A Functional Analysis of the Influence of β_3 -adrenoceptors on the Rat Micturition Cycle

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ABSTRACT

Dysfunctions of the lower urinary tract, such as overactive bladder syndrome and incontinence, are the product of storage failure. Spontaneous regional bladder wall movements [non-micturition contractions (NMCs)] are proposed to generate afferent activity that signals volume status to the central nervous system. The sympathetic nervous system, via activation of β -adrenoceptors (β -ARs), causes bladder relaxation and promotes urine storage. We hypothesized that β -AR regulation of micturition is mediated by suppression of NMCs. We used an unanesthetized, decerebrate, artificially perfused rat preparation that allows simultaneous cystometry with external urethral sphincter and pelvic afferent nerve recordings. Systemic isoprenaline (10 nM to 1 μ M) increased intervold interval and bladder compliance accompanied by a decrease in NMC amplitude, voiding pressure, and voiding threshold. Isoprenaline also reduced arterial pressure and increased heart rate. The β_3 -AR agonist mirabegron (10–100 nM) increased intervold interval

and bladder compliance and reduced NMC amplitude, yet preserved active voiding function and had no effect on arterial pressure or heart rate. All of these effects of mirabegron were blocked by the selective β_3 -AR antagonist *N*-[[3-[(2*S*)-2-hydroxy-3-[[2-[4-[(phenylsulfonyl)amino]phenyl]ethyl]amino]propoxy]phenyl]methyl]-acetamide (L748,337), which alone shortened intervold interval and decreased bladder compliance—suggesting the presence of a basal β_3 -AR-mediated sympathetic tone. Similar effects of mirabegron were seen in an acetic acid-sensitized bladder preparation and in preparations after loss of spinobulbar reflex bladder control. The β_3 -AR-mediated increase in intervold interval correlated with increased bladder compliance but not with the decrease in NMC amplitude. These findings indicate that β_3 -adrenoceptors have a selective effect that improves urine storage by increasing compliance without affecting the active components of voiding.

Introduction

Dysfunctions of the lower urinary tract (LUT), such as overactive bladder (OAB), urgency, incontinence, and nocturia, signify failures of urine storage (Abrams et al., 2002). The mechanisms by which the bladder signals its volume status to the central nervous system are key to understanding such failures of storage. During the storage phase of micturition, a progressive increase in sympathetic activity releases nor-adrenaline that is believed to act on β -adrenoceptors (β -ARs) 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 et al., 2008). When a storage threshold is reached (and the environmental context is permissive), parasympathetic muscarinic receptor activation evokes detrusor contraction and emptying of bladder contents.

The major roles of β -AR and muscarinic receptors in regulating bladder function in health and disease have made them attractive drug targets for treatment of bladder pathophysiology (Andersson and Wein, 2004; Hood and Andersson, 2013). It is worth noting that voiding dysfunctions, such as poor stream and incomplete bladder emptying, can arise in conjunction with storage problems. Thus, the development of therapies for storage dysfunction needs to consider the potential adverse effect of impaired voiding function.

All of the known β -AR subtypes (1, 2, and 3) have been localized in human and rat bladders (Yamaguchi and Chapple, 2007), and detrusor relaxant actions have been described particularly for β_2 - and β_3 -AR, with some variation across species. Importantly, the relatively restricted distribution of the β_3 -AR subtype on the bladder has led to the development and recent licensing of selective agonists as treatments for OAB (Michel et al., 2011). However, it remains unclear how β_3 -AR activation functionally improves urine storage—via direct relaxant effects on bladder smooth muscle (Takeda et al., 2000) or via changes in afferent transmission (Aizawa et al., 2012) with or without urothelial mediation.

We have developed and characterized a novel model for integrated functional physiological and pharmacological studies

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ABBREVIATIONS: ANOVA, analysis of variance; β -AR, β -adrenoceptor; DAPR, decerebrate artificially perfused rat; EMG, electromyogram; EUS, external urinary sphincter; L748,337, *N*-[[3-[(2*S*)-2-hydroxy-3-[[2-[4-[(phenylsulfonyl)amino]phenyl]ethyl]amino]propoxy]phenyl]methyl]-acetamide; LUT, lower urinary tract; NMC, non-micturition contraction; ns, not significant; OAB, overactive bladder; PAN, pelvic afferent nerve.

of the complete rat micturition cycle in an unanesthetized preparation: the decerebrate artificially perfused rat (DAPR) (Pickering and Paton, 2006; Sadananda et al., 2011), a variant of the working heart brainstem preparation (Paton, 1996). This has intact LUT-spinal-brainstem reflex loops and demonstrates autonomic tone, the presence of which is crucial for normal bladder function. It also facilitates both nerve and sphincter recordings and enables the precise control of the composition of the perfusate for pharmacological investigations—analogue to in vitro conditions.

