

REVIEW ARTICLE

β_3 -Adrenoceptors in the normal and diseased urinary bladder—What are the open questions?

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β_3 -Adrenoceptor agonists are used in the treatment of overactive bladder syndrome. Although the relaxant response to adrenergic stimulation in human detrusor smooth muscle cells is mediated mainly via β_3 -adrenoceptors, the plasma concentrations of the therapeutic dose of mirabegron, the only clinically approved β_3 -adrenoceptor agonist, are considerably lower than the EC_{50} for causing direct relaxation of human detrusor, suggesting a mechanism of action other than direct relaxation of detrusor smooth muscle. However, the site and mechanism of action of β_3 -adrenoceptor agonists in the bladder have not been firmly established. Postulated mechanisms include prejunctional suppression of ACh release from the parasympathetic nerves during the storage phase and inhibition of micro-contractions through β_3 -adrenoceptors on detrusor smooth muscle cells or suburothelial interstitial cells. Implications of possible desensitization of β_3 -adrenoceptors in the bladder upon prolonged agonist exposure and possible causes of rarely observed cardiovascular effects of mirabegron are also discussed.

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1 | INTRODUCTION

The presence of a third subtype of **β -adrenoceptor** was proposed long ago, for example, in adipose tissue (Harms, Zaagsma, & Van der Wal, 1974), but its existence was only established unequivocally when a human gene encoding the **β_3 -adrenoceptor** was cloned (Emorine et al., 1989). β_3 -adrenoceptors exhibit a restricted expression pattern in humans (Michel & Gravas, 2016), with a somewhat broader but still restricted expression in rats (Roberts, Papaioannou, Evans, & Summers, 1999) and mice (Nahmias et al., 1991). A potential role of β_3 -adrenoceptors in the treatment of obesity and type 2 diabetes had been proposed based on the stimulation of lipolysis and thermogenesis in rats (Arch et al., 1984), but corresponding studies in humans

failed, presumably because of a lack of expression of β_3 -adrenoceptors in human adipose tissue (Michel & Korstanje, 2016).

The relaxation of human urinary bladder by β -adrenoceptor agonists and its inhibition by antagonists could not be explained by the known **β_1 -** and **β_2 -adrenoceptors** in early studies (Nergardh, Boreus, & Naglo, 1977), but unequivocal evidence for the involvement of β_3 -adrenoceptors in the regulation of human bladder smooth muscle tone only came much later (Igawa et al., 1998; Igawa et al., 1999; Takeda et al., 1999). This led to the identification of β_3 -adrenoceptors as a target for the treatment of bladder disease (Michel & Korstanje, 2016).

β_3 -adrenoceptor agonists are primarily used in the treatment of overactive bladder syndrome (OAB). OAB is a symptom complex characterized by urgency, usually associated with frequency and nocturia, with or without urgency incontinence, in the absence of any other local pathology (Abrams et al., 2002). It is a common chronic condition with a prevalence of 10–20% and has a substantial negative impact on the

Abbreviations: BOO, bladder outlet obstruction; DO, detrusor overactivity; ENT, equilibrative nucleoside transporter; IC, interstitial cell; NDO, neurogenic detrusor overactivity; NVC, non-voiding contraction; OAB, overactive bladder syndrome; SAA, single-unit afferent activity.

quality of life of patients. While β_3 -adrenoceptor agonists have proven effective and safe in the treatment of OAB (Chapple, Cardozo, Nitti, Siddiqui, & Michel, 2014; Ohlstein, von Keitz, & Michel, 2012; Yoshida, Takeda, Gotoh, Nagai, & Kurose, 2018), several important questions remain for a sound understanding of their mechanism of action. Against this background, we first summarize current knowledge on the expression and function of β_3 -adrenoceptors in the normal and diseased bladder and the characteristics of β_3 -adrenoceptor agonists for OAB treatment. Thereafter, we discuss key unknowns in relation to the use of β_3 -adrenoceptor agonists in human bladder pathology.

2 | EXPRESSION OF β_3 -ADRENOCEPTORS IN THE BLADDER

2.1 | mRNA level

The urinary bladder expresses mRNA for all three β -adrenoceptor subtypes (Michel & Vrydag, 2006). While all three subtypes have similar abundance in rat bladder (Barendrecht et al., 2009), it has been reported that the β_3 -adrenoceptor accounts for more than 95% of all β -adrenoceptor mRNA in the human detrusor (Nomiya & Yamaguchi, 2003). However, others reported a comparable expression of all three subtypes using whole bladder rather than only detrusor tissue (Uhlen et al., 2015). The localization of β_3 -adrenoceptor mRNA in the human detrusor has been explored in *in situ* hybridization studies (Takeda et al., 1999). However, the predictive value of β_3 -adrenoceptor mRNA for the presence of functional receptor protein remains uncertain.

2.2 | Protein level

Although many antibodies have limited selectivity for β_3 -adrenoceptors (Cernecka, Ochodnický, Lamers, & Michel, 2012), some antibodies have been validated as selective (Chamberlain et al., 1999; De Matteis et al., 2002; Guillaume et al., 1994). While the latter antibodies are still not perfect, it has been proposed that a combination of two antibodies, one directed against the N-terminus and one against the C-terminus of the receptor, may be informative if they yield the same staining pattern (Cernecka et al., 2012). This approach has successfully been applied (Coelho, Antunes-Lopes, Gillespie, & Cruz, 2017; Silva et al., 2017).

A recent immunohistochemistry study in the human bladder detected β_3 -adrenoceptors in smooth muscle fibres and, to a lesser extent, in urothelium and suburothelium (Silva et al., 2017). In contrast, another immunohistochemical study demonstrated β_3 -adrenoceptors colocalized with the vesicular ACh transporter and primarily in nerve fibres in the mucosa and muscular layers of the bladder but not in urothelium or smooth muscle; the cholinergic fibres expressing β_3 -adrenoceptors were found mostly in the suburothelium (Coelho et al., 2017). These two studies have used the same approach of concomitant labelling with multiple antibodies targeting different epitopes in the β_3 -adrenoceptor, even with the same antibodies. Earlier studies based on validated antibodies have reported β_3 -adrenoceptor expression to a greater extent in urothelium than

smooth muscle of the human bladder and also in suburothelial myofibroblast-like cells, intramural ganglions, Schwann cells, and intramural nerves (Limberg et al., 2010). Thus, various investigators using similar approaches and antibodies have obtained at least in part different results regarding the localization of β_3 -adrenoceptors in the human urinary bladder. The reasons for these divergent results are not fully clear. However, it should be noted that sensitivity and specificity of immunohistological staining depend not only on the antibody being used but also on other factors, including thickness of slices, fixation and denaturalization protocols (Jositsch et al., 2009), and type of microscopy. Therefore, it is possible that rather minor differences in experimental protocol may have led to major differences in staining pattern, making it difficult to determine which cell types within the urinary bladder β_3 -adrenoceptor are expressed at the protein level (Okeke, Gravas, & Michel, 2017).

