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Modulation of non-voiding activity by the muscarinergic antagonist tolterodine and the β_3-adrenoceptor agonist mirabegron in conscious rats with partial outflow obstruction

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OBJECTIVE

- To investigate the hypothesis that tolterodine and the β_3-adrenoceptor agonist mirabegron exert their actions on the motor component of the motor/sensory system in the bladder wall: non-voiding activity (NVA).

MATERIALS AND METHODS

- The present study used standard cystometric techniques and a conscious rat model of partial bladder outflow obstruction (BOO). A single dose of either tolterodine (0.01, 0.1 0.3 or 1.0 mg/kg) or mirabegron (0.03, 0.1, 0.3, 1.0 or 3.0 mg/kg) was given i.v. to each animal.

RESULTS

- In the dose ranges used, tolterodine reduced the voiding contraction amplitude, whereas mirabegron did not.
- Non-voiding activity consisted of small (<0.6 mmHg) and large (>0.6 mmHg) transients.
- As a fill progressed, both tolterodine and mirabegron reduced the cumulative activity of the large non-voiding contractions, but had little effect on the small transients.
- Tolterodine affected both the amplitude and frequency of NVA, whereas mirabegron affected primarily the frequency.

What's known on the subject? and What does the study add?
Experimental urethral obstruction in rats alters micturition patterns with non-voiding activity (NVA) during filling cystometry, showing similarity to that observed in human detrusor overactivity. Several drug classes with therapeutic potential in overactive bladder in humans have been tested in this model in rats, rabbits or guinea pigs, but no detailed analysis of drug effects on cystometric patterns has been published.

The present study uses a rat model of overactivity with partial bladder outflow obstruction (BOO) in combination with the procedures to analyse NVA to study the effects of the anticholinergic drug tolterodine and the novel β_3-adrenoceptor agonist mirabegron. The current data for the first time show that NVA in rats with BOO is sensitive to both the muscarinergic antagonist tolterodine and the β_3-adrenoceptor agonist mirabegron, but with clear differences between the two drugs: during progression of bladder filling, tolterodine affected both the amplitude and frequency of NVA whereas mirabegron affected primarily the frequency. In addition, tolterodine dose-dependently reduced voiding contractions, while mirabegron did not. A model is proposed to account for these observations where both agents act on a 'pacemaker-like' mechanism which is sensitive to cholinergic excitatory and beta-adrenergic inhibitory inputs. Such concepts could provide insights into the nature of overactive bladder and the site of action of key therapeutic drugs.

CONCLUSIONS

- Non-voiding activity is sensitive to muscarinergic antagonists and β_3-adrenoceptor agonists, but there are clear differences between the two drugs.
- A model is proposed to account for these observations where both agents act on a 'pacemaker-like' mechanism with cholinergic excitatory and adrenergic inhibitory inputs.
- Such concepts may provide insights into the nature of overactive bladder and the site of action of key therapeutic drugs.

KEYWORDS

rat, chronic urethral obstruction, non-voiding activity, tolterodine, mirabegron

INTRODUCTION

Overactive bladder (OAB) is a common and chronic symptom complex that increases in prevalence with advancing age. The International Continence Society definition [1] is based on symptoms and does not require urodynamic investigation. However, urodynamics is a useful tool to diagnose detrusor overactivity (DO) before initiating treatment [2].

Experimental urethral obstruction in rats is known to cause increases in bladder weight and altered micturition patterns associated with changes in both the central and peripheral nervous control of the lower urinary tract. It serves as a model for human DO [3,4]. For urethral obstruction models to be useful to assess potential pharmacotherapeutic concepts for DO in humans, it is necessary to establish functional changes leading to quantifiable cystometric patterns of DO. In addition, evidence of amelioration and/or reversion of such patterns by drugs with proven clinical efficacy in the overactive bladder syndrome is needed. The procedure and timing of urethral obstruction in animals are critical. In rats that underwent urethral obstruction for 6 weeks, experimental DO was clearly established and found to be inhibited by the non-selective muscarine antagonist drug tolterodine, and a phosphodiesterase type-4 inhibitor [5], as well as by adenosine triphosphate (ATP)-dependent potassium channel agonists [6]. For ATP-dependent potassium channel agonists, proof of concept in DO is generated both in isolated human detrusor [7,8] and in small-scale experiments in patients [7]; for the phosphodiesterase type-4 inhibitor rolipram, inhibition of DO was shown only in human detrusor strips *in vitro* [9] and because of unfavourable efficacy/side-effect balance no drugs in these pharmacological classes have become licensed drugs for treating OAB.