We previously observed that loss of autonomic tone in the DAPR (both pharmacological with hexamethonium ganglion blockade and pathological with loss of brainstem function) enhanced nonmicturition contraction (NMC) amplitude and reduced intervoid interval (Sadananda et al., 2011). This led us to hypothesize that the sympathetic drive acts to suppress NMCs—which are proposed to be part of a volume assessment reflex mechanism (Drake et al., 2001), and hence facilitate urine storage. We aimed to test this proposal by investigating the role of β -AR agonists on the micturition cycle with a focus on the functional effect of β_3 -agonism, given the predominant role of the β_3 subtype in mediating bladder relaxation (Igawa et al., 1999).

We show that both nonselective β -AR and selective β_3 -AR agonism with mirabegron prolonged the intervoid interval and increased bladder compliance while suppressing NMC amplitude but not frequency. The β_3 -AR-mediated effects promote storage without associated impairment of voiding function and are also seen in an acid-sensitized bladder pathological model. However, counter to our original hypothesis, we show that the increase in the intervoid interval correlates with the degree of increase in bladder compliance produced by β_3 -AR activation, but not with the reduction in NMC amplitude, nor with changed afferent sensitivity.

Materials and Methods

All experiments and procedures conformed to the UK Animals (Scientific Procedures) Act 1986 and were approved by our institutional ethics review committee.

Preparation. The procedures for the DAPR preparation were as previously described (Pickering and Paton, 2006; Sadananda et al., 2011) and are outlined here in brief. Female Wistar rats (40–90 g, P20–P28) were deeply anesthetized with halothane until loss of paw withdrawal reflex. Following a midline laparotomy, the stomach, spleen, and free intestine were vascularly isolated with ligatures and removed. The animals were precollicularly decerebrated and the halothane anesthesia was withdrawn. The preparation was chilled in ice-cold Ringer's solution and a midline sternotomy was performed. The lungs were removed and both atria were incised to avoid venous congestion during subsequent arterial perfusion. An incision at the apex of the heart allowed insertion of the perfusion cannula ($\emptyset$ 1.25 mm; DLR-4 Braintree Scientific, Braintree, MA) into the ascending aorta. The preparation was arterially perfused with carbogen-gassed Ringer's solution (composition described in "Drugs and Solutions"), containing Ficoll-70 (1.25%; Sigma-Aldrich, St. Louis, MO), at 32°C at ~30 ml/min. The arterial perfusion pressure was monitored via the second lumen of the cannula. Once perfusate flow was initiated, the heart resumed beating and rhythmic respiratory muscle contractions were seen within minutes, signaling the return of brainstem function (typically as arterial perfusion pressure reached 60–80 mm Hg).

Recordings. Simultaneous recordings of phrenic nerve, bladder pressure, electromyogram (EMG) of the external urinary sphincter (EUS), and pelvic afferent nerve (PAN) activity were recorded using

custom built amplifiers (Electronic Workshop, School of Physiology & Pharmacology), digitized using a micro1401 (Cambridge Electronic Design, Cambridge, UK) and analyzed using Spike2 software (Cambridge Electronic Design). Rhythmic, ramping phrenic nerve activity gave a continuous physiological index of brainstem viability (Paton, 1996). The ECG was visible on the phrenic nerve recording, and heart rate was derived from the ECG. To allow EUS-EMG recording, the pubic symphysis was cut in the midline, and a glass suction electrode (tip diameter 200 μ m) was placed slightly lateral to the midline directly below the bladder neck under visual control. The bladder afferent nerve was identified on the left and recorded proximal to the major pelvic ganglion, where it became the PAN. Both phrenic nerve and PAN were recorded with glass suction electrodes. A 30G needle was inserted into the bladder dome for pressure monitoring and fluid infusion.

