Enhanced sensitivity to afferent stimulation and impact of overactive bladder therapies in the conscious, spontaneously hypertensive rat

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Running Title Page

Running title: Characterization of the SHR overactive bladder model

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Abbreviations:
SHR - spontaneously hypertensive rat
PGE$_2$ – prostaglandin E$_2$
AA - acetic acid
VV – void volume
MI – micturition interval
MP – micturition pressure
SD - Sprague-Dawley
Abstract

The spontaneously hypertensive rat (SHR) has been proposed as an overactive bladder model, driven, at least partially, by alterations in bladder innervation. To assess the functional role of sensory bladder afferents we evaluated the conscious cystometric response to prostaglandin E$_2$ (PGE$_2$) or acetic acid (AA) bladder infusion. SHR demonstrated a hypersensitivity to PGE$_2$ and AA, as indicated by a greater reduction in both void volume (VV) and micturition interval (MI) compared to Sprague-Dawley controls. The heightened PGE$_2$ and AA responses in the SHR were inhibited by capsaicin desensitization, supporting a role for bladder afferents in facilitating the hypersensitivity. Furthermore, we characterized the SHR pharmacologically using overactive bladder therapeutic agents. In the SHR, both darifenacin and oxybutynin (M$_3$- and non-selective muscarinic antagonists, respectively) reduced micturition pressure (MP) and functional bladder capacity (VV and MI). In sharp contrast, functional bladder capacity was significantly enhanced by beta3-adrenoceptor agonism (CL316243), and by gabapentin, without effect on MP. These data provide the first functional evidence for hypersensitive bladder afferents in the SHR, and provide a pharmacological benchmark in this model for overactive bladder therapeutics. These data also support the idea that beta3-adrenoceptor agonism and gabapentin may provide a more effective overactive bladder therapy than muscarinic antagonism.
Introduction

The current approved pharmacological treatments for overactive bladder / urinary incontinence are limited, with the predominant first-line therapy represented by muscarinic antagonists (Andersson et al., 2009). These agents are contraindicated in some patient populations and have poor patient compliance, due to limited effectiveness and significant side effects, such as dry mouth, constipation and cognitive interference. In recent years clinical benefit against neurogenic bladder with intravesicular administration of botulinum toxin or desensitizing TRPV1 channel activators (capsaicin, resiniferatoxin) has been observed, which function to de-innervate the bladder (Cruz and Dinis, 2007). Although these intravesicular therapies are somewhat effective, patients can require bladder catheterization resultant from a complete inhibition of their voiding reflex. Therefore, a large unmet need remains for therapeutics free of unwanted side effects that maintain normal bladder voiding, yet are efficacious in reducing bladder overactivity.

The spontaneously hypertensive rat (SHR) has been reported by multiple groups to demonstrate a reduced void volume (VV) and micturition interval (MI) consistent with bladder overactivity (Clemow et al., 1998; Persson et al., 1998; Patra et al., 2007; Jin et al., 2009). It has been indicated that the afferent and efferent bladder nerves are hypertrophied in the SHR (Clemow et al., 1997), with a concomitant enhancement of nerve growth factor production (Clemow et al., 1998). Consistent with an underlying role for nerve growth factor in SHR bladder overactivity, nerve growth factor increases nerve activity and voiding frequency in
Sprague-Dawley (SD) rats (Lamb et al., 2004) and increased nerve growth factor levels have been reported in many other pre-clinical overactive bladder models (Steers et al., 1991; Dupont et al., 2001). This enhancement of nerve growth factor also appears to translate to some clinical conditions of bladder dysfunction, as observed in bladder outlet obstruction (reviewed by Steers et al., 1991; Steers and Tuttle, 2006). In addition, spinal mechanisms and/or an altered urethral regulation may also contribute to the SHR phenotype, which include changes in alpha-adrenergic signaling (Tong et al., 1996; Perrson et al., 1998). These findings may also be linked to clinical pathophysiology as alpha-adrenergic antagonists are effective in males with urine storage disorders (Patel and Chapple, 2008). Together these reports suggest that the enhanced bladder activity in the SHR is at least partially driven by alterations in neuronal control of micturition, and suggest that overlap may exist in the mechanisms underlying bladder overactivity in the SHR and those driving urological dysfunction in patients.

