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On the Site and Mechanism of Action of β_3-Adrenoceptor Agonists in the Bladder

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The clinical success of mirabegron as the first β_3-adrenoceptor (AR) agonist for treatment of the overactive bladder (OAB) syndrome, has resulted in substantial interest in its site and mechanism of action. Even if the adrenergic innervation of the bladder and urethra has been well studied, the location(s) of β_3-ARs in different structures within the bladder wall and urethra, and the mode(s) of action of β_3-AR stimulation have still not been established. The recent demonstration of β_3-ARs on cholinergic nerve terminals with no immunoreactivity in urothelium or detrusor smooth muscle, is not in agreement with previous morphological studies, and functional data strongly suggest that β_3-ARs can be found these structures. However, recent studies suggest that the β_3-ARs on detrusor smooth muscle may not be the functionally most relevant. The assumption that β_3-AR activation during bladder filling inhibits acetylcholine release from parasympathetic neurons by a prejunctional mechanism and that this decreases bladder micromotions that generate afferent activity, is an attractive hypothesis. It does not exclude that other mechanisms may be contributing, and supports combined approaches to reduce afferent activity for treatment of the OAB syndrome.

Keywords: Lower Urinary Tract Symptoms; Adrenergic Nerves; Detrusor Smooth Muscle; Urothelium; Relaxation

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INTRODUCTION

Mirabegron has been successfully introduced into clinical practice as the first selective β_3-adrenoceptor (AR) agonist for treatment of the overactive bladder (OAB) syndrome [1-3], and its clinical success has resulted in an increased interest in the site and mechanism of action of β_3-AR agonists. β-AR agonists, including mirabegron, have been suggested to relieve OAB symptoms through various mechanisms, e.g., direct relaxation of detrusor muscle by stimulation of cyclic AMP (cAMP) generation [4,5], opening of potassium channels [6,7], inhibition of spontaneous contractile activity in the bladder [8,9], and reduction in bladder afferent activity [9,10]. However, the site(s) and mechanism(s) of action of β_3-AR agonists have not been established and has been the subject of several recent studies.

ADRENERGIC NERVES IN THE BLADDER AND URETHRA

It seems reasonable to assume that there should be a relation between the distribution of adrenergic nerves and the location of β_3-ARs. The sympathetic innervation to the urinary tract consists of short preganglionic neurons and long postganglionic neurons originating from the paravertebral and prevertebral

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ganglia [11]. Efferent sympathetic and parasympathetic fibres are conveyed to the genitourinary organs via the hypogastric and pelvic splanchnic nerves, respectively. The hypogastric and pelvic splanchnic nerves of either side meet and branch to form the pelvic plexus. The part of the plexus related to the urinary bladder — the vesical plexus — contains both sympathetic and parasympathetic neurons. The vesical plexus lies adjacent to the posterior and lateral walls of the urinary bladder. Similar mixed autonomic ganglia occur throughout all regions of the human bladder wall, both within and on the outer surface of the detrusor muscle with occasional ganglia being found in the lamina propria [11].

Bladder Body

The body of the human bladder receives a relatively sparse innervation by noradrenergic nerves [11], and Coelho et al. [12] observed that numerous smooth muscle areas were without any tyrosine-hydroxylase (key enzyme involved in noradrenaline synthesis) immunoreactive fibres. The density of noradrenergic nerves increases markedly toward the bladder neck, where the smooth muscle receives a dense noradrenergic nerve supply, particularly in the male [11]. Noradrenergic nerves can be found also in the lamina propria of the bladder, only some of which are related to the vascular supply [11,12].

The bladder and urethra form a functional unit, but the adrenergic nerves innervating these structures have different distribution and mediate different effects. It is generally considered, based on animal studies, that the activity in the adrenergic nerves keeps the bladder relaxed and the urethra contracted during filling [4,9]. However, the importance of the sympathetic input for human bladder function during the storage phase has not been established. Sympathectomy has no distinct effect on bladder filling in humans, and neither has blockade of β-ARs [13]. Furthermore, since patients with deficiency of dopamine β-hydroxylase, the enzyme that converts dopamine to noradrenaline, void normally [14], the sympathetic nervous system may not be essential for urine storage in humans. The density of noradrenergic nerves increases markedly towards the bladder neck, the majority of these nerves being intramuscular in location. The noradrenaline content of the bladder neck is higher than that of the bladder dome and the noradrenergic nerves have been shown to cause smooth muscle contraction and thus closure of the bladder neck. The trigone has a different embryological origin from the detrusor muscle and its pattern of innervation is readily distinguished from the bladder body. The superficial trigone receives a rich innervation by noradrenergic nerves although the precise function of this muscle layer is not established [11].