Cystometry Protocol. For cystometry, both ureters were cut and ligated to stop natural filling from the kidneys. The bladder was filled by constant infusion with an artificial urine saline solution (Sadananda et al., 2011) at a flow rate of 20 μ l/min, which was chosen to produce relatively frequent (every 3–4 minutes) and stable voids. For all drugs, at least four stable control voiding contractions (typically over a period of 20 minutes) were elicited before systemic administration of the drug. The following micturition parameters were measured from a minimum of four cycles under each condition (see Fig. 1):

1. Baseline pressure: The lowest bladder pressure at the start of the filling phase.
2. Threshold pressure: The bladder pressure at the point where the EUS-EMG changes in activity from tonic to bursting, indicating the initiation of voiding (Maggi et al., 1986).
3. Voiding pressure: The peak bladder pressure achieved during the bursting phase of the EUS-EMG.
4. Voiding period: Determined from the duration of EUS-EMG bursting.
5. Intervoid interval: Time between voids.
6. Compliance: Obtained from the linear regression fit to the volume-infused versus bladder pressure relationship during the filling phase from the end of one void to the initiation of the next (at the threshold point).
7. NMCs: The peak to trough amplitude and inter-event interval of successive bladder pressure waves that were not associated with EUS-EMG bursting. The NMCs were identified (using a Spike2 function) by searching the intervoid regions for pressure waves of duration >5 seconds and amplitude >0.1 mm Hg and were manually verified.

Acute, acetic acid bladder sensitization was performed by switching to continuous intravesical infusion of acid at a pH of 5.5 (Zhang et al., 2003; Yamaguchi and Chapple, 2007; Sadananda et al., 2009; Parsons and Drake, 2011). The change in micturition parameters was observed within minutes of starting the infusion, and the sensitization continued for the remaining duration of the experiment.

Drugs and Solutions. The composition of the modified Ringer's solution used for arterial perfusion was as follows (in mM): NaCl (125), NaHCO_3 (24), KCl (3), CaCl_2 (2.5), MgSO_4 (1.25), KH_2PO_4 (1.25), and dextrose (10); pH 7.3 after carbogenation (95% O_2 /5% CO_2). All drugs and salts were from Sigma-Aldrich unless otherwise stated. β -Adrenergic agents were the nonselective agonist isoprenaline (1 nM to 1 μ M), the β_1 receptor-selective antagonist metoprolol (10 nM), the β_3 receptor-selective agonist mirabegron (1 nM to 1 μ M; Astellas Pharma), and β_3 -selective antagonist *N*-[3-[(2*S*)-2-hydroxy-3-[[2-[4-[(phenylsulfonyl)amino] phenyl]ethyl]amino]propoxy]phenyl]methyl]-acetamide (L748,337, 3 μ M; Tocris, Bristol, UK). Isoprenaline (100 μ M stock) was dissolved with 1 mM ascorbic acid to prevent oxidation (final pH 7.25 when diluted to 1 μ M in carbogenated modified Ringer's). Mirabegron (100 μ M stock) was dissolved in 20% 2-hydroxypropyl- β -cyclodextrin solvent. L748,337 (30 mM stock) was dissolved in

Fig. 1. Micturition parameters recorded in DAPR preparation. (A) Cystometrogram showing two voiding contractions under control conditions (continuous infusion at 20 μ l/min). The start of voiding is signaled by the onset of EUS bursting [seen in (B) on an expanded time base] from which the threshold pressure is derived. The intervoid interval is measured between the threshold points of successive voids. Voiding pressure is the maximal pressure during the EUS bursting phase. The baseline pressure is the trough pressure after a void once the bladder has relaxed, allowing filling to recommence. During the filling phase, the bladder pressure gradually ramps, with superimposed nonmicturition contractions whose peak-trough amplitude and period were quantified. The pelvic nerve recording showed characteristic responses during the filling and voiding phases, with bursts of activity corresponding to the NMCs, correlated with changes in bladder pressure and EUS activity. Note the preparation also showed increases in heart rate, arterial perfusion pressure, and phrenic nerve activity during each void, indicating global autonomic changes during micturition. (EUS, phrenic, and pelvic nerve recordings rectified and integrated with a time constant of 200 ms.)

dimethylsulfoxide. For all other agents, aqueous stock solutions were prepared and diluted in Ringer's solution prior to administration. All drugs were added systemically to the perfusate at the final stated concentrations. Perfusion of Ringer's containing either 2-hydroxypropyl- β -cyclodextrin, dimethylsulfoxide, or ascorbic acid alone (0.2%, 0.03%, and 10 μ M, respectively) was without effect on micturition parameters.