Therefore, there has been interest around the SHR as a model of bladder dysfunction, as reflected by attempts to identify novel targets (Yono et al., 2010), and its use in urodynamic validation studies with novel pharmacological modulators (Leon et al., 2008; Su et al., 2008). Recent functional analyses of afferent mechanosensitivity in the anesthetized SHR did not detect any alteration in responses to increased bladder pressure (Leon et al., 2008; Su et al., 2008). It has been shown previously that hyperactivity induced by intravesicular bladder infusion of acetic acid (AA) or prostaglandin E₂ (PGE₂) is capsaicin-sensitive and
dependent on sensory afferent nerves in the rat (Maggi et al., 1988; Mitsui et al., 2001). We therefore evaluated SHR bladder afferent sensitivity in the conscious state using these chemical stimuli. In addition, we evaluated the conscious SHR model pharmacologically, by assessing the response to overactive bladder agents: oxybutynin, darifenacin, beta3-adrenoceptor agonism, and gabapentin.
Methods

Chemicals and reagents: Oxybutynin, CL-316243 (5-[(2R)-2-][(2R)-2-(3-chlorophenyl-2-hydroxyethyl)amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate), capsaicin and PGE₂ were purchased from Sigma. Gabapentin was obtained from Ryan Scientific, and Darifenacin from Matrix Laboratories (India). Gelucire (10%) was utilized as vehicle for intraduodenal dosing. PGE₂ was dissolved in ethanol. Female SHRs (n=193; ~11 weeks of age) and SD rats (n=23; ~8 weeks of age) 180-240 gm were purchased from Charles River Laboratories. Since it has been established that the bladder:body weight ratio is unchanged in the SHR (Clemow et al., 1997, 1998; Perrson et al., 1998), SHR and SD rats were weight-matched to facilitate comparison. All animal experiments adhered to National Institute of Health guidelines and were approved by the institutional animal care and use committee (IACUC) of GlaxoSmithKline Pharmaceuticals.

Conscious, continuous-filling cystometry: Cystometry was performed as previously described (Jugus et al., 2009). Female rats were received from Charles River Surgical Services with urinary bladder and intraduodenal catheters surgically implanted. After a 5 day minimum post-surgical recovery period rats were placed in restraints (Braintree Scientific). Urinary bladder catheters were connected via a T connector to a pressure transducer and a saline infusion pump (Harvard Instruments) was used to continuously fill the bladder at a rate of 100 μl min⁻¹. Bladder pressure was viewed and recorded via Chart software through a PowerLab data acquisition system (ADI Instruments), and void volumes were measured by a digital balance located beneath the restraint. After an infusion...
equilibration period (~2 hours), an hour of control/basal urodynamics was obtained and compound or vehicle was then dosed via the intraduodenal (id) catheter (CL316243, gabapentin, darifenacin, oxybutynin), or added to the continuous intravesicular saline infusion (AA, PGE₂). Urodynamics were continually assessed for an additional 2 hour period post-dosing. In experiments involving pretreatment of rats with capsaicin a single 75 mg/kg subcutaneous dose of capsaicin or vehicle was administered 7 days prior to cystometry, similar to previously described (Mitsui et al., 2001).

