

Tissue functions mediated by β_3 -adrenoceptors—findings and challenges

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Abstract As β_3 -adrenoceptor agonists metamorphose from experimental tools into therapeutic drugs, it is vital to obtain a comprehensive picture of the cell and tissue functions mediated by this receptor subtype in humans. Human tissues with proven functions and/or a high expression of β_3 -adrenoceptors include the urinary bladder, the gall bladder, and other parts of the gastrointestinal tract. While several other β_3 -adrenoceptor functions have been proposed based on results obtained in animals, their relevance to humans remains uncertain. For instance, β_3 -adrenoceptors perform an important role in thermogenesis and lipolysis in rodent brown and white adipose tissue, respectively, but their role in humans appears less significant. Moreover, the use of tools such as the agonist BRL 37344 and the antagonist SR59230A to demonstrate functional involvement of β_3 -adrenoceptors may lead in many cases to misleading conclusions as they can also interact with other β -adrenoceptor subtypes or even non-adrenoceptor targets. In conclusion, we propose that many responses attributed to β_3 -adrenoceptor stimulation may need re-evaluation in the light of the development of more selective tools. Moreover, findings in experimental animals need to be extended to humans in order to better understand the potential additional indications and side effects of the β_3 -

adrenoceptor agonists that are beginning to enter clinical medicine.

Keywords β_3 -Adrenoceptor · BRL 37,344 · SR59230A · CL 316,243 · L-748,337 · Vasodilatation

While β_1 - and β_2 -adrenoceptor ligands have long assumed key roles in the treatment of various conditions such as coronary heart disease or obstructive airway disease, compounds acting on β_3 -adrenoceptors are only now undergoing a metamorphosis from experimental tools into therapeutic drugs, e.g., in the treatment of the overactive bladder syndrome (Chapple et al. 2008). The introduction of a new class of drugs is exciting but also generates uncertainty about possible safety and tolerability issues associated with this drug class. The determination of tissues where β_3 -adrenoceptors play a role has long been hampered by the lack of highly selective agonists and antagonists (Vrydag and Michel 2007). In this issue of the journal, Mori et al. report β_3 -adrenoceptor-mediated vasodilatation in rat retinal blood vessels *in vivo* (Mori et al. 2010). Their study expands our knowledge of tissue functions mediated by β_3 -adrenoceptors but also highlights the methodological challenges in this field. Against this background, we will briefly mention those tissues in which functional β_3 -adrenoceptors have been demonstrated with various degrees of certainty and discuss the implications for the therapeutic use of agonists acting at these receptors. This discussion will largely be based on examples and does not attempt to be comprehensive. Where possible, we will primarily focus on human tissues. Fields that have been extensively reviewed recently will only be mentioned briefly.

At the mRNA level, β_3 -adrenoceptors have been found in a range of human tissues including brown and white

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adipose tissue, small and large intestine, gall bladder, urinary bladder, and brain with low levels in heart and colon; no mRNA was detected in quadriceps and abdominal muscle, liver, lung, kidney, thyroid, or lymphocytes (Berkowitz et al. 1995; Krief et al. 1993; Otsuka et al. 2008). Studies in rats have detected β_3 -adrenoceptor mRNA mainly in brown and white adipose tissue, in various segments of the gastrointestinal tract, and in the urinary bladder (Cohen et al. 1995; Evans et al. 1996; Fujimura et al. 1999; Roberts et al. 1999), but as in humans, it is also present in brain (Summers et al. 1995). Antibody-based detection of β_3 -adrenoceptor expression at the protein level has been reported in human gall bladder, colon, prostate, right atrium, and gastrocnemius muscle, whereas no labelling was detected in lung, left ventricle, appendix, uterus, or thyroid (Chamberlain et al. 1999). Detection in adipose tissue from breast, perirenal, and axillary sites proved inconclusive due to problems of interpreting labelling of the thin-walled adipocytes (Chamberlain et al. 1999). While this study provided some validation of antibody selectivity, more recent data raise doubts about the validity of many other receptor antibodies (Michel et al. 2009), including those acting on β -adrenoceptor subtypes (Hamdani and van der Velden 2009; Pradidarcheep et al. 2009).

Based on rodent data, β_3 -adrenoceptors have long been associated with the promotion of lipolysis in adipocytes, mostly in brown adipose tissue. These findings have prompted drug discovery programmes in the fields of obesity and type 2 diabetes that have yielded disappointing results (Arch 2008) at least partly due to the distinct difference between the rodent and human pharmacophore, which led to the development of several drugs (e.g., BRL 37,344, CL 316,243) that were highly effective and selective in rodents (Arch et al. 1984; Bloom et al. 1992) but with little selectivity or efficacy in humans. The explanation that was adopted initially was that β_3 -adrenoceptors play an important role in rodent lipolysis but have a much smaller role in humans (Arner et al. 1991; Thomas and Liggett 1993). However, recent findings question this assumption and strongly suggest that there is metabolically active brown fat in humans (Nedergaard et al. 2007). Nevertheless, there is still debate as to whether and to what extent the metabolic effects of catecholamines in humans are mediated through β_1 - or β_3 -adrenoceptors (Nedergaard and Cannon 2010). If anything, the metabolic effects of β_3 -adrenoceptor agonists are likely to be beneficial in humans, but whether the extent of such effects is clinically relevant cannot be determined with certainty based upon the present data.