The effects of noradrenaline on structures in the bladder body are presumably mediated via β_3-ARs (see below). However, the transmitter has different effects on different parts of the bladder and on the various structures in the bladder wall. Based on animal data, it has been assumed that there is a release of noradrenaline during filling. If this is the case also in humans, it is unclear how this affects the different structures in the bladder wall, e.g., the urothelium, interstitial cells, intramural ganglia, and bladder vasculature.

Urethra

There are well known anatomical differences between the male and female urethra, and this is also reflected in the innervation. In the human male, the smooth muscle surrounding the preprostatic part of the urethra is richly innervated by both cholinergic and adrenergic nerves [11]. This part is believed to serve as a sexual sphincter, contracting during ejaculation and thus preventing retrograde transport of sperm. The role of this structure in maintaining continence is unclear, but probably not essential. In the human female, there is no anatomical urethral smooth muscle sphincter, and the muscle bundles run obliquely or longitudinally along the length of the urethra. In the whole human female urethra, and in the human male urethra below the preprostatic part, there is a scarce supply of adrenergic nerves along the bundles of smooth muscle cells. Adrenergic terminals can also be found around blood vessels.

β-ARS: LOCATION IN THE BLADDER AND URETHRA

Human bladder tissue, taken from the anterior portion of the bladder dome of male patients undergoing radical cystectomy because of malignancy, predominantly expressed β_3-AR mRNA (97% β_3; 1.5% β_1 and 1.4% β_2, respectively) [15]. However, all 3 subtypes of β_3-ARs (β_1, β_2, and β_3) has been demonstrated in the detrusor muscle of most species [16], and also in the human urothelium [17].

The direct effect of noradrenaline on the detrusor, mediated mainly by β_3-ARs, is inhibitory [4]. This does not exclude the possibility that released noradrenaline exerts an inhibitory effect on bladder function by other indirect mechanisms. β_3-ARs can be found also in other bladder structures than detrusor and

urothelium [12,18]. A recent study [12] demonstrated that β_3-ARs are abundantly located in acetylcholine (ACh)-containing nerve fibres in the the mucosa and muscular layers of the human bladder. No β_3-AR immunoreactivity was detected on urothelial, suburothelial interstitial cells or smooth muscle cells, which is in contrast to previous findings [17,18]. However, the authors did not exclude the possibility that the receptors on the urothelium or on smooth muscle cells were too few to be detected. Their findings thus suggest that the main site of action of β_3-AR agonists may not be the detrusor, which is in support of findings in several functional studies [19-25].

The main effect of noradrenaline on the smooth muscle of the human urethra is to mediate contraction [4]. However, this action is mediated by β_1-ARs [26,27], and the role of the urethral β_3-ARs is unclear. Radioligand studies shown the presence of β_3-ARs in the human external urethral sphincter [28], but the role of these receptors has not been established.

β-AR AGONISTS: MECHANISM OF ACTION

Activation of β_3-ARs is associated with relaxation of the bladder during the storage phase of micturition [9,29,30]. The generally accepted mechanism by which β_3-ARs induce direct detrusor relaxation in most species, is activation of adenylyl cyclase with the subsequent formation of cAMP [4]. However, studies with adenylyl cyclase or protein kinase A inhibitors have detected only a small, if any, role for this pathway in bladder relaxation [6]. There is compelling evidence suggesting that β-ARs can also stimulate large-conductance Ca^{2+}-activated K^+ (BKCa) channels in bladders from several animal species [31-34] and humans [7,35-37]. It is thus well established that these mechanisms can mediate relaxation of detrusor smooth muscle. However, the concentrations of the drugs needed to produce the effects are not always in the range obtained in the human plasma after administration of clinically used doses of e.g., mirabegron [20]. The plasma concentrations of therapeutic doses of mirabegron are in the order of 60–115 nM [38]. In this concentration range, little relaxation can be seen in carbachol-induced contractions in isolated strips of human bladder [39]. Experiments have also been done on isolated strips of the rat bladder demonstrating little or no effect of mirabegron [20,21]. β_3-AR agonists have a conspicuous effect on spontaneous bladder contractions in isolated bladder strips from humans [8,9] and several animal species, and on nonvoiding contractions in animal models of increased bladder activity [20,21,24,40].