3 | ROLES OF β_3 -ADRENOCEPTORS IN THE BLADDER PHYSIOLOGY

3.1 | Detrusor smooth muscle

Human detrusor smooth muscle expresses predominantly β_3 -adrenoceptor mRNA (Nomiya & Yamaguchi, 2003). Numerous studies show that relaxation of human bladder smooth muscle is mediated predominantly, if not exclusively, by the β_3 -adrenoceptor subtype; however, the situation is different in other species, for example, a mix of β_2 - and β_3 -adrenoceptors is involved in rat detrusor, which can complicate the interpretation of animal data (Igawa & Michel, 2013; Michel & Vrydag, 2006). There is no consistent evidence that **isoprenaline** can cause greater maximum bladder relaxation than selective β_2 - or β_3 -adrenoceptor agonists in any species. Potential reasons for this observation include the possibility that activation of one of the two receptor subtypes is sufficient for a maximum effect, indicating a possible redundancy of their effects. However, this is not applicable to the human bladder where the contribution of β_2 -adrenoceptors to relaxation is negligible (Michel & Vrydag, 2006). Secondly, β_2 -adrenoceptor agonists may act via β_3 -adrenoceptors because of limited selectivity. Thus, it has been proposed that **fenoterol** may cause relaxation of rat bladder smooth muscle via β_3 -adrenoceptors (Palea et al., 2012), but this has not been confirmed by other investigators. In addition, β -adrenoceptor agonists not only cause direct smooth muscle relaxation but also counteract contraction by stimulation of muscarinic receptors (Ehlert et al., 2007; Klausner, Rourke, Miner, & Ratz, 2009). β_3 -adrenoceptor agonists inhibited the contractile responses evoked by electrical field stimulation to a much greater extent than those evoked by exogenous application of ACh in isolated human detrusor smooth muscle strips (Rouget et al., 2014). Such additional action of β_3 -adrenoceptor agonists may be supported by a recent histological demonstration that an abundance of β_3 -adrenoceptor immunoreactivity was observed on ACh-containing nerve fibres coursing the suburothelium and the detrusor of the human bladder (Coelho et al., 2017). Work in rat detrusor has shown

that β -adrenoceptor agonists are more potent and more effective against any contractile stimulus other than muscarinic agonists (Cernecka, Pradidarcheep, Lamers, Schmidt, & Michel, 2014; Michel & Sand, 2009), which is in line with observations from various other types of smooth muscle (Dale et al., 2014). This raises the possibility that β_3 -adrenoceptor agonists can inhibit detrusor contractions induced by pathological stimuli such as bradykinin, but largely spare those involved in physiological voiding and mediated by muscarinic receptors.

3.2 | Urothelium

The urothelium actively participates in sensory functions, expressing various receptors for neurotransmitters and releasing neurotransmitters in response to various stimuli (Sellers, Chess-Williams, & Michel, 2018). At the mRNA level, all three β -adrenoceptor subtypes are expressed in the urothelium (Ochodnický et al., 2012; Otsuka, Shinbo, Matsumoto, Kurita, & Ozono, 2008; Tyagi, Thomas, Yoshimura, & Chancellor, 2009). Immunohistochemical studies suggest that β_3 -adrenoceptors are more abundant in the urothelium as compared to detrusor but β_2 -adrenoceptors appear more relevant for the regulation of urothelial function (Sellers et al., 2018). The cellular mechanisms of β_3 -adrenoceptor-mediated effects of the urothelium on bladder function are not fully clear but appear to involve the afferent pathways innervating the bladder via release of NO or an unidentified inhibitory factor (urothelial-derived inhibitory factor) from the urothelium (Figure 1).

3.3 | Suburothelial interstitial cells

One report, based on a poorly validated antibody, described no major difference in the staining intensity of β_3 -adrenoceptor between the

interstitial cells (ICs) and detrusor smooth muscle in the human urinary bladder, but both structures exhibited greater staining than the urothelium (Otsuka et al., 2013). The bladder suburothelium is abundant in sensory nerves and microvasculature, which may contribute to the maintenance of the function of the mucosa. Previous studies have demonstrated that the mucosa of guinea pig and pig bladders show spontaneous contractile activity, and one possible origin for these activities may be the suburothelial ICs (Bialosterski, van Koeveringe, van Kerrebroeck, Gillespie, & de Wachter, 2011; Hashitani, Takano, Fujita, Mitsui, & Suzuki, 2011; Heppner, Layne, Pearson, Sarkissian, & Nelson, 2011; Kushida & Fry, 2016; Moro, Leeds, & Chess-Williams, 2012; Moro, Uchiyama, & Chess-Williams, 2011). **Mirabegron**, a β_3 -adrenoceptor agonist, reduced the frequency of non-voiding contractions (NVCs), similar to spontaneous contractile activities, in rats with bladder outlet obstruction (BOO; Gillespie et al., 2012). These previous reports propose that β_3 -adrenoceptor agonists can inhibit spontaneous contractile activities in the urinary bladder via β_3 -adrenoceptors in ICs, and such spontaneous contractile activities may partly contribute to the development of bladder afferent hyperactivity (Aizawa et al., 2017). This would fit the theory of an urothelium-associated sensory web linking sensory function as well as voiding function (Figure 1; Apodaca, 2004). However, in another recent study (Silva et al., 2017) very different findings were reported, that is, that β_3 -adrenoceptors in human bladder were detected in smooth muscle fibres and, to a lesser extent, in the urothelium and suburothelium, and this may need further investigation.