**Data and statistical analysis:** Averages of cystometric parameters were obtained for 30 minute time periods post dosing. These values were normalized to the control period and expressed as a percentage of control. Average control / baseline urodynamic values for each group are shown in Tables 1 and 2. This approach reduced numerical variability between groups and facilitated unpaired comparisons (ie: compound versus vehicle). In the cases where single time point data are shown: 30-60 minute averages (labeled 60 min in time course data) were utilized for comparison of muscarinic antagonist effects, and 90-120 min averages were used for AA and PGE₂ studies. Micturition interval (MI) was measured as the time between micturition events and void volume (VV) as the volume of urine collected per micturition. Peak micturition pressure (MP) was measured as the maximum pressure reached during urine voiding. Changes in ‘functional bladder capacity’ were indicated by alterations in both average VV and MI.
Data are expressed as averages with associated standard errors, and statistics were performed using GraphPad Prism version 4.02 and 5.00. Student’s unpaired t-tests for single time points were used. Time course data were analyzed by 2-way repeated measures ANOVA, followed by a Bonferroni post-hoc analysis to compare equivalent time points.
Results

Enhanced sensitivity to AA and PGE$_2$ in the SHR: We evaluated the response to AA and PGE$_2$ in the SHR and compared the response to that observed in SD controls, as SD rats have historically been utilized for cystometric pharmacology studies. In both rat strains initiation of PGE$_2$ (120 uM) infusion resulted in a time-dependent reduction in both VV and MI reaching a maximum effect at 60-90 min. However the reduction in both VV and MI was significantly greater in the SHR (Fig. 1). MP was not significantly affected by PGE$_2$ (%control: SD veh 90±10%, n=8; SD PGE$_2$ 123±12%, n=8; SHR veh 113±5%, n=6; SHR PGE$_2$ 111±20%, n=4). Likewise, SHR hypersensitivity was observed with AA infusion (Fig. 2). SHR demonstrated a dose dependent reduction in VV and MI with AA (0.1-0.4%). Both 0.25% and 0.4% AA in the SHR provided a significantly greater decrease in VV and MI than SD rats receiving 0.4% AA. Whereas 0.1% AA in the SHR provided approximately the same reduction in VV and MI as 0.4% in SD rats (Fig. 2). MP tended to increase with AA in both SD and SHR, however the response was variable between rats and was not significantly different between strains (%control: SHR 0.1%AA 105±7%, n=5; SHR 0.25%AA 119±15%, n=6; SHR 0.4%AA 119±11%, n=4; SD 0.4%AA 147±12%, n=7).

As capsaicin-sensitive, transient receptor potential vanilloid 1 channels are preferentially expressed on sensory afferents, including those innervating the urinary bladder (Cruz and Dinis, 2007) we evaluated whether the enhanced response to PGE$_2$ and AA in the SHR was inhibitable by peripheral capsaicin administration, and therefore mediated via activation of bladder afferents. SHR
were pretreated with a single capsaicin dose (75 mg/kg sc) 7 days prior to performing irritant challenge cystometry. Capsaicin pretreatment alone significantly increased both VV and MI in the SHR under control conditions (Fig. 3A and B). In addition, we also observed an increase in MP in response to capsaicin pretreatment (vehicle 21±3 mmHg, n=16; capsaicin 31±3 mmHg, n=13; p=0.01). In a number of capsaicin pretreated SHR it was clear that distinct micturition events were absent (5 of 18). In these 5 rats saline infusion resulted in an overflow incontinence phenotype characterized by urethral drip, which was absent in all vehicle pretreated controls (0 of 16). Capsaicin pretreatment significantly inhibited the PGE$_2$ effect on VV and MI, and completely eliminated the AA (0.25%) response on VV and MI (Fig. 3C and D). Capsaicin pretreated SHR with overflow incontinence were excluded from these assessments of PGE$_2$ or AA responsiveness. These data indicate that the SHR response to AA and PGE$_2$ involves activation of capsaicin-sensitive afferent nerves.