β_3-Adrenoceptor agonists are proposed as a new drug class in treating OAB. The selective β_3-adrenoceptor agonist mirabegron is an example that has been shown to be clinically effective in randomized placebo-controlled phase 2 and phase 3 studies in OAB [10–12]. The precise therapeutic mode of action of these drugs is unknown at the present time.

During filling, the human urinary bladder is under control of the sympathetic nerve system activating β-adrenoceptors in the bladder [13]. mRNA expression data as well as functional pharmacology data have shown that the β_3-adrenoceptor is the most dominant β-adrenoceptor involved in relaxation of the human urinary bladder in both healthy bladder specimens and those with LUTS [14–16]. In animal studies with the β_3-adrenoceptor agonists FK175 and CL-316243, there is evidence that activation of β_3-adrenoceptors promoted urine storage in rats by increasing bladder capacity [17,18]. In addition, CL-316243 increased urinary bladder capacity in models for induced bladder hyperactivity conditions in rats [19,20]. Mirabegron showed a high degree of β_3-adrenoceptor selectivity and a higher agonist activity than CL-316243 for human and rat β_3-adrenoceptors and had similar effects on urine storage [21].

In a study using restrained conscious rats after 6 weeks of urethral obstruction, altered patterns of activity in the filling phase of micturition cycles were reported: non-voiding activity (NVA) [4]. However, no quantitative analysis was presented. Subsequently, an approach has been described to analyse NVA during micturition cycles in conscious rats [22].

The present study uses a rat model of overactivity with partial bladder outflow obstruction (BOO) [4] in combination with the procedures to analyse NVA [22] to study the effects of the anticholinergic drug tolterodine and the novel β_3-adrenoceptor agonist mirabegron. The objective was to develop hypotheses to account for the mode of action of these different drug classes on bladder overactivity.

MATERIALS AND METHODS

All experimental protocols were carried out in accordance with the European Community Council Directive 86/609/EEC and French legislation and licensing was by the French Ministry for Agriculture and Fisheries.

Female Sprague–Dawley rats (190–250 g) were used in the experiments. Four groups were studied: control (six animals); sham operated (six animals); operated with mirabegron (five doses, 29 animals); and operated with tolterodine (four doses, 26 animals). The total number of animals used in the present study was 67.

Rats were anaesthetized with isoflurane (3%). For the BOO group, the abdominal wall was opened though a midline incision and the bladder and urethra were exposed. A constant degree of urethral obstruction was produced by a ligature around the urethra and a metal rod with an outer diameter of 1 mm. The abdominal wall was closed. Food and water were given *ad libitum*. After obstruction, animals were treated with one injection of long-lasting 5% terramycine (w/v) to prevent infections. During the first 3 days after obstruction, the urinary bladder was squeezed once a day to avoid urinary retention and renal failure (two major causes of death for acutely obstructed rats) and each animal received an injection of 1% ketofen (w/v, 0.1 mL/rat daily s.c.). The sham group received the same surgery and treatment without urethral ligation.

After 6 weeks, rats were anaesthetized with isoflurane (3%) and the urethral ligature was removed. Polyethylene catheters (or 0.58 and 0.96 mm internal and outer diameter, respectively) were implanted through the bladder dome and in the jugular vein for drug administration. Catheters were exteriorized at the scapular level.