3.4 | Afferent nerves

Myelinated A δ -fibres are located primarily within the detrusor smooth muscle layer, whereas unmyelinated C-fibres are more widespread and can be found not only in the detrusor but also in the lamina propria

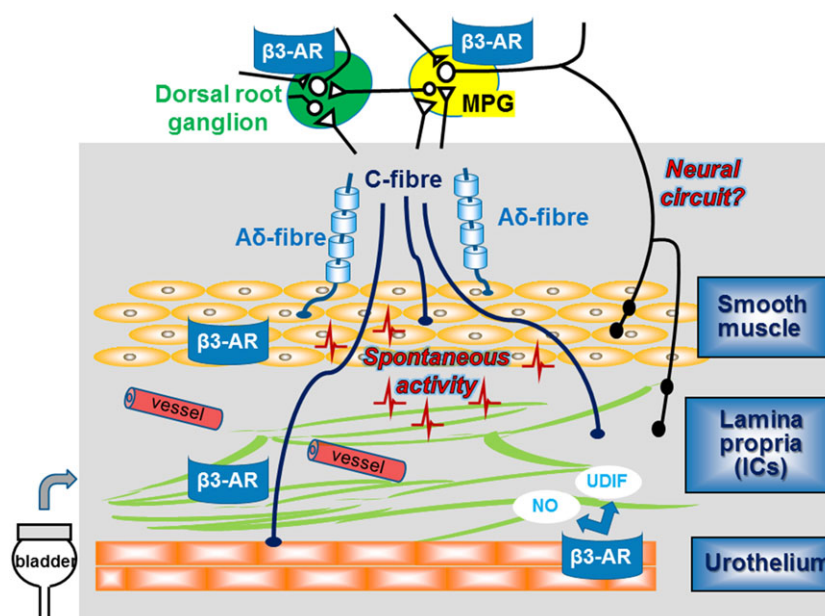


FIGURE 1 Expression of β_3 -adrenoceptors (β_3 -AR) and possible mechanisms that might be involved in the afferent pathway and modulation of spontaneous activity in the bladder

(including ICs), and often directly adjacent to the urothelial cells themselves (de Groat, 2004; Gabella & Davis, 1998; Ouslander, 2004; Vera & Nadelhaft, 2000). Previous studies indicated that β_3 -adrenoceptors are expressed on bladder afferent nerves (Coelho et al., 2017) and dorsal root ganglion neurons (Kanai et al., 2012). Furthermore, afferent activities of both A δ - and C-fibres were intermittently enhanced by propagation of bladder myogenic micro-contractions in BOO rats (Aizawa et al., 2017). Mirabegron can inhibit the mechanosensitive A δ -fibres, which may be related to suppression of the bladder micro-contractions even in normal rats (Aizawa, Homma, & Igawa, 2012). Moreover, another study has identified a population of small-diameter neurons in the major pelvic ganglion of the rat that express β_3 -adrenoceptor immunoreactivity (Eastham, Stephenson, Korstanje, & Gillespie, 2015), suggesting a possibility of involvement of β_3 -adrenoceptors on neural circuits in the regulation of afferent outflow and sensation (Figure 1).

3.5 | Bladder vasculature

Blood vessels providing perfusion to the bladder possibly contribute to the regulation of bladder function. In a rat model of bladder hypoperfusion caused by experimental atherosclerosis, the relaxation responses of isolated detrusor strips to isoprenaline and salbutamol remained unchanged whereas those to [BRL 37,344](#) were reported to be increased (Bayrak et al., 2015). In a different rat model of chronic bladder ischaemia induced by atherosclerosis, chronic treatment with mirabegron prevented bladder hyperactivity and collagen deposition in the bladder wall, suggesting β_3 -adrenoceptor agonism may be a potential treatment target for chronic ischaemia-related bladder dysfunction (Sawada et al., 2013).

3.6 | In vivo studies

The in vivo effects of β_3 -adrenoceptor agonists on bladder function have been studied mainly in rodents. β_3 -adrenoceptor agonists increase bladder capacity without changes in micturition pressure and the residual volume during the voiding phase (Fujimura et al., 1999; Hicks et al., 2007; Kaidoh et al., 2002; Takeda et al., 2002; Woods, Carson, Norton, Sheldon, & Argentieri, 2001). Similarly, mirabegron decreased the frequency of rhythmic bladder contraction without suppressing its amplitude under isovolumetric conditions (Takasu et al., 2007). Thus, activation of β_3 -adrenoceptors is associated with relaxation of the bladder during the storage phase of the micturition cycle without an effect on the voiding phase (Michel, Ochodnický, Homma, & Igawa, 2011; Nitti et al., 2013). While not covered here in detail, β_3 -adrenoceptors may also be involved in the regulation of other genitourinary tissues including the ureter (Matsumoto et al., 2013), prostate (Calmasini et al., 2015; Suzuki, Otsuka, Matsumoto, Furuse, & Ozono, 2016), and urethra (Alexandre et al., 2016).

β_3 -adrenoceptors have been proposed to inhibit spontaneous myogenic contractions that may possibly generate afferent activity

(Aizawa et al., 2017). Mirabegron can inhibit mechanosensitive bladder afferent activity, especially of myelinated A δ -fibres, which may be related to suppression of bladder myogenic spontaneous contractile activities at least under normal conditions (Aizawa et al., 2012). In addition, β_3 -adrenoceptor agonists reduced bladder myogenic activity in rats with BOO (Gillespie et al., 2012; Hatanaka et al., 2013; Woods et al., 2001). This may lead to inhibition of abnormal bladder sensation related to enhanced myogenic spontaneous contractile activities in pathophysiological situations.

4 | ROLES OF β_3 -ADRENOCEPTORS IN PATHOPHYSIOLOGY OF OAB

Physiological detrusor contraction during the voiding phase is predominantly mediated by muscarinic M₃ receptors in humans, but in OAB, additional mediators may contribute (Ouslander, 2004). The expression level of β -adrenoceptor subtype mRNA was not significantly different in the human detrusor muscle with or without BOO condition (Nomiya & Yamaguchi, 2003), and mirabegron concentration-dependently decreased [carbachol](#)-induced detrusor smooth muscle tone in bladder samples obtained from healthy subjects and patients with BOO and BOO with detrusor overactivity (DO) to a similar extent (Svalo et al., 2013). β_3 -adrenoceptor agonists may be more potent in decreasing nerve-evoked ACh release than in producing direct relaxation of human bladder smooth muscle (D'Agostino, Maria Condino, & Calvi, 2015; Rouget et al., 2014). This is in line with the observed abundance of β_3 -adrenoceptor immunoreactivity on ACh-containing nerve fibres coursing the suburothelium and the detrusor of the human bladder, leading to the hypothesis that β_3 -adrenoceptor agonists act, at least in part, through inhibition of ACh release from cholinergic, most probably parasympathetic, nerve terminals through a prejunctional mechanism (Coelho et al., 2017). According to this theory, β_3 -adrenoceptor agonists would preferentially inhibit pathologically-increased detrusor tone during bladder filling over physiological detrusor contraction during voiding and enabling them to inhibit an increased cholinergic tone in OAB. If so, combining them with a muscarinic antagonist would be promising therapeutic. While initial data support this idea (Abrams et al., 2017), dedicated clinical trials are required to confirm it.