**Muscarinic antagonism reduces MP and functional bladder capacity:** A representative cystometric response to 2 mg kg$^{-1}$ id oxybutynin, a non-selective muscarinic antagonist, is shown in Figure 4A. At doses of 2 and 3 mg kg$^{-1}$ id MP was significantly reduced as compared to vehicle (Fig. 4D). At these doses a significant reduction in both the MI and VV were also observed (Fig. 4B and C, respectively). The decrease in MP, MI and VV produced by oxybutynin appeared to be dose dependent as 1 mg kg$^{-1}$ produced a smaller reduction in these parameters. However, the response for all three parameters with 1 mg kg$^{-1}$ was not deemed significant. We hypothesized that doses of ≥1 mg kg$^{-1}$ id may be
therapeutically super-maximal yielding the deleterious decrease on functional bladder capacity. Therefore we evaluated a 10 fold lower dose (0.1 mg kg\(^{-1}\) oxybutynin) to determine if a beneficial functional response was attainable. However the response to 0.1 mg kg\(^{-1}\) was no different than that observed with vehicle (Fig 4). Scatter plots of MP versus VV, or MP versus MI for all 25 rats that received oxybutynin (using individual data points from the data shown in Fig. 4B-D) were fit by linear regression analysis and yielded p values of 0.03 and 0.02, respectively. This indicates that a correlation exists between the drop in MP and decrease in both VV and MI.

We also evaluated an M\(_3\)-selective muscarinic antagonist darifenacin (Chapple et al., 2005), as M\(_2\) and M\(_3\) receptors demonstrate some divergence in signaling in the lower urinary tract (reviewed by Giglio and Tobin, 2009). Darifenacin was found to produce a similar effect as oxybutynin (representative recording Figure 5A). Darifenacin at doses of 3, 10 and 30 mg kg\(^{-1}\) significantly reduced MI (Fig. 5B), and VV (Fig. 5C), whereas the reduction in peak pressure reached significance at 30 mg kg\(^{-1}\) (Fig. 5D). Lower doses, as low as 0.1 mg kg\(^{-1}\) darifenacin, were ineffective at altering MI, VV or MP. A correlation analysis of MP versus VV, and MP versus MI for darifenacin yielded similar results as observed for oxybutynin, however p values were not significant. This may reflect the smaller decrease in MP in response to darifenacin (Fig. 5) as compared to oxybutynin (Fig 4).

**Beta3-adrenoceptor activation enhances functional bladder capacity:** A number of beta3-adrenoceptor agonists are currently under development for overactive
bladder (Andersson et al., 2009). The beta3-adrenoceptor agonist CL316243 (Mori et al., 2010) provided a dose-dependent increase in VV and MI reaching ~150% of control with 3 mg kg\(^{-1}\). CL316243 3 mg kg\(^{-1}\) had a significant effect on both VV and MI (Fig. 6) and no effect on MP. CL316243 at 1 mg kg\(^{-1}\) significantly increased the MI and increased VV, but the effect on VV did not reach significance compared to vehicle control.

**Gabapentin enhances functional bladder capacity:** We also evaluated gabapentin currently under clinical development for overactive bladder (Kim et al., 2004; Carbone et al., 2006). Representative cystometrograms before and after gabapentin (30 mg kg\(^{-1}\)) are shown in Figure 7A. Gabapentin at doses of 30 and 300 mg kg\(^{-1}\) significantly enhanced functional bladder capacity reaching an increase of ~50% in the VV and MI at 120 minutes post-dosing (Fig. 7B and C). Gabapentin did not significantly alter MP (Fig 7D). Gabapentin at 3 mg kg\(^{-1}\) was deemed an ineffective dose as it was not significantly different from vehicle control for all cystometric parameters.
Discussion

Based on analogous observations between the SHR and clinical conditions of urological dysfunction, the SHR has been considered as a pre-clinical overactive bladder model with an altered bladder innervation. Although it is clear that the SHR demonstrates an enhanced urination frequency and reduced functional bladder capacity (Clemow et al., 1998; Perrson et al., 1998; Patra et al., 2007; Jin et al., 2009), there is no urodynamic evidence reported to support a heightened bladder afferent sensitivity. In this study, we probed the sensitivity to intravesicular irritants (PGE$_2$ and AA) known to activate bladder afferents. In addition, we assessed the urodynamic response in the conscious SHR to a number of overactive bladder agents.