Analysis. The effect of all of the agents was assayed against the micturition parameters (listed previously) as well as on integrated EUS-EMG, pelvic afferent nerve, and phrenic nerve activity alongside heart rate and arterial perfusion pressure (Fig. 1). Results are expressed as the mean $\pm$ S.E., where normally distributed, or as the median [interquartile range]. Data were compared by paired *t* test or repeated-measures analysis of variance (ANOVA) with Dunnett's or

Bonferroni's post-hoc analysis and a significance threshold of $P < 0.05$. The relationship between changes in NMC amplitude, compliance, and intervoid intervals was explored using linear regression. All data analyses were performed using Prism 5 (GraphPad software, La Jolla, CA).

Results

Cystometry in the DAPR under control conditions (with a continuous infusion of artificial urine at $20 \mu\text{l}/\text{min}$) showed a baseline pressure of $2.9 \pm 0.4 \text{ mm Hg}$ with threshold and peak voiding pressures of 7.9 ± 0.8 and $22.4 \pm 1.0 \text{ mm Hg}$, respectively ($n = 15$). The intervoid interval was 181 seconds [130–211] ($n = 18$), and in time control experiments, these pressures and intervals remained stable for up to 3 hours ($n = 7$).

Actions of Systemic β -AR Agonists on the Micturition Cycle. Arterial infusion of isoprenaline (1 nM to $1 \mu\text{M}$) caused a concentration-dependent decrease in threshold pressure and voiding pressure at all doses from 100 nM (Fig. 2, A–C; $n = 4$). At the highest concentration ($1 \mu\text{M}$; $n = 5$), isoprenaline reduced threshold pressure by $28 \pm 4.2\%$ ($P < 0.02$) and voiding pressure by $21 \pm 3.1\%$ ($P < 0.008$). Isoprenaline increased both intervoid interval (by $231 \pm 63\%$ of control) and bladder compliance ($265 \pm 77\%$ of control; $P = 0.03$) but did not significantly change baseline bladder pressure. The duration of the voiding phase was reduced by $30 \pm 8\%$ ($P = 0.03$; $n = 6$), but voiding remained complete. Notably, isoprenaline also caused a dose-dependent

decrease in arterial perfusion pressure (Fig. 2D; from control of 70.8 ± 3.6 to $54.0 \pm 3.1 \text{ mm Hg}$ at 100 nM isoprenaline; $P = 0.0006$) and an increase in heart rate (from 327 ± 19 to 355 ± 23 beats per minute; $P = 0.008$; $n = 6$).

In a further set of experiments, repetition of the same incrementing isoprenaline dose protocol in the presence of metoprolol (10 nM; β_1 -AR antagonist) produced a similar profile of effects on bladder/micturition parameters in all six preparations, including reducing threshold pressure by $25 \pm 6.3\%$ ($P < 0.04$) and voiding pressure by $24 \pm 7.1\%$ ($P = 0.02$; $n = 6$) and increasing compliance ($260 \pm 78\%$ of control; $P = 0.04$) and intervoid interval ($180\% \pm 42\%$ of control; $P < 0.05$) in all preparations. Isoprenaline still decreased perfusion pressure in the presence of metoprolol but no longer caused a significant change in heart rate, providing evidence of its β_1 -AR antagonist activity.

The metoprolol-resistant effects of isoprenaline on micturition suggested that they may be mediated by a β_2 - and/or β_3 -AR mechanism, so we tested the actions of incremental dosing with mirabegron, a β_3 -selective agonist (1 nM to $1 \mu\text{M}$). Mirabegron (100 nM) increased the intervoid interval ($135 \pm 15\%$; $P = 0.04$; $n = 7$; Fig. 3, A and B), increased bladder compliance in all preparations ($293 \pm 57\%$; $P = 0.01$; $n = 7$; Fig. 3C), and produced a decrease in threshold pressure (Fig. 3D). However, at this concentration, there was no change in voiding pressure (Fig. 3E), baseline pressure, or duration of voiding phase, nor was there a change in arterial perfusion

Fig. 2. Dose-response to systemic isoprenaline infusion. (A) Incremental dosing with isoprenaline (10 nM to $1 \mu\text{M}$ in the perfusate) prolonged the intervoid interval, reduced voiding pressure, and lowered arterial perfusion pressure. The pooled data showed significant reductions in voiding pressure (B) at isoprenaline $\geq 100 \text{ nM}$, threshold pressure (C) at isoprenaline $\geq 100 \text{ nM}$, and arterial perfusion pressure (D) at isoprenaline $\geq 10 \text{ nM}$. (Repeated-measures ANOVA with Dunnett's post-hoc tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