5 | CHARACTERISTICS OF β_3 -ADRENOCEPTOR AGONISTS FOR OAB TREATMENT

Potency and efficacy at stimulating cAMP formation via β_1 -, β_2 -, and β_3 -adrenoceptor for each of the compounds mentioned in this section is summarized in Table 1.

5.1 | Mirabegron

Mirabegron (YM-178) has higher affinity and greater intrinsic activity at β_3 - as compared to β_1 - and β_2 -adrenoceptors (Table 1; Takasu et al.,

TABLE 1 EC₅₀ values for cAMP formation by β_3 -adrenoceptor (AR) agonists in CHO cells transfected with human β -adrenoceptor subtypes

Drugs	EC ₅₀			References
	β_1 -AR (nM)	β_2 -AR (nM)	β_3 -AR (nM)	
Mirabegron (YM-178)	>10,000	>10,000	22.4	Takasu et al. (2007)
Ritobegron (KUC-7483)	22,000	2,300	73	Maruyama et al. (2012)
Solabegron (GW427353)	3,980	1,260	123.98	Uehling et al. (2006)
Vibegron (MK-4618)	>20,000	>20,000	1.1	Edmondson et al. (2016)

2007). The efficacy and safety of mirabegron has been shown in numerous randomized placebo-controlled studies in OAB (Chapple et al., 2014), leading to it being the first β_3 -adrenoceptor agonist for the treatment of OAB.

5.2 | Ritobegron

The selectivity of ritobegron (KUC-7483) for β_3 -adrenoceptors was 301 and 32 times higher than that for β_1 - and β_2 -adrenoceptors, respectively (Table 1; Maruyama et al., 2012). In its first Phase III study, ritobegron did not significantly improve the mean number of micturitions per 24 hr compared to placebo. A long-term safety and efficacy study was subsequently withdrawn, and ritobegron does not seem to have been developed further (Thiagamorthy, Giarenis, & Cardozo, 2015).

5.3 | Solabegron

Solabegron (GW427353) had an EC₅₀ value of 1.9 nM in human bladder strips pre-contracted with KCl, whereas isoprenaline had an EC₅₀ value of 8.3 mM (Tyagi et al., 2009). In a Phase II multicentre, randomized, proof-of-concept trial in 258 women with wet OAB, the drug produced a statistically significant difference in percent change from baseline to Week 8 in incontinence episodes over 24 hr (primary outcome) when compared with placebo ($P = 0.025$) and was well tolerated (Ohlstein et al., 2012). An additional Phase II dose-ranging study with a new formulation is currently ongoing.

5.4 | Vibegron

Vibegron (MK-4618) is a β_3 -adrenoceptor agonist, potently activates human β_3 -adrenoceptors with an EC₅₀ value of 1.1 nM, and vibegron is also highly selective over β_1 - and β_2 -adrenoceptors versus β_3 -adrenoceptors across multiple species (Table 1). Vibegron did not show any stimulating or inhibitory effects on cytochrome P450 enzymes, suggesting a low risk of drug–drug interaction (Edmondson et al.,

2016). A recent randomized placebo-controlled Phase III study showed that the 12-week treatment with vibegron (50 or 100 mg once daily) resulted in a significant improvement over the placebo in changes in the mean number of micturitions per day at Week 12 from baseline (primary endpoint) and changes from baselines in OAB symptom variables (daily episodes of urgency, urgency incontinence, incontinence, and nocturia, and voided volume/micturition) as the secondary endpoints. Vibegron also significantly improved quality of life, with high patient satisfaction. Incidence of drug-related adverse events with vibegron 50 and 100 mg were similar to placebo, and less than imidafenacin, an anti-muscarinic agent (Yoshida, Takeda, et al., 2018). In addition, a 1-year, multicentre, open-label, non-controlled study confirmed that vibegron 50 and 100 mg have favourable safety profiles for the 52-week treatment and improved OAB symptoms and quality of life, and a dose increase to 100 mg improved OAB symptoms without increasing adverse events in those patients who did not respond well to 50 mg vibegron (Yoshida, Kakizaki, Takahashi, Nagai, & Kurose, 2018).

6 | KEY QUESTIONS FOR FUTURE RESEARCH

6.1 | Does an endogenous β -adrenoceptor agonist tone exist in the bladder, and if so, does it change in pathological conditions?

The presence of endogenous β -adrenoceptor-mediated tone to the bladder detrusor is controversial. Systemic sympathetic activity increases during the storage phase of the micturition cycle, and the **noradrenaline** released is believed to act on β -adrenoceptors on the bladder wall to produce smooth muscle relaxation. This increases bladder compliance and enables continued low pressure filling of the bladder (reviewed in Fowler, Griffiths, & de Groat, 2008). Both nonselective β -adrenoceptor and selective β_3 -adrenoceptor agonism with mirabegron prolonged the inter-void interval and increased bladder compliance while suppressing amplitude of NVCs but not their frequency (Sadananda, Drake, Paton, & Pickering, 2013). The β_3 -adrenoceptor-mediated effects promote storage without associated impairment of voiding function and are also seen in an acid-sensitized bladder pathological model. The increase in the inter-void interval correlates with the degree of increase in bladder compliance produced by β_3 -adrenoceptor activation, but not with the reduction in NVC amplitude, nor with changed afferent sensitivity. Moreover, **L 748,337**, a selective β_3 -adrenoceptor antagonist, shortened inter-micturition interval and decreased bladder compliance, suggesting the presence of a basal β_3 -adrenoceptor-mediated sympathetic tone, or inverse agonistic activity. However, sympathetic innervation of the human detrusor is sparse (Gosling, Dixon, & Jen, 1999) and sympathectomy has no distinct effect on bladder filling (Andersson, 1986). Furthermore, a deficiency of dopamine β -hydroxylase, the enzyme that converts dopamine to noradrenaline, does not lead to abnormal voiding in patients (Gary & Robertson, 1994). Additional investigations are necessary to better understand the role of endogenous

noradrenaline, the sympathetic tone and its alteration in pathophysiological settings for urine storage in humans.