We observed a striking hypersensitivity in the SHR to AA infusion, with the 0.1% AA induced decrease in VV and MI in the SHR being similar to that observed with 0.4% AA in SD rats. Overall AA effects were dose-dependent in the SHR with 0.25% and 0.4% driving dramatic reductions in functional bladder capacity. The SD was chosen as a control rat strain for this study as it has historically been utilized in cystometric pharmacology studies. In addition, it appears that WKY rats, used as normotensive controls for SHR cardiovascular studies, may demonstrate a certain degree of bladder overactivity, including an apparent intermediate bladder capacity between that of the SD and SHR (Patra et al., 2007; Jin et al., 2010).

The AA response in the SHR was completely inhibited by peripheral capsaicin desensitization, as shown in other AA studies with TRPV1
desensitizers (capsaicin and resiniferatoxin) in conscious control rat strains (Mitsui et al., 2001; Zhang et al., 2003). These data strongly implicate capsaicin-sensitive bladder afferents in the SHR as a mediator of the AA hypersensitivity. In a similar fashion to AA, the response to infusion of PGE$_2$ was enhanced in the SHR and was significantly inhibited by peripheral capsaicin pretreatment. However, the PGE$_2$ response reported here in the SHR was incompletely blocked by capsaicin pretreatment, unlike observations in Wistar rats (Maggi et al., 1988). This may reflect a lack of complete capsaicin desensitization in the current SHR study. However, this seems unlikely as 5 of 18 SHRs treated with capsaicin had a complete loss of voiding events. This is consistent with robust capsaicin desensitization in the protocol utilized for these studies. Alternatively, it may reflect capsaicin insensitive effects of PGE$_2$ acting on smooth muscle (Palea et al., 1998) or other tissues, such as the urothelium (Wang et al., 2008), capable of modulating bladder function. Capsaicin pretreatment alone enhanced basal VV and MI in SHR consistent with previous studies in control rats (Maggi et al., 1988; Mitsui et al., 2001). However, capsaicin also significantly enhanced the MP in the SHR. This effect was not reported previously in control rats and is likely due to the reduced MP in the SHR (Patra et al., 2007) that conceivably may reflect alterations in the urethral outflow resistance (Persson et al., 1998). Overflow incontinence was induced by peripheral capsaicin pretreatment in a minority of SHR. This is consistent with patients treated with intravesicular capsaicin or resiniferatoxin, a number of which require bladder catheterization due to loss of their micturition reflex (Cruz and Dinis, 2007).
The enhanced functional response shown here with PGE$_2$ and AA, agents known to target sensory afferents in the bladder, are consistent with biochemical and histological findings of nerve growth factor upregulation (Clemow et al., 1998) and bladder afferent hypertrophy (Clemow et al., 1997) in the SHR. This suggests that the SHR does present with a hypersensitive afferent innervation that may also contribute to its basal bladder overactivity. Although we have clearly detected an enhanced sensitivity to chemical irritants in the conscious SHR, sensitivity to bladder distention (pressure) appears to be unaffected based on visceromotor reflex and afferent nerve recording studies performed under urethane anesthesia (Leon et al., 2008; Su et al., 2008). Taken together this suggests that SHR afferent hypersensitivity is only observed in response to chemical and not mechanical stimuli, or alternatively that anesthesia may have interfered with detecting the SHR afferent hypersensitivity in early studies in which the effect of pressure was evaluated.