Cystometric investigation was performed in conscious rats 48 h after catheter implantation. Animals were placed in restraining cages. An intravesical catheter was connected via a T-tube to a strain gauge MX 860 Novatrans III Gold (Medex Medical SARL, Nantes-Carquefou, France) and a syringe pump (70-2208 Model II plus, Havard Appartus, Les Ullis, France). The intravesical pressure was recorded continuously using a PowerLab interface (ADInstruments Pty Ltd, Castle-Hill, Australia). The bladder was continuously infused with saline at the rate of 10 mL/h (1 mL/h for sham). The rate of infusion of saline was different in animals with BOO from that in sham animals so as to obtain similar intervals between micturitions in obstructed and control rats [4]. After a stabilization period of about 30 min, control-filling cycles were recorded. Subsequently, vehicle, tolterodine (0.01, 0.1, 0.3 and 1 mg/kg) or mirabegron (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg) were administered

FIG. 1. Demonstration of the effects of i.v infusion of tolterodine (0.3 mg/kg) on the voiding pattern of an awake rat with a surgically induced partial BOO (see the 'Materials and Methods' section). ***A****, A section of record illustrating four void cycles. Where indicated by the horizontal bar, tolterodine was infused. Voiding contractions are easily identified and NVA is clearly seen during each filling phase.* ***B****, The isolated phasic activity during each filling phase. This trace was derived from the record in (****A****). The original record was subjected to smoothing to remove the transient activity but retain the slow rise in pressure. This record was then subtracted from the original record to produce the transient component.* ***C****, An analysis of the isolated phasic activity. It shows an integration of the phasic activity (60 s integration periods) during each fill cycle.* ***D****, Data extracted from (****C****) for the second control fill (green symbols) and the final fill in the presence of tolterodine (red symbols).*