6.2 | How can mirabegron relax human detrusor if plasma concentrations after therapeutic dosing do not exceed 100 nM but most studies find EC₅₀ values in the low micromolar range?

Most studies using isolated bladder strips have reported a potency of mirabegron for causing relaxation in the low micromolar range (Igawa & Michel, 2013) but there are a few exceptions where an EC₅₀ of about 100 nM has been reported (D' Agostino et al., 2015). However, plasma concentrations observed upon therapeutic doses of mirabegron typically do not exceed 100 nM (Krauwinkel et al., 2012). Based on this discrepancy, it has been proposed that the cellular target of β_3 -adrenoceptor agonists in the treatment of OAB may not be the smooth muscle cell of the urinary bladder (Eastham et al., 2015) but rather a different cell type such as urothelium, ICs of Cajal, efferent, afferent nerves, the major pelvic ganglion, and/or blood vessels supplying the urinary bladder (Okeke et al., 2017). However, except for inhibition of ACh release from cholinergic nerves (D' Agostino et al., 2015; Silva et al., 2017), a submicromolar potency of mirabegron has not been shown in any of these alternative cell types. Inhibition of neuronal ACh release is an unlikely mechanism of the therapeutic effects in OAB because neuronal ACh is implicated in physiological voiding but less so if at all in the DO typical for OAB (Michel & Chapple, 2009).

This raises the question whether the focus on bladder smooth muscle or some of our analytical approaches has been wrong. While we do not have a definitive answer to this question, several considerations apply. First, the potency of β -adrenoceptor agonists to cause smooth muscle relaxation depends on the contractile agent against which they are tested; they are about 10-fold less potent against muscarinic agonists than against other stimuli (Dale et al., 2014). Second, mirabegron is less potent against the rodent as compared to the human β_3 -adrenoceptor (Igawa & Michel, 2013). Third, less than 50% of receptors need to be occupied to achieve a half-maximal response for many agonist effects, a phenomenon called spare receptors or non-linear stimulus effect coupling (Brown et al., 1992). Fourth, and perhaps most importantly, drug concentrations in plasma may underestimate those in the micro-environment in which they are acting. Enrichment within target tissue has been shown for other drugs used in the treatment of lower urinary tract function (Korstanje, Krauwinkel, & van Doesum-Wolters, 2011).

6.3 | Do prejunctional β_3 -adrenoceptors contribute to treatment effects on OAB?

Mirabegron had high potency for inhibiting the nerve-evoked contraction and ACh release in human bladder preparation, with EC₅₀ value of 123 and 129 nM respectively (D' Agostino et al., 2015). Inhibition of neuronal ACh release is so far the only cellular response to mirabegron in the human urinary bladder that has been consistently

reported to occur at concentrations that are found in plasma of mirabegron-treated subjects. The activation of β_3 -adrenoceptors inhibits neurogenic contractions of both rat and human urinary bladders. β_3 -adrenoceptor agonism also inhibits contractions induced by exogenously applied ACh. However, the effect is clearly less than that on neurogenic contractions (particularly in human), presuming that in addition to a direct effect on smooth muscle, activation of prejunctional β_3 -adrenoceptors may inhibit ACh release from parasympathetic cholinergic nerve terminals. Supporting the inhibitory action of β_3 -adrenoceptor agonists on nerve-evoked ACh release, a recent immunohistochemistry study on human bladder tissue indicated that β_3 -adrenoceptors are expressed only on cholinergic nerve fibres but are not present on urothelial or smooth muscle cells (Coelho et al., 2017). However, another study showed a controversial finding that β_3 -adrenoceptors are diffusely distributed among detrusor smooth muscle cells but are not present on cholinergic nerve fibres, while adenosine **A₁ receptors** are predominantly expressed on cholinergic nerve fibres (Silva et al., 2017). Indeed, both isoprenaline and mirabegron decreased ACh release induced by electrical field stimulation in the human detrusor preparation, which was prevented by β_3 -adrenoceptor antagonists and **DPCPX**, an A₁ receptor antagonist (Silva et al., 2017). Because β_3 -adrenoceptors, similar to other β -adrenoceptor subtypes, couple to stimulation of cAMP formation and cAMP is degraded to adenosine, Silva et al. tested whether adenosine may mediate the inhibition of ACh release observed with β_3 -adrenoceptor agonists. In fact, isoprenaline and mirabegron increased extracellular adenosine concentrations in the detrusor strips. Antagonism of A₁ receptors by DPCPX or blockade of the equilibrative nucleoside transporters (ENT) with dipyrindamole prevented inhibition of ACh release, suggesting that it did not necessarily occur via a β_3 -adrenoceptor located in the nerve ending but rather indirectly by intermediate formation of adenosine and subsequent activation of inhibitory A₁ receptors. The authors demonstrated positive ENT1 and ENT2 immunoreactivities in human detrusor strips. These findings suggest that β_3 -adrenoceptor agonists, including therapeutically-achieved concentrations of mirabegron, can inhibit neuronal ACh release in human detrusor. They support a hypothesis that (indirect) inhibition of ACh release may be the mechanism for detrusor smooth muscle relaxation. However, it is not yet fully clear whether such inhibition of ACh release indeed occurs exclusively indirectly via adenosine formation and A₁ receptor activation or whether it may also involve a neuronally-expressed β_3 -adrenoceptor. However, translating the cellular activity of β_3 -adrenoceptor agonists to tissue and whole-body systems is not straightforward and can often be misleading (Figure 2).

6.4 | Do β_3 -adrenoceptor agonists inhibit bladder mechanosensitive afferent activity, and if so, how do they act?