Fig. 3. Influence of β_3 -adrenoceptors on storage and voiding. (A) Incremental dosing with mirabegron in the perfusate (steps from 10 nM to 1 μ M shown) increased the intervoid interval (100 nM; pooled data in red; $n = 7$; $P < 0.05$) (B), bladder compliance (100 nM; pooled data in red; $n = 7$; $P < 0.01$) (C), and threshold pressure (D) without changing the voiding pressure (E). (F) Application of the β_3 -adrenoceptor antagonist L748,337 (3 μ M) alone had the opposite effect—shortening the intervoid interval—suggesting the presence of a basal sympathetic tone to the bladder. (Repeated-measures ANOVA with Dunnett's post-hoc tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

pressure or heart rate. Only at the highest concentration (1 μ M) did mirabegron reduce arterial perfusion pressure (from 71.3 ± 4.9 to 64.3 ± 3.7 mm Hg; $P = 0.01$; Fig. 3A), although heart rate was still unchanged.

The actions of mirabegron (100 nM) to prolong the intervoid interval and increase compliance were blocked by the coapplication of the β_3 -AR antagonist L748,337 (3 μ M, $n = 3$; Table 1), and mirabegron now appeared to shorten the intervoid interval as well as increase voiding pressure. However, L748,337 alone was noted to reduce the intervoid interval (from 177 ± 44 to 105 ± 28 seconds; $P < 0.05$) and increase voiding pressure (24.5 ± 1.2 to 26.5 ± 1.7 mm Hg; $P < 0.05$) and baseline pressure (2.4 ± 0.06 to 3.5 ± 0.3 mm Hg;

$P < 0.05$) (Fig. 3F). These findings indicate that there is ongoing sympathetic tone in the DAPR which acts via β_3 -ARs to promote urine storage. There was no significant additional effect of mirabegron (100 nM) upon these changes evoked by the β_3 -AR antagonist.

Actions of Systemic β -AR Agonists on Nonmicturition Contractions. NMCs were readily apparent in most preparations with an amplitude of 0.88 ± 0.12 mm Hg and inter-NMC interval of 25.9 ± 5.4 seconds ($n = 17$) in controls. Within preparations, NMC amplitude and frequency were maintained throughout the course of the experiment as long as brainstem control remained intact (as reflected by the eupnoic pattern of phrenic nerve activity; Paton, 1996; Pickering and

TABLE 1

Effect of mirabegron in the presence of L748,337

	Control	Mirabegron + L748,337
IVI ($n = 3$)	177 $\pm$ 44	133 $\pm$ 44*
Voiding pressure ($n = 3$)	24.5 $\pm$ 1.2	26.2 $\pm$ 1.6*
Threshold pressure ($n = 3$)	3.7 $\pm$ 0.3	4.9 $\pm$ 1.1
Baseline pressure ($n = 3$)	2.4 $\pm$ 0.06	3.5 $\pm$ 0.3
Compliance ($n = 3$)	41.37 $\pm$ 6.3	34.2 $\pm$ 6.8

IVI, intervoid interval.

* $P < 0.05$, paired t test.

Paton, 2006), but as we have noted previously (Sadananda et al., 2011), they showed a progressive increase in amplitude as bladder volume increased during the filling cycle (see Fig. 4A).

The amplitude of NMCs was reduced by both isoprenaline ($-73 \pm 9\%$; $P = 0.02$; $n = 6$; $1 \mu\text{M}$; Fig. 4C) and mirabegron ($-23 \pm 2.4\%$; $P < 0.0005$; $n = 5$; 100 nM ; Fig. 4, A and D) in all preparations (Fig. 4A). NMC frequency was not significantly altered in the presence of mirabegron [inter-NMC interval for

control vs. mirabegron: 21.0 ± 4.7 vs. 23.8 ± 8.3 seconds, not significant (ns); Fig. 4D] or isoprenaline (Fig. 4E). The β_3 -AR antagonist, L748,337 increased NMC amplitude by $144 \pm 11\%$ ($P = 0.01$; $n = 3$) but had no effect on NMC frequency. The level of pelvic afferent nerve activity during NMCs and during voids was not altered by mirabegron (Fig. 4B) with maintained afferent sensitivity to bladder pressure [0.12 ± 0.02 control vs. $0.14 \pm 0.02 \mu\text{V.s/mm Hg}$ in the presence of mirabegron; $n = 3$, ns).