Gillespie et al. [20,21] have questioned the accepted view on the mode and site of action of β_3-AR agonists, and suggested that effects on neither spontaneous contractions, nor on nonvoiding contractions in e.g., obstructed rats, can fully explain the effects of β_3-AR agonist stimulation (mirabegron). Since there is evidence that there is a release of ACh during bladder filling [41,42], the finding that activation of prejunctional β_3-ARs can decrease ACh release resulting in an inhibitory control of parasympathetic activity may be of interest [22,23]. Electrical stimulation of nerves in the bladder releases ACh from the bladder cholinergic nerves, thus mimicking parasympathetic outflow from the spinal cord. However, during bladder filling, there is no parasympathetic outflow from the spinal cord, which means that the source of ACh, which may be neurogenic or nonneurogenic, is not known. The fact that antimuscarinics works during the filling phase in humans strongly favours that there in fact is a release. It may be speculated that small amounts of ACh are "leaking" from cholinergic nerves and that this amount is sufficient to enhance spontaneous, myogenic contractions and the generation of afferent activity ("afferent noise"). This release may be inhibited by β_3-AR agonist stimulation, but it seems unlikely that the massive release of ACh that occurs in the detrusor during voiding can be prevented by the inhibitory action of β_3-AR agonists on cholinergic nerve endings. This is in accordance with studies showing that the capacity of β_3-AR agonists to inhibit electrically induced ACh release is only partial [22,23], and with the finding that β_3-AR agonists have little or no effect on the voiding contraction in humans.

β_3-ARS IN THE UROTHELIUM

Since β_3-ARs are present in the urothelium, their possible role in bladder relaxation has been investigated [17,43,44]. Murakami et al. [43] found that the relaxation responses of the detrusor were not influenced by the urothelium. However, isoprenaline was more potent at inhibiting carbachol contractions in the presence of the urothelium than in its absence. It was suggested that this might reflect the release of an inhibitory factor from the urothelium. Further support for this hypothesis was given by Otsuka et al. [17]. However, to what extent a urothelial signaling pathway contributes *in vitro* and *in vivo* to the relaxant effects of β-AR agonists in general, and β_3-AR agonists specifically, remains to be elucidated. Birder et al. [45] showed that activation of β-AR by isoproterenol in rat urothelial cells can release nitric oxide through an increase in intracellular Ca^{2+} by

cAMP accumulation. However, activation of urothelial cells also release a urothelial-derived factor that inhibits contractions induced by carbachol in the pig detrusor [43]. The β-AR involved in the release of a urothelial-derived inhibitory factor was shown to be a β_3-AR [46]. However, the role of urothelial β_3-ARs in bladder relaxation during filling is unclear and has to be established.

β_3-AR AGONISTS: PROFILE OF MIRABEGRON

Mirabegron is a selective β_3-AR agonist whose preclinical pharmacological profile has been well described *in vitro* and *in vivo* [5,47,48]. In animal models, mirabegron increases bladder capacity without decreasing the amplitude of the voiding contraction. It increases intervoid intervals, bladder compliance, and reduces nonmicturition contractions, while preserving active voiding function [9,48]. Mirabegron is by far the best investigated β_3-AR agonist, and has been used as an important tool for elucidation of β_3-AR mediated effects. However, the β_3-AR selectivity of the drug has been questioned [49]. Alexandre et al. [50] showed that in addition to its major β_3-AR agonistic effect, promoting urethral relaxation in mice, mirabegron exhibited selective α1A- and α1D-AR antagonistic actions that could be expected to contribute to this relaxation. The importance of this additional effect of mirabegron is unclear. The study findings seem to have little, if any, clinical relevance for the effects of mirabegron on the human lower urinary tract, or the use of the drug for the treatment of OAB [51].

β_3-AR agonists, including mirabegron, are an alternative therapeutic option for the treatment of urgency and their clinical efficacy (mirabegron) is well documented. β_3-AR agonists have a different mode of action from antimuscarinic agents, but both classes of drugs decrease bladder afferent activity, which would make combination therapy an attractive approach for treatment of OAB [52].

SUMMARY AND CONCLUSIONS

The location(s) of β_3-ARs in different structures within the bladder wall, and the mode(s) of action of β_3-AR stimulation have still not been established. The recent demonstration of β_3-ARs on cholinergic nerve terminals with no immunoreactivity in urothelium or detrusor smooth muscle, is not in agreement with previous morphological studies and has to be confirmed. Functional studies strongly suggest that β_3-ARs can be found on the detrusor muscle and on the urothelium. With respect to the mode of action of β_3-ARs in the mediation of bladder relaxation during filling and decreasing afferent output, recent data suggest that the receptors on detrusor smooth muscle may not be the functionally most relevant. The assumption that β_3-AR activation during bladder filling inhibits ACh release from parasympathetic neurons by a prejunctional mechanism and that this decreases bladder micromotions that generate afferent activity, is an attractive hypothesis. It does not exlude that other mechanisms may be contributing, and supports combined approches to reduce afferent activity for treatment of the OAB syndrome.

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