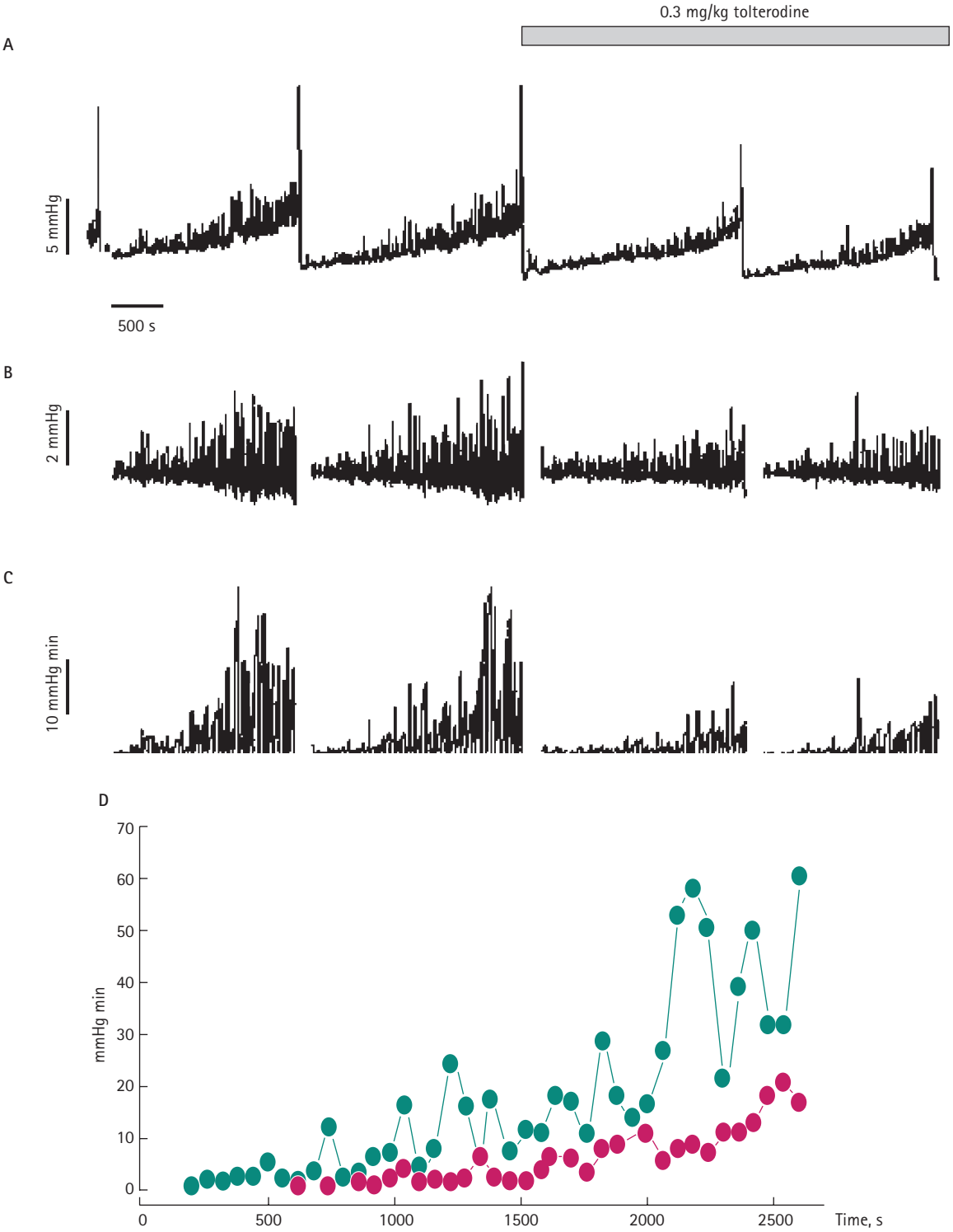


FIG. 2. The effects of i.v infusion of mirabegron (0.3 mg/kg) on the voiding pattern of an awake rat with a surgically induced partial BOO (see the 'Materials and Methods' section). ***A****, Section of record illustrating four void cycles. Where indicated by the horizontal bar, mirabegron was infused. Voiding contractions are easily identified and NVA is clearly seen during each filling phase.* ***B****, The isolated phasic activity during each filling phase (see the 'Materials and Methods' section).* ***C****, An analysis of the isolated phasic activity. It shows an integration of the phasic activity (60 s integration periods) during each fill cycle.* ***D****, Data extracted from (****C****) for the second control fill (green symbols) and the final fill in the presence of mirabegron (red symbols).*