β_3 -AR Agonism in Pathological Models of Micturition. We have previously noted that NMCs are enhanced when spinal-brainstem autonomic control of the lower urinary tract is lost (Sadananda et al., 2011), which mimics the situation after spinal injury or autonomic neuropathy. Under these conditions, the NMCs changed in morphology with abnormally high amplitude ($3.9 \pm 0.4 \text{ mm Hg}$; $n = 6$). In such preparations, mirabegron (100 nM) still reduced NMC amplitude by $-36 \pm 5.5\%$ ($n = 4$; $P = 0.003$; see example in Fig. 5). The basal pelvic afferent nerve activity level diminished with the decrease in baseline bladder pressure on

Fig. 4. Nonmicturition contractions are attenuated by β_3 -adrenoceptor agonists. (A) Application of mirabegron (100 nM) increased the intervoid interval and decreased the amplitude of NMCs. [Pelvic nerve activity was rectified and integrated—time constant 200 ms (purple) and 2 seconds (black).] (B) Pelvic afferent nerve activity retained the same sensitivity to bladder pressure in the presence of mirabegron, still showing marked increases in activity during NMCs and during voids. (Correlation analysis using rectified and integrated pelvic nerve activity with time constant 500 ms.) Pooled data showed that both isoprenaline ($1 \mu\text{M}$; $n = 4$; $P < 0.01$) (C) and mirabegron (100 nM ; $n = 5$; $P < 0.01$) (D) significantly reduced NMC amplitude (individual paired data in black and means in red) without an action on the frequency of occurrence of the NMCs (E and F). (Paired t test: ** $P < 0.01$.)

mirabegron administration, but the amplitude of the bursts of increased pelvic nerve activity associated with each NMC was preserved ($n = 4$; see Fig. 5).

Acute acetic acid sensitization (intravesical application at pH 5.5; $n = 7$) produced the anticipated increase in voiding frequency (181 ± 27 to 129 ± 25 seconds; $P = 0.01$; see Fig. 6A) accompanied by a decrease in compliance (32.9 ± 9.0 to $23.0 \pm 9.7 \mu\text{l/mm Hg}$; $P < 0.05$). Acetic acid had no effect on NMC amplitude (control vs. acid: 1.2 ± 0.13 vs. 1.6 ± 0.12 mm Hg) or frequency. Mirabegron (100 nM; $n = 5$ preparations; Fig. 6, B and C) reversed the acid-induced shortening of the intervoid interval (125 ± 17 to 262 ± 37 seconds; $P = 0.01$) and the decreased bladder compliance (25.2 ± 12.8 to $85.4 \pm 13.8 \mu\text{l/mm Hg}$), but had no effect upon NMC amplitude (acid 1.6 ± 0.1 vs. acid+mirabegron 1.9 ± 0.3 mm Hg, ns).

The restoration of the intervoid interval by mirabegron in the acid-sensitized preparation without an action on NMCs suggested that the β_3 receptor agonist might be exerting its main beneficial effect by increasing bladder compliance. Therefore, we performed correlation analysis on the data set from naive preparations to look at the relationships between mirabegron (100 nM)-induced changes in intervoid interval, NMC amplitude, and bladder compliance (Fig. 7). A positive correlation was found between the intervoid interval and compliance (linear regression; $R^2 = 0.45$; $P < 0.05$), but there was no evident relationship to NMC amplitude. Furthermore, there appeared to be no significant relationship between compliance and NMC amplitude, suggesting that they may be independently regulated.

Discussion

We have undertaken a detailed examination of the mechanism of action of β -AR agonists on micturition in a functionally preserved, nonanesthetized rat preparation. We have shown that systemic administration of nonselective β -AR- and β_3 -AR-selective agents promotes storage by increasing bladder capacity and hence prolongation of the intervoid interval. This action was also seen following acute acetic acid bladder sensitization. These β -AR-mediated effects are associated with

increased bladder compliance and a decrease in the amplitude of non-micturition contractions. The selective β_3 -AR agonist mirabegron (Takasu et al., 2007) produced these effects on filling without changing active voiding parameters (unlike the nonselective β -AR agonist) or producing hemodynamic disturbances (see also Takeda et al., 2000). Selective β_3 -AR antagonism reduced bladder capacity and intervoid interval, indicating that there is a basal sympathetic tone acting via β_3 -AR to promote urine storage.

These findings are consistent with an existing body of literature indicating that sympathoactivation via β -AR promotes urine storage both in rodents and in humans (reviews: Furuta et al., 2006; Yamaguchi and Chapple, 2007; Michel et al., 2011). These studies have shown roles for both β_2 - and β_3 -AR-mediated effects on the detrusor (Longhurst and Levendusky, 1999; Morita et al., 2000; Michel and Vrydag, 2006), on signaling from the urothelium (Otsuka et al., 2008; Deba et al., 2009; Masunaga et al., 2010; Kullmann et al., 2011), and, latterly, afferent nerve signaling (Kanai et al., 2011; Aizawa et al., 2012). Much of these data have been gathered from in vitro studies or in vivo studies without intact micturition cycles, and as such, the importance of each mechanism in the regulation of the micturition cycle has been difficult to ascertain.

