candidates for clinical development. Application of these ideas may help to prevent late-stage attrition, as recently reported for a $\beta_3$-adrenoceptor agonist developed by Kissei. While other compound classes and/or other therapeutic areas may have distinct consideration for specific aspects of these ideas, we feel that the general principles we discuss are likely to be applicable to many other areas of drug development.
REFERENCES


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