In contrast, β_3 -adrenoceptors play an important role in the urinary bladder of humans, likely to an even greater extent than in some animal species (Michel and Vrydag

2006). Within the urinary bladder, they mediate smooth muscle relaxation (Michel and Parra 2008), but they may also affect the function of the urothelium (Masunaga et al. 2010; Otsuka et al. 2008) and afferent nerves (Aizawa et al. 2010). Accordingly, the β_3 -adrenoceptor agonist mirabegron, previously known as YM-178, has shown efficacy in a clinical proof of concept study in patients with overactive bladder (Chapple et al. 2008) and is now in the late stages of clinical development for this indication. β_3 -Adrenoceptors may also play a role in the relaxation of human ureter (Park et al. 2000; Tomiyama et al. 2003; Wanajo et al. 2004), urethra (Yamanishi et al. 2003), and penis smooth muscle (Cirino et al. 2003).

β_3 -Adrenoceptors have also been proposed to play a role in the cardiovascular system, but the evidence for their role remains equivocal, particularly in humans. Thus, several studies have proposed functional β_3 -adrenoceptors in the rodent heart, which in contrast to β_1 - and β_2 -adrenoceptors may mediate negative inotropic effects (Rozec and Gauthier 2006). β_3 -Adrenoceptors mediating positive inotropic effects have been proposed in the human heart (Gauthier et al. 1996; Pott et al. 2003; Skeberdis et al. 2008), but other studies have failed to demonstrate this finding (Christ et al. 2010; Ikezono et al. 1987). On the other hand, reports of vasodilatation mediated by β_3 -adrenoceptors are more consistent (Guimaraes and Moura 2001; Rozec and Gauthier 2006), although the vast majority of these findings come from experimental animals that exhibit major inter-tissue and interspecies differences that make extrapolation to humans difficult. The study by Mori et al. (2010) adds interesting information in this regard as it demonstrates β_3 -adrenoceptor-mediated vasodilatation in retinal vessels based on drugs with validated selectivity at human subtypes such as the antagonist L-748,337. On the other hand, the same study also shows that concomitant systemic vasodilatation by putative β_3 -adrenoceptor agonists such as BRL 37,344 or CL 316,243, assessed as blood pressure reductions, is largely, if not completely, mediated by β_1 - or β_2 -adrenoceptors, being inhibited by propranolol but not L-748,337 (Mori et al. 2010). While these data suggest that there may be therapeutic potential for β_3 -adrenoceptor agonists in the treatment of diabetic retinopathy, it remains to be confirmed whether this can be extrapolated to humans. Moreover, based upon the expression of functional β_3 -adrenoceptors in retinal endothelial cells (Steinle et al. 2003), it remains to be determined whether vasodilatation of retinal vessels primarily involves smooth muscle or endothelium. Potential effects of β_3 -adrenoceptor agonists on the systemic circulation and the heart remain to be studied in more detail in humans, particularly with regard to a possible risk of hypotension and/or arrhythmia.

In line with the β_3 -adrenoceptor mRNA expression in the gastrointestinal tract of rats (Evans et al. 1996) and

humans (Roberts et al. 1997), various investigators have proposed the presence of functional receptors (largely based on rat and guinea pig studies) in the esophagus (de Boer et al. 1995; Lezama et al. 1996; Oostendorp et al. 2004), stomach (McLaughlin and MacDonald 1991) (Cohen et al. 1995; Horinouchi and Koike 2001), and small and large intestine (Bond and Clarke 1988; Hoey et al. 1996; Horinouchi and Koike 2001; Roberts et al. 1997; Roberts et al. 1999). Generally, β_3 -adrenoceptor agonists mediate smooth muscle relaxation in these tissues including human colon (Roberts et al. 1997). Accordingly, in *in vivo* studies, β_3 -adrenoceptor agonists slowed transit time in wild-type but not β_3 -adrenoceptor knockout mice (Fletcher et al. 1998). On the other hand, the β_3 -adrenoceptor agonist solabegron did not significantly alter human colonic transit during a 7-day administration (Grudell et al. 2008). Additional effects on the gastrointestinal tract included reports of enhanced gastric blood flow (Kuratani et al. 1994) and reduced gastric acid secretion (Coruzzi and Bertaccini 1997), leading to protection against gastric ulcers in experimental animals. Moreover, a β_3 -adrenoceptor agonist was reported to improve colitis in a rat model (Vasina et al. 2008). Functional β_3 -adrenoceptors have also been proposed in the gall bladder (Oriowo and Thulesius 1999) and in pancreas (Atef et al. 1996). Based on these results, some β_3 -adrenoceptor agonists may cause constipation, but they may also have protective effects against gastric ulcers and/or colitis.