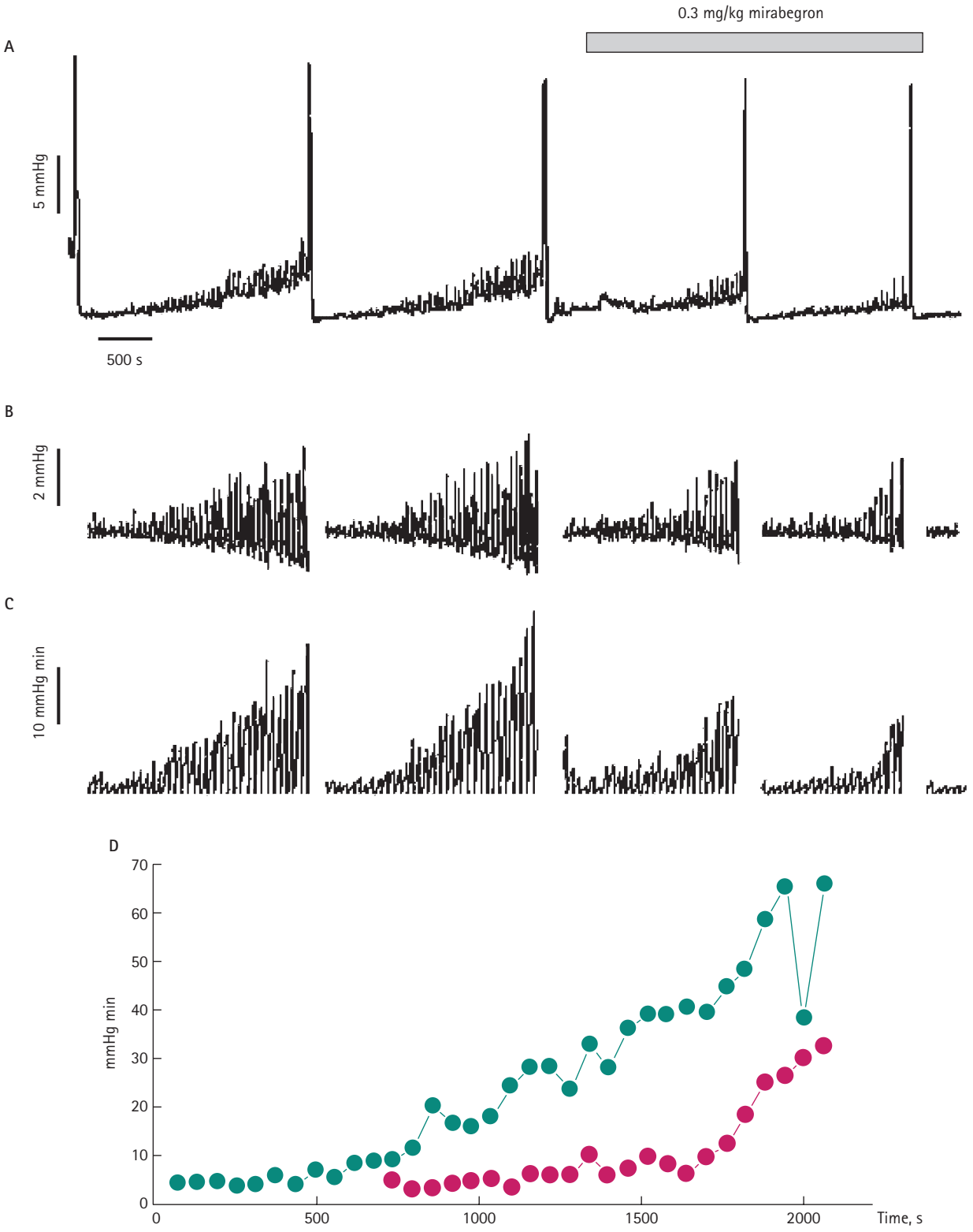
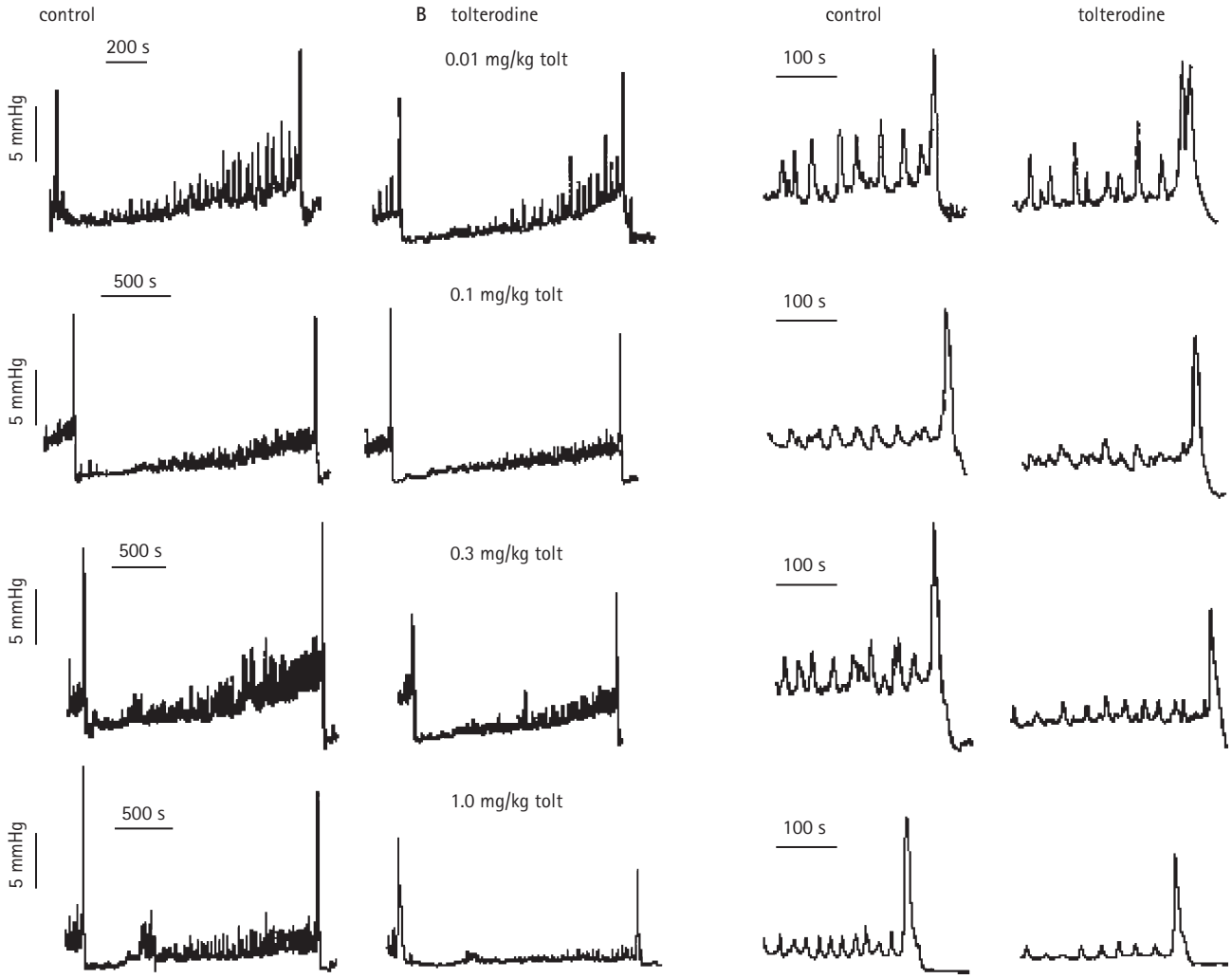