Several studies have reported that detrusor smooth muscle show spontaneous contractile activity. In women with overactive bladder, these localized contractile activities increase during occurrence of

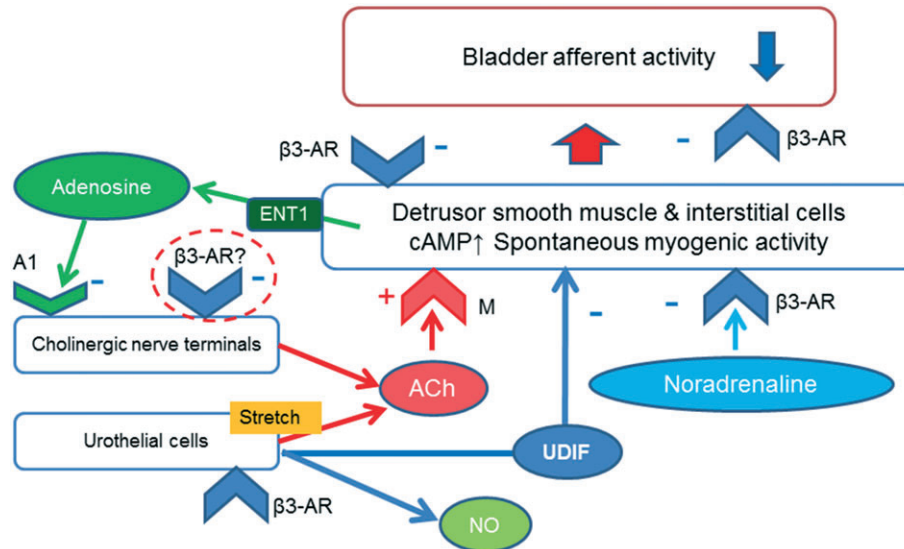


FIGURE 2 Hypothetic mechanism of mirabegron's inhibitory action on ACh release through the A₁ receptors

urinary urgency without increasing intravesical pressure (Drake, Harvey, Gillespie, & Van Duyl, 2005). Sadananda et al. demonstrated in vivo cystometry in the decerebrated rat model that intravesical administration of the selective β_3 -adrenoceptor agonist mirabegron increased inter-voiding intervals and compliance and decreased the amplitude of NVCs (Sadananda et al., 2013).

Studies in which bladder mechanosensitive single-unit afferent activities (SAAs) were monitored in rats revealed that the afferent activities of both A δ - and C-fibres decreased after mirabegron administration in a dose-dependent manner, which was more marked for A δ -fibres than C-fibres; moreover, the inhibition of afferent activities appeared to synchronize with the decrease in fluctuation on bladder pressure (micro-contractions), whereas bladder compliance did not change significantly from the baseline value with the mirabegron treatment at the doses used (Aizawa et al., 2012). In order to determine the relation of afferent activities with micro-contractions and the effect of mirabegron on them, the authors further investigated these in an isovolumetric condition and confirmed the suppression of micro-contractions concomitantly with A δ -fibre activity but not C-fibre activity with 0.3-mg·kg⁻¹ mirabegron (Aizawa, Homma, & Igawa, 2015). I.v. administration of another β_3 -adrenoceptor agonist, **CL316,243** (10 μ g·kg⁻¹), similarly decreased A δ -fibre, but not C-fibre, activities in response to bladder filling with saline. Intravesical instillation of **PGE₂** significantly increased C- but not A δ -fibre activities. The PGE₂-induced increase in C-fibre activities was inhibited by pretreatment with CL316,243 (Aizawa, Igawa, Nishizawa, & Wyndaele, 2010). A recent study with similar monitoring bladder mechanosensitive SAAs in rats with BOO showed a higher number of micro-contractions and lower SAAs of A δ -fibres, but SAAs of both A δ - and C-fibres were intermittently enhanced by micro-contractions. These pathophysiological findings may contribute to the development of OAB associated with BOO (Aizawa et al., 2017).

Taken all together, the possible mechanisms of the inhibition of urgency induced by the β_3 -adrenoceptor agonist may include (a) relaxing detrusor muscle-decreasing bladder tone, (b) inhibiting micro-contractions/A δ -fibre activity, and (c) inhibiting urotheliogenic afferent activation-C-fibre activity (Figure 3).

6.5 | Do β_3 -adrenoceptor agonists inhibit neurogenic detrusor overactivity?

While several β_3 -adrenoceptor agonists appear effective in the treatment of OAB (Chapple et al., 2014; Ohlstein et al., 2012; Yoshida, Takeda, et al., 2018), only limited information, largely based on animal models, is available for neurogenic DO (NDO). An early study used the experimental β_3 -adrenoceptor agonist FK175 in a rat model of DO based on ibotenic acid-induced brain lesions (Fujimura et al., 1999). Acute administration of FK175 dose-dependently increased bladder capacity without major changes in micturition or threshold pressure. In DO induced by cerebral infarction in rats, acute administration of the experimental β_3 -adrenoceptor agonist CL316,243 also increased bladder capacity without changing voiding pressure or post-voiding residual volume (Kaidoh et al., 2002). In contrast, the β_2 -adrenoceptor agonist **procatenol** increased bladder capacity and residual volume but did not change voiding pressure. While CL316,243 had only minimal effects on cardiovascular function, procatenol lowered blood pressure and increased heart rate. Two studies have explored effects of β_3 -adrenoceptor agonists in rat models of spinal cord injury. Acute administration of CL316,243 similarly increased inter-contraction intervals in conscious sham and spinal cord injury rats and decreased micturition frequency without affecting amplitude of micturition (Beauval et al., 2015); moreover, CL316,243 reduced the frequency of NVCs in spinal cord injury rats by about half without major effects on amplitude of contractions.

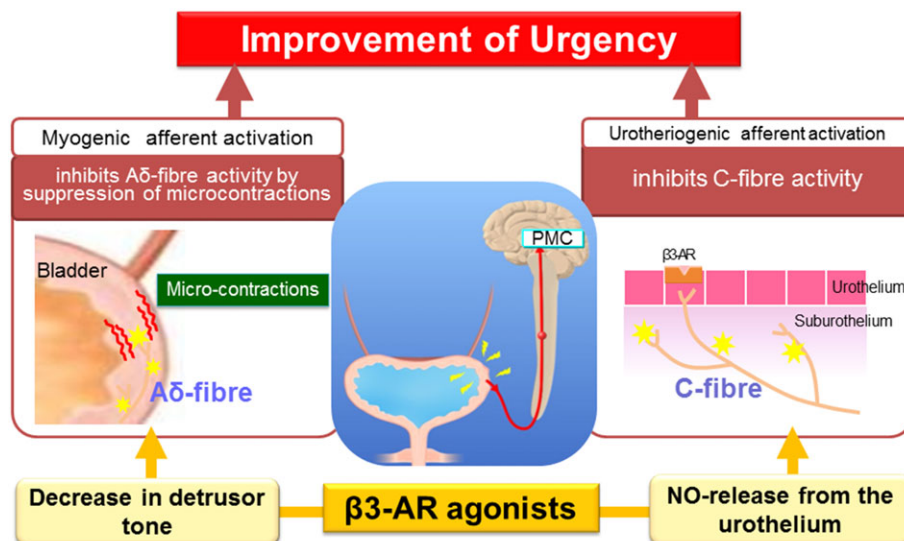


FIGURE 3 Hypothetic mechanisms involved in urgency improvement by β_3 -adrenoceptor agonists

Others have explored effects of a 2- to 4-week treatment with **oxybutynin**, mirabegron, and their combination in spinal cord injury rats (Wada et al., 2017). Both monotherapies tended to decrease NVCs with statistical significance being reached with the combination treatment. Combination treatment also improved bladder compliance and increased inter-contraction intervals, voided volume, and bladder capacity.