We have taken advantage of the decerebrate, arterially perfused rat preparation (Pickering and Paton, 2006; Sadananda et al., 2011) which allows cystometry to assess both filling and voiding parameters while enabling in vitro-like control over dosing of β -AR agonists and antagonists. In particular, we have been able to focus on the differential effects of these agents on bladder compliance, contractility, NMCs, and afferent signaling, and how these changes influence volume sensing and voiding frequency.

As well as increasing intervoid interval, we observed that β -AR agonists consistently reduced NMC amplitude (but not frequency). Such spontaneous bladder motions may form part of a detection mechanism that signals bladder fullness to the central nervous system (Drake et al., 2001). Based on our previous observations that NMC amplitude was increased following loss of autonomic control (pharmacological or pathological; Sadananda et al., 2011), we hypothesized that

Fig. 5. Pathologically augmented nonmicturition contractions are suppressed by β_3 -adrenoceptor agonist. After loss of brainstem-spinal control, micturition ceased, and the amplitude and morphology of NMCs and pelvic afferent activity were pathologically increased (shown on left, bladder at constant volume). Systemic infusion of mirabegron (100 nM) decreased both the amplitude of the NMCs and the pelvic afferent nerve activity (mirroring the reduction in bladder pressure) without a change in bladder volume. Note that the smaller NMCs in the presence of mirabegron still evoked similar-sized bursts of afferent discharge. Pelvic nerve activity was rectified and integrated, shown here with two time constants: 200 ms (purple) and 2 seconds (black).

Fig. 6. Intravesical acetic acid sensitization of the lower urinary tract is reversed by β_3 -adrenoceptor agonist. (A) Intravesical infusion of acetic acid (pH 5.5) shortened the intervoid interval and decreased bladder compliance. Systemic infusion of mirabegron (10 and 100 nM), in the continued presence of intravesical acetic acid, prolonged the intervoid interval (≥ 10 nM; $P < 0.05$; $n = 7$) (B) and increased bladder compliance (100 nM; $P < 0.01$; $n = 7$) (C) without significantly changing NMC amplitude. (Paired t test: $*P < 0.05$, and repeated-measures ANOVA with Dunnett's multiple comparison test versus acid-sensitized condition: $\#P < 0.05$; $##P < 0.01$.)

β -AR sympathomimetic agents would decrease NMC amplitude and thereby promote urine storage. We observed that β -AR agonists both reduced NMC amplitude and increased the intervoid interval. However, contrary to our original hypothesis, the increase in intervoid interval was not correlated with the degree of change in amplitude of NMCs, suggesting that these mechanisms are likely to be independent. The β -AR agonist-evoked increase in intervoid interval was, instead, correlated with an increase in bladder compliance. In the case of β_3 -AR agonism, this increase in compliance was not accompanied by an alteration in the active voiding parameters, suggesting it did not produce a change in detrusor contractility (unlike the nonselective β -AR agonists). This result indicates that the effects of β_3 -AR agonism are not achieved by directly opposing parasympathetic actions. Thus, the mirabegron-induced change in compliance is mediated independent of an action on active contractility.

An important caveat to these conclusions regarding the mechanism of action of β_3 -AR on urine storage should be noted: namely, that the intravesical pressure change of the NMCs may not be the physiologically relevant feature of the event. It may be that the movement-induced localized change in bladder wall stretch is the relevant signal to the bladder afferents (Drake et al., 2001)—indeed, afferent nerve recordings in the presence of mirabegron suggested that this afferent signaling was preserved even though the amplitude of the NMCs was smaller.

It is also worth noting that the threshold pressure for the initiation of voids was lowered by the β_3 -AR agonists, suggesting that there was no attenuation in the afferent signaling of bladder pressure. This was supported by our pelvic

afferent nerve recordings that showed preserved afferent signaling in response to dynamic changes in bladder pressure (both NMCs and during voiding contractions) in the presence of β_3 -AR agonist. This appears to contrast with recent reports from in vitro (Kanai et al., 2011) and in vivo (Aizawa et al., 2012) studies showing a decrease in afferent nerve activities by β_3 -AR agonists. This disparity will require further investigation, but our findings may be explained by the presence of dynamic bladder motions seen in our preparation with intact micturition that may provide a more physiological afferent activation.