Outside the abovementioned systems, functional β_3 -adrenoceptors have also been proposed in myometrium (Bardou et al. 2000; Bardou et al. 2007; Rouget et al. 2004). Whether responses to β_3 -adrenoceptor agonists are less prone to desensitization than β_2 -adrenoceptor agonists when used for tocolytic treatment remains to be established. β_3 -Adrenoceptors were also proposed in skeletal muscle (Roberts et al. 1993) and in the brain, where they apparently can mediate important effects in animal models of memory, anxiety, and depression (Gibbs et al. 2010; Hutchinson et al. 2007; Stemmelin et al. 2008). The latter effect may become useful therapeutically for agonists with good penetration into the brain.

Of note, most of the above studies have relied on the use of agonists and/or antagonists with little selectivity for this subtype such as SR59230A (Vrydag and Michel 2007). As highlighted by the study in this issue (Mori et al. 2010) and other recent data (Ngala et al. 2009), agonists such as BRL 37,344 or CL 316,243 can activate not only β_3 -adrenoceptors but also other β -adrenoceptor subtypes and possibly nonadrenoceptor targets. While SR59230A has relatively high affinity for β_3 -adrenoceptors in contrast to many classical β -adrenoceptor antagonists, it has at least similar, if not slightly higher, affinity for human β_1 - and β_2 -adrenoceptors and can be a partial agonist at β_3 -

adrenoceptors (Vrydag and Michel 2007). Therefore, most of the above studies have to be interpreted carefully in the light of the selectivity of the tools on which they are based. At present, L-748,337 appears to be the only widely available antagonist with a well-validated selectivity for human β_3 -adrenoceptors. Thus, several tissue functions that have until now been assigned to β_3 -adrenoceptors may require confirmation with these more selective tools. Another issue is that findings in rodents and other experimental animals are an important step but may not necessarily reliably predict findings in humans. For example, β_3 -adrenoceptors appear to be the predominant if not the only subtype-mediating relaxation of the human urinary bladder, whereas in rats and some other species, this function involves at least one other subtype (Michel and Vrydag 2006). Conversely, β_3 -adrenoceptors play an important role in rodent lipolysis, but their role is much less clear-cut in humans (Arch 2008).

In addition to off-target effects due to the presence of β_3 -adrenoceptors in tissues that are not being targeted for a therapeutic effect, it is also increasingly evident that drugs acting at this receptor cannot be classified simply as agonists, partial agonists, or antagonists, and that ligands can induce unique ligand-specific receptor conformations that can result in differential activation of particular signal transduction pathways (Urban et al. 2007), the phenomenon known (amongst other terms) as ligand-directed signaling bias. At the β_3 -adrenoceptor, L-748,337 is a competitive antagonist for cAMP accumulation but has high agonist potency and efficacy for ERK1/2 phosphorylation. Zinterol, which has agonist properties at the human β_3 -adrenoceptor (Hutchinson et al. 2006), has high efficacy for cAMP accumulation but lower efficacy than L-748,337 for both ERK1/2 and p38 MAPK phosphorylation (Sato et al. 2008). A similar reversal of efficacy was also seen with CL 316,243 and SR59230A acting at the mouse β_3 -adrenoceptor (Sato et al. 2007). When the functional readout is cAMP, CL 316,243 is a full agonist and SR59230A either a partial agonist or antagonist depending on the level of receptor expression. In the identical cells, but using extracellular acidification rate as the functional measure, both CL 316,243 and SR59230A are full agonists at all levels of receptor expression. Further analysis with selective MAPK inhibitors confirmed that SR59230A has much higher efficacy than CL 316,243 for MAPK signaling. These examples of reversal of efficacy provide strong support for the concept of ligand-directed signaling (Evans et al. 2010) and suggest that other factors may have to be taken into account when optimizing clinical efficacy of new drugs acting at β_3 -adrenoceptors.

A final aspect of the study by Mori et al. (2010) merits comment. They show that β_2 -adrenoceptor-mediated vasodilatation in the general circulation apparently undergoes

some desensitization in diabetes, whereas β_3 -adrenoceptor-mediated vasodilatation in retinal vessels does not. Indeed, the expression and function of β_1 - and β_2 -adrenoceptors undergoes extensive desensitization in disease states such as heart failure (Brodde 2007) or upon agonist treatment, e.g., in the context of tocolysis (Frambach et al. 2005), whereas β_3 -adrenoceptor responses may be much less susceptible to such regulation in most but not all cases (Chaudhry and Granneman 1994; Vrydag et al. 2009).

In conclusion we propose that many responses attributed to β_3 -adrenoceptor stimulation may need revalidation using truly selective tools. Moreover, findings in experimental animals need to be extended to humans in order to better understand the safety profile of β_3 -adrenoceptor agonists likely to enter clinical medicine soon.

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