*FIG. 3. Illustration of the effects of different concentrations of tolterodine on a single fill cycle in different rats each with parital BOO. **A**, The control cycle; **B**, the corresponding cycle in the same animal in the presence of the concentration of tolterodine (tolt) used. The two columns on the right illustrate the final 300 s of the control records and the tolterodine records in (**A**) and (**B**) on an expanded timescale.*



i.v. (1 mL/5 min). The dose range selected for the drugs was based on published *in vivo* studies of bladder function in rats for mirabegron [21] and tolterodine [5]. Cystometrograms for analysis of vehicle and drug effects were taken 10–20 min after infusion of drugs or vehicle.

At the end of an experiment, animals were killed and their bladder weight measured.

The vehicle used to dissolve mirabegron and tolterodine was a mixture of 10% N,N dimethylacetamide (VWR, Fontenay/s bois, France), 5% Cremophor (Sigma, Saint Quentin Fallavier, France) and 85% saline (NaCl 0.9% from Centravet, Lapalisse, France). Tolterodine and mirabegron were provided from Astellas Pharma Europe. Isoflurane was purchased from Baxter (Maurepas, France). Terramycine and ketofen were purchased from Centravet (Lapalisse, France).

The variables analysed were voiding frequency, voiding contraction amplitude, voiding threshold, voiding pressure, NVA frequency and amplitude. Amplitude distribution histograms were constructed for NVA in control and drug-treated animals. Bin widths for the amplitude analysis were optimalized to obtain maximal distinction between drug-treated and control animals and were set at 0.2 mmHg.

In both drug-treated and control animals, transient amplitudes showed a maximum at ≈0.6 mmHg, so small (<0.6 mmHg) and larger (>0.6 mmHg) transients were isolated for further analysis. In the P3 phase (the last phase before micturition [22]) NVA frequency and amplitude were analysed for both drug treatment groups vs control