There are few clinical studies on the efficacy of mirabegron for either NDO or low-compliance bladder. Wöllner and Pannek recently reported that 15 patients with NDO treated with mirabegron for a period of at least 6 weeks showed a significant reduction in the frequency of bladder evacuation per 24 hr (8.1 vs. 6.4, $P = 0.003$) and of incontinence episodes per 24 hr (2.9 vs. 1.3, $P = 0.027$; Wollner & Pannek, 2016). Furthermore, urodynamic studies revealed improvements in bladder capacity (from 365 to 419 ml), compliance (from 28 to 45 $\text{ml}\cdot\text{cmH}_2\text{O}^{-1}$), and detrusor pressure during storage phase (45.8 vs. 30 cmH_2O). At follow-up, 9/15 patients were satisfied with the therapy.

Wada et al. retrospectively examined the efficacy of combination therapy with mirabegron in seven patients (five men and two women) with NDO or low-compliance bladder ($<10 \text{ ml}\cdot\text{cmH}_2\text{O}^{-1}$) refractory to anticholinergic treatment (Wada et al., 2015). Underlying diseases were spinal cord injury in three patients, spina bifida in two, spinal cord infarction in one, and post-radical hysterectomy in one. After mirabegron, urinary incontinence was improved in all patients. G3 bladder deformity was improved to G2 and G1 in one patient each, and vesicoureteral reflux disappeared in all three patients. DO disappeared in two of the five patients, and bladder compliance was improved in all four patients with low-compliance bladder.

Due to the limited number of patients and the retrospective nature of these studies, prospective, placebo-controlled studies are required to confirm the beneficial effects of mirabegron on NDO suggested by these studies.

6.6 | Do β_3 -adrenoceptor agonists ameliorate poorly compliant detrusor?

Other than DO, a poorly compliant detrusor can be a cause of OAB-like symptoms. There is only one clinical study available focusing on the effect of mirabegron on poor compliant detrusor. Kamei et al. investigated video-urodynamic effects of mirabegron in nine patients (three men and six women, age 17–68 years) with low-compliance bladder including seven patients with neurological diseases (three spinal cord injury, four myelomeningocele, and one post-radical hysterectomy; Kamei et al., 2015). Mirabegron treatment significantly increased first desire to void and cystometric capacity with an average increment of 80 ml ($P = 0.027$) and 123 ml ($P = 0.005$) respectively. Bladder compliance also significantly increased (mean value $8.1 \text{ ml}\cdot\text{cmH}_2\text{O}^{-1}$ before, $18.2 \text{ ml}\cdot\text{cmH}_2\text{O}^{-1}$ after, $P = 0.024$). In the six patients who had been taking anticholinergic agents at a baseline video-urodynamic study and then switched to mirabegron, mean cystometric capacity and bladder compliance were also increased significantly from 208.3 to 346.8 ml ($P = 0.015$) and from 7.2 to $17.5 \text{ ml}\cdot\text{cmH}_2\text{O}^{-1}$ ($P = 0.047$) respectively. Vesicoureteral reflex grade was improved in three of the four patients who had shown vesicoureteral reflex on cystography before treatment. Further prospective studies are needed to confirm the effect of β_3 -adrenoceptor agonists on poor compliant detrusor.

6.7 | Does desensitization of β_3 -adrenoceptors exist in the bladder upon prolonged agonist exposure?

β_3 -adrenoceptor agonists can effectively reduce OAB symptoms (Chapple et al., 2014; Ohlstein et al., 2012; Yoshida, Takeda, et al., 2018). As they are not curative, it is assumed that prolonged, possibly life-long treatment is required for sustained symptom control. Data with β_2 -adrenoceptors show that prolonged treatment can cause

desensitization, for instance, in the treatment of preterm labour (Engelhardt et al., 1997; Frambach et al., 2005; Michel, Pingsmann, Nohlen, Siekmann, & Brodde, 1989) and this can become treatment-limiting in patients (Canadian Preterm Labor Investigators, 1992) and animal models thereof (Lye, Dayes, Freitag, Brooks, & Casper, 1992). Whether this also applies to the use of β_3 -adrenoceptor agonists in the treatment of OAB remains unclear because the earliest time points of symptom assessment after initiation of treatment have been 2–4 weeks in clinical studies (Chapple et al., 2014; Ohlstein et al., 2012; Yoshida, Takeda, et al., 2018), that is, a time point where desensitization may already have happened.

Many investigators have studied agonist-induced desensitization of β_3 -adrenoceptors in a wide range of animal tissues and of the human subtypes upon endogenous expression and transfection in various cell lines, as reviewed in detail elsewhere in this issue (Okeke, Angers, Bouvier, & Michael, 2019). While there is consensus that β_3 -adrenoceptors are less sensitive to agonist-induced desensitization as compared to β_1 - and β_2 -adrenoceptors, whether they exhibit agonist-induced desensitization and which mechanisms are involved appears to be highly cell type-dependent. This necessitates studying β_3 -adrenoceptor desensitization directly in the urinary bladder.

We are aware of only one study on the urinary bladder (Michel, 2014) in which, in contrast to the human bladder, relaxation is a mixed β_2/β_3 response (Michel & Vrydag, 2006). Following pretreatment of isolated rat bladder strips for 6 hr with the reference agonist isoprenaline, the β_2 -selective fenoterol or the β_3 -selective CL316,243 or mirabegron and subsequent washout, concentration–response curves were generated for relaxation by freshly added agonists. Pretreatment with isoprenaline or fenoterol markedly reduced relaxation to freshly added agonist, demonstrating desensitization of the β_2 -component by agonists stimulating that subtype. In contrast, pretreatment with CL316,243 or mirabegron caused a much smaller reduction of isoprenaline- or fenoterol-induced relaxation, which did not reach statistical significance. Pretreatment with isoprenaline reduced the response to CL316,243 but not to mirabegron; similarly, pretreatment with CL316,243 reduced relaxation to freshly added agonist (although not reaching statistical significance), whereas pretreatment with mirabegron did not (Table 2). Pretreatment with all four agonists

attenuated contractile responses to the muscarinic agonist carbachol or to KCl.