To our knowledge this is the first report to evaluate the effect of non-selective and M$_3$-selective muscarinic receptor antagonism (oxybutynin and darifenacin, respectively) in the SHR. In both cases antagonism decreased MP and functional bladder capacity. The deleterious effects on SHR functional bladder capacity do not reflect an effect of overdosing, as considerably lower doses (10-30 fold) of both oxybutynin, or darifenacin, did not provide an enhancement of VV or MI. The ability of muscarinic antagonism to reduce MP in multiple species, including rats, is well documented (Angelico et al., 2005; Ney et al., 2008; McCafferty et al., 2009). Likewise, MI shortening and VV reduction in
the SHR in response to muscarinic antagonism is consistent with previous rat studies (Igawa et al., 1993; Takeda et al., 2000, 2002; Ney et al., 2008). Overall, our urodynamic observations in response to muscarinic antagonism in the SHR are consistent with previous findings in cystometric studies of normal rats. These findings in rats with muscarinic antagonism contrast with clinical studies reporting an enhancement of functional bladder capacity (increased VV) and a reduced urinary frequency (increased MI), at doses that typically do not reduce MP (Chapple et al., 2005; Finney et al., 2006; Andersson et al., 2009). The reason for this discrepancy between the effect of muscarinic antagonism on rat and human bladder function is incompletely understood, but likely involves complex species differences in the mechanistic contribution of muscarinic receptors to bladder control, including alterations in muscarinic receptor isoform expression/function with pathology (reviewed by Giglio and Tobin, 2009). Although muscarinic antagonists have been utilized clinically for many years the precise mechanism of action responsible for their clinical benefit remains unclear (Finney et al., 2006; Andersson et al., 2009). This discrepancy between the impact of muscarinic antagonists in rat and human would suggest that rat cystometry may not be a good model for clinical bladder overactivity, at least for agents targeting muscarinic mechanisms.

In contrast to the urodynamic effects of muscarinic antagonism, gabapentin and beta3-adrenoceptor agonism with CL316243 demonstrated an enhancement of MI and VV with no effect on MP, similar to our observations in this model with PTGER3 (EP3) antagonists (Jugus et al., 2009). Beta3-
adrenoceptors are proposed to function at least partially through relaxation of the urinary bladder smooth muscle (Clouse et al., 2007), a mechanism involving activation of large conductance, Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (Hristov et al., 2008), whereas gabapentin and PTGER3 antagonists are likely active in modulating afferent nerve sensitivity and/or central nervous system signaling (Su et al., 2008; Jugus et al., 2009; Taylor, 2009). Our current observations using CL316243 to activate beta3-adrenoceptors (Mori et al. 2010) in the conscious SHR, demonstrating an enhanced functional bladder capacity are consistent with findings in the anesthetized SHR rhythmic contraction model in which CL316243 reduced contraction frequency (Leon et al., 2008). In addition, studies in SD rats using urethral obstruction, AA, PGE\textsubscript{2} and cerebral infarction to induce bladder overactivity have all demonstrated an enhancement of bladder capacity in response with CL316243 (Takeda et al., 2000, 2002; Kaidoh et al, 2002). This beneficial response on bladder function in response to beta3-adrenoceptor activation reported here in the SHR is also observed in the dog (Hicks et al., 2007), and has recently demonstrated translation into humans (Andersson et al, 2009). Interestingly, although significant clinical interest has been generated around the use of gabapentin to treat overactive bladder through small clinical studies demonstrating a urological benefit of gabapentin (Kim et al., 2004; Carbone et al., 2006), to our knowledge this is the first report of efficacy in a preclinical in vivo urodynamic model. Although the exact mechanism of action for gabapentin is unclear it is suggested to work by modulation of the neuronal control of bladder function. This may occur through both central and peripheral
mechanisms, including interactions with gamma aminobutyric acid receptors and the ancillary voltage-gated Ca\(^{2+}\) channel subunit alpha\(_{2}\)delta (Taylor, 2009).

This is the first \textit{in vivo} evidence for a functional afferent hypersensitivity in the SHR and is consistent with prior reports of afferent hypertrophy and increased nerve growth factor production. Muscarinic antagonists demonstrated deleterious effects on SHR bladder function by reducing MP, VV and MI. The enhancement of VV, MI without significant effect on MP observed with beta3-adrenoceptor activation or gabapentin are strikingly different and reflect a much preferred urodynamic response. These findings suggest that beta3-adrenoceptor antagonism and gabapentin may provide an enhanced clinical efficacy over muscarinic antagonism.
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Authorship Contributions

Participated in research design: Patra, Thorneloe.

Conducted experiments: Patra.