The selective β_3 -AR agonist mirabegron improved urine storage and bladder compliance without attenuating voiding pressure, unlike the nonselective β agonist isoprenaline. Thus, it is plausible that the effect of isoprenaline on voiding pressure is mediated via β_1/β_2 -AR receptors to oppose parasympathetic contractile responses. In addition to the reduced voiding pressure, we also noted that isoprenaline shortened the duration of the voiding phase (unlike mirabegron). Although we did not directly study urethral outflow function, this shortening of the voiding phase may be consistent with activation of β_2 -adrenoceptors that have a dilator function in the rat urethra (unlike β_3) (Michel and Vrydag, 2006)—such dilation may allow more rapid evacuation of bladder contents. This lowered LUT outflow resistance could in itself account for the lowered voiding pressure in the presence of isoprenaline rather than a direct action on the detrusor, but this will require further experimental verification. Nonetheless, the features of selective β_3 -AR agonism are clinically relevant to OAB, as they suggest that storage function can be improved

Fig. 7. Mirabegron-induced increase in the intervoid interval is correlated with the degree of increase in compliance. (A) Linear regression analysis showed a positive correlation between the change in compliance and the degree of increase in the intervoid interval for each preparation ($R^2 = 0.45$; $P < 0.05$; $n = 7$). (B) In contrast, there was no significant correlation between the change in NMC amplitude and the intervoid interval ($n = 5$). (C) The NMC amplitude change was also independent of the mirabegron-induced changes in bladder compliance ($n = 5$).

without affecting voiding characteristics. This is an important aspect, as storage disorders can coexist with impaired voiding function.

Given this profile of action under physiological conditions, we next investigated the effect of β_3 -AR agonism in pathological models. First, mirabegron reversed the abnormally high amplitude of NMCs seen after loss of brainstem control in the DAPR preparation. Second, following acid sensitization to mimic acute bladder overactivity (Zhang et al., 2003), mirabegron increased the intervoid interval and compliance without an effect on NMC amplitude. This observation again implies that the effects of β_3 -AR agonism on storage are not mediated through a reduction in NMC amplitude. Although it has previously been noted in chronic models of lower urinary tract dysfunction such as bladder outlet obstruction (Hood and Andersson, 2013) that β_3 -AR agonism suppresses NMCs, it has not yet been demonstrated whether this action produces an improvement in urine storage in this pathological model.

Technical Aspects of the Use of the DAPR. An advantage of the DAPR illustrated in this study is the ability to assay the systemic effects of pharmacological agents on multiple organ systems (relatively independent of preparation viability) and thus detect potential side effects. This was illustrated in the current investigation in which isoprenaline was shown to increase heart rate (via β_1 -AR) and reduce arterial perfusion pressure. These actions were not seen with mirabegron except at the highest dose, where it caused a mild hypotensive effect.

The DAPR approach requires the use of young rats typically younger than 4 weeks of age. Although there is some debate over the age at which rat micturition is fully mature (Yamaguchi and Chapple, 2007; Kanai et al., 2011; Zvarova and Zvara, 2012), we found coordinated and complete bladder emptying in all our preparations as described previously (Sadananda et al., 2011). It should be noted that, for cystometry, we chose to use a bladder infusion rate (20 μ l/min) that produced a relatively short intervoid interval (typically less than 5 minutes) to allow drug responses to be assayed expeditiously, permitting repeated dosing studies with washouts.

Conclusions

Treatments for bladder storage dysfunctions such as OAB must possess a number of specific features: they must enhance bladder capacity and/or compliance while preserving the active voiding phase, and they should be well tolerated systemically. Previous therapies have focused on direct relaxation of the detrusor muscle, resulting in improved storage, but potentially adversely affecting voiding function, when contraction is required. In our current study, we found β_3 -AR agonism promoted urinary storage without impairing the voiding phase, and was relatively free of “off-target” cardiorespiratory side effects. Intriguingly, we also demonstrate a basal tone of sympathetically mediated β_3 activation that regulates lower urinary tract function and speculate that loss of such tone could play a role in the pathogenesis of conditions such as OAB.

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Authorship Contributions

Participated in research design: Sadananda, Pickering, Paton, Drake.

Conducted experiments: Sadananda.

Performed data analysis: Sadananda, Pickering.

Wrote or contributed to the writing of the manuscript: Sadananda, Pickering, Paton, Drake.

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