These data confirm in rat bladder that β_3 -adrenoceptors are less sensitive to desensitization than other β -adrenoceptor subtypes. They raise three new questions, which remain to be answered: Is the numerical reduction of relaxation not reaching statistical significance indicating a lack of desensitization or rather an underpowered study? Do β_3 -adrenoceptor agonists differ in their ability to cause desensitization of bladder relaxation? Can these findings with rat bladder be extrapolated to the human bladder?

6.8 | What is the cause of (rarely observed) cardiovascular effects of mirabegron?

β_3 -adrenoceptor agonists as a class appear to be well-tolerated and have little effect on the cardiovascular system (Rosa et al., 2018). While this general conclusion is largely based on studies with mirabegron, more limited data with [solabegron](#) (Ohlstein et al., 2012) and [vibegron](#) (Yoshida, Takeda, et al., 2018) confirm it. The molecular basis for the good tolerability appears to be the rather restricted expression of β_3 -adrenoceptors in the human body, with their largely lack of expression in the heart (Michel & Gravas, 2016). Nonetheless, some cases of severe hypertension and associated cerebrovascular and cardiac effects have been observed and have led to corresponding warnings in the prescribing information (Medicines and Healthcare Product Regulatory Agency, 2015). While convincing evidence for functional β_3 -adrenoceptors in the heart of experimental animals such as rats has been presented (Arioglu-Inan, Ozakca, Kayki-Mutlu, Sepici-Dincel, & Altan, 2013), the data available do not support their presence in the human heart (Michel, Harding, & Bond, 2011). Correspondingly, positive inotropic effects of mirabegron in isolated human atrium have been shown to be resistant to inhibition by β_3 -adrenoceptor antagonists (Mo, Michel, Lee, Kaumann, & Molenaar, 2017). Similarly, increases in blood pressure and heart rate induced by supra-therapeutic doses of mirabegron in healthy volunteers were blocked by [propranolol](#) or [bisoprolol](#) (van Gelderen et al., 2017). This raises the question what mediates the observed rare but severe cases of hypertension in patients receiving mirabegron treatment.

TABLE 2 Effects of pretreatment on relaxation responses of isolated rat bladder to β -adrenoceptor (AR) agonists

Relaxing agonist Pretreatment	Isoprenaline		Fenoterol		CL316,243		Mirabegron	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Vehicle	7.34 ± 0.06	49 ± 5	6.99 ± 0.23	42 ± 3	8.45 ± 0.24	30 ± 5	5.55 ± 0.17	45 ± 4
Isoprenaline	7.01 ± 0.09	26 ± 4*	8.39 ± 0.33*	19 ± 2*	8.65 ± 0.35	12 ± 3*	5.52 ± 0.49	40 ± 4
Fenoterol	7.46 ± 0.16	24 ± 5*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CL316,243	7.36 ± 0.02	38 ± 3	6.63 ± 0.17	33 ± 4	8.82 ± 0.25	20 ± 2	n.d.	n.d.
Mirabegron	7.08 ± 0.13	35 ± 4	6.63 ± 0.10	34 ± 3	n.d.	n.d.	4.84 ± 0.13	45 ± 4

Note: β -adrenoceptor agonist potency (pEC₅₀) is given in $-\log$ molar units and maximum relaxation (E_{max}) in %. Data are means ± SEM of 3–8 experiments.

* $P < 0.05$ versus vehicle pretreatment in one-way ANOVA followed by Dunnett's multiple comparison tests; n.d.: not determined. Reproduced with permission from Michel (2014).

One possibility would be direct effects of mirabegron on β_1 - or β_2 -adrenoceptors. Of note, spare receptors for β -adrenoceptor-mediated positive inotropic effects have been reported in the human heart (Brown et al., 1992), indicating that even occupation of a few β_1 - or β_2 -adrenoceptors by an agonist could increase cardiac contractility. However, we consider this unlikely for mirabegron because it not only is selective for β_3 -adrenoceptors based on affinity but also has a very low intrinsic efficacy at the human β_1 - and β_2 -adrenoceptors (Igawa & Michel, 2013). It has been reported that mirabegron has α_1 -adrenoceptor antagonist effects in concentrations comparable to its affinity measured at the β_3 -adrenoceptors (Alexandre et al., 2016; U.S. Food and Drug Administration, 2012). However, it is questionable whether such concentrations can be reached therapeutically; even if they were, inhibition of α_1 -adrenoceptors, if anything, should lower and not increase blood pressure (Michel, 2016).

A recent study has reported that high concentrations of mirabegron can have positive inotropic effects in isolated human right atrium (Mo et al., 2017). These were not affected by the β_3 -adrenoceptor antagonist L 748,337 but abolished by the β_1 -adrenoceptor antagonist **CGP 20,712**, indicating the involvement of β_1 -adrenoceptors. Given the poor intrinsic efficacy of mirabegron at β_1 -adrenoceptors (Igawa & Michel, 2013), the authors investigated other possible underlying mechanisms. Because the neuronal uptake blockers **desipramine** and **phenoxybenzamine** also inhibited positive inotropic effects of mirabegron and because mirabegron belongs to the chemical class of phenylethanolamines, many of which are indirect sympathomimetics, they proposed that high concentrations of mirabegron may promote noradrenaline release which in turn may stimulate cardiac β_1 -adrenoceptors (Mo et al., 2017). As it remains unclear whether these high concentrations are reached upon administration of therapeutic doses and because no data on human ventricle have been reported, this proposal remains to be proven. However, it remains the only plausible interpretation of the clinically-observed rare occurrence of severe hypertension. Irrespective of such mechanistic considerations, it is interesting that a pilot study in a small number of patients with congestive heart failure has shown that a 6-month treatment with mirabegron has a beneficial effect as compared to placebo (Bundgaard et al., 2017).

7 | CONCLUSION

The β_3 -adrenoceptor agonist mirabegron has become a widely used drug for the treatment of OAB, and other β_3 -adrenoceptor agonists such as solabegron and vibegron have shown similar effects in clinical studies. In contrast to the original smooth muscle-centric view that β_3 -adrenoceptors should improve OAB symptoms by directly acting on the detrusor muscle, more recent data suggest that several other cell types may be involved. However, the relative role of the various cellular targets of β_3 -adrenoceptor agonists related to bladder function remains to be elucidated.

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

CONFLICT OF INTEREST

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