Performed data analysis: Patra, Thorneloe

Wrote or contributed to the writing of the manuscript: Thorneloe


the selective beta(3)-adrenoceptor agonist, CL316, 243, and various smooth muscle relaxants. *J Pharmacol Exp Ther* **293**:939-45.


Footnotes

This research was funded by GlaxoSmithKline Pharmaceuticals. The studies were completed by the authors as employees of GlaxoSmithKline.
Figure Legends

**Figure 1: Comparison of intravesicular PGE₂ infusion responses in SD and SHR.** PGE₂ (120 uM) infused intravesicularly into the bladder provides a significantly greater reduction in VV (A) and MI (B) in SHR than in SD rats. Vehicle infusion (ctrl) is without effect in either rat strain. (**p<0.01 vs. SD, unpaired t-test).

**Figure 2: Comparison of intravesicular AA infusion responses in SD and SHR.** Intravesicular AA provides a dose-dependent reduction in VV (A) and MI (B) in the SHR. The response in SHR is significantly greater than in SD rats (*, **p<0.05 and p<0.01 vs. SD respectively).

**Figure 3: Capsaicin pretreatment in the SHR inhibits the responses to PGE₂ and AA infusion.** Capsaicin pretreatment (75 mg/kg sc single dose, 7 days prior to cystometry) significantly increased basal VV (A), MI (B). The PGE₂ and AA (0.25%) effects on VV (C) and MI (D) were significantly reduced by capsaicin pretreatment (*, **p<0.05 and p<0.01 vs. ctrl, unpaired t-test).

**Figure 4: Effect of muscarinic antagonist oxybutynin on SHR urodynamics.**

A. Representative cystometrograms before and after oxybutynin (2 mg kg⁻¹ id).

B, C and D. Dose response to oxybutynin on MI, VV, and MP, respectively, measured 30-60 min post-dose (*p<0.05, **p<0.01, unpaired test vs. vehicle; n=19, 7, 3, 8, 7 rats for vehicle, 0.1, 1, 2, 3 mg kg⁻¹, respectively).
Figure 5: Effect of M₃-selective muscarinic antagonist darifenacin on SHR urodynamics. A. Representative cystometrograms before and after darifenacin (3 mg kg⁻¹ id). B, C and D. Dose response to darifenacin on MI, VV, and MP, respectively, measured 30-60 min post-dose (*p<0.05, **p<0.01, unpaired test vs. vehicle; n=8, 8, 4, 3, 8, 9 rats for vehicle, 0.1, 0.3, 1, 3, 10, 30 mg kg⁻¹, respectively).

Figure 6: Effect of beta3-adrenoceptor agonist CL316243 on SHR urodynamics. CL316243 at 3 mg kg⁻¹ significantly increased VV (A) and MI (B), whereas the effect of CL316243 at 1 mg kg⁻¹ was significant on the MI. Data shown is the effect as assessed at 90-120 min post intraduodenal dosing. (*, ** p<0.05 and p<0.01 vs vehicle unpaired t-test).

Figure 7: Effect of gabapentin on SHR urodynamics. A. Representative cystometrograms before and after gabapentin (30 mg kg⁻¹ id). B, C and D. Dose response to gabapentin on VV, MI and MP, respectively. For each dose the response was averaged for 30 min periods over a total post-dose time of 120 min. 3 mg kg⁻¹ gabapentin was without effect and no effect was observed on MP at any doses (p=0.009, p=0.01 and p=0.3 VV, MI and MP respectively by 2 way RM-ANOVA for an effect of gabapentin; *, **, *** p<0.05, p<0.01, and p<0.001 vs. vehicle Bonferroni post-hoc).
### Table 1: Irritant Studies Baseline Cystometric Parameters

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<tr>
<th>Group</th>
<th>Void Volume (ml)</th>
<th>Interval (min)</th>
<th>Peak Pressure (mmHg)</th>
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<tr>
<td>Control SD (n=8)</td>
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Table 2: Therapeutic Agent Studies Baseline Cystometric Parameters

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<td>Vehicle (n=19)</td>
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<td>Oxybutynin 0.1 mg/kg (n=7)</td>
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<td>Oxybutynin 1 mg/kg (n=3)</td>
<td>0.60 ± 0.09</td>
<td>6.0 ± 1.0</td>
<td>18.6 ± 0.8</td>
</tr>
<tr>
<td>Oxybutynin 2 mg/kg (n=8)</td>
<td>0.67 ± 0.07</td>
<td>5.7 ± 0.5</td>
<td>17.3 ± 4.3</td>
</tr>
<tr>
<td>Oxybutynin 3 mg/kg (n=7)</td>
<td>0.57 ± 0.08</td>
<td>5.4 ± 0.7</td>
<td>13.2 ± 2.2</td>
</tr>
<tr>
<td>Vehicle (n=8)</td>
<td>1.09 ± 0.12</td>
<td>10.7 ± 1.3</td>
<td>22.6 ± 1.8</td>
</tr>
<tr>
<td>Darifenacin 0.1 mg/kg (n=8)</td>
<td>1.31 ± 0.19</td>
<td>14.3 ± 2.3</td>
<td>19.6 ± 1.7</td>
</tr>
<tr>
<td>Darifenacin 0.3 mg/kg (n=8)</td>
<td>1.31 ± 0.17</td>
<td>13.5 ± 1.0</td>
<td>19.5 ± 2.6</td>
</tr>
<tr>
<td>Darifenacin 1 mg/kg (n=4)</td>
<td>1.22 ± 0.17</td>
<td>12.5 ± 2.3</td>
<td>21.2 ± 1.2</td>
</tr>
<tr>
<td>Darifenacin 3 mg/kg (n=3)</td>
<td>1.70 ± 0.34</td>
<td>17.6 ± 3.0</td>
<td>24.4 ± 7.5</td>
</tr>
<tr>
<td>Darifenacin 10 mg/kg (n=8)</td>
<td>0.88 ± 0.14</td>
<td>9.7 ± 1.4</td>
<td>19.6 ± 1.5</td>
</tr>
<tr>
<td>Darifenacin 30 mg/kg (n=9)</td>
<td>1.19 ± 0.11</td>
<td>12.1 ± 0.9</td>
<td>22.2 ± 2.2</td>
</tr>
<tr>
<td>Vehicle (n=10)</td>
<td>1.16 ± 0.16</td>
<td>11.6 ± 1.6</td>
<td>18.8 ± 1.6</td>
</tr>
<tr>
<td>Gabapentin 3 mg/kg (n=4)</td>
<td>1.19 ± 0.27</td>
<td>11.5 ± 2.6</td>
<td>15.9 ± 0.4</td>
</tr>
<tr>
<td>Gabapentin 30 mg/kg (n=4)</td>
<td>1.06 ± 0.15</td>
<td>10.5 ± 1.6</td>
<td>15.2 ± 3.1</td>
</tr>
<tr>
<td>Gabapentin 300 mg/kg (n=8)</td>
<td>1.33 ± 0.14</td>
<td>13.3 ± 1.4</td>
<td>18.5 ± 2.0</td>
</tr>
<tr>
<td>Vehicle (n=11)</td>
<td>0.68 ± 0.12</td>
<td>7.3 ± 1.1</td>
<td>11.8 ± 2.0</td>
</tr>
<tr>
<td>CL316243 1 mg/kg (n=4)</td>
<td>0.73 ± 0.20</td>
<td>7.1 ± 1.7</td>
<td>13.7 ± 3.9</td>
</tr>
<tr>
<td>CL316243 3 mg/kg (n=6)</td>
<td>0.43 ± 0.07</td>
<td>4.6 ± 0.7</td>
<td>5.3 ± 0.5</td>
</tr>
</tbody>
</table>
Figure 1

(A) Void Volume

(B) Micturition Interval
Figure 2
Figure 3

A. Void Volume

B. Micturition Interval

C. Void Volume

D. Micturition Interval
Figure 4
Figure 5
Figure 6

(A) Void Volume

(B) Micturition Interval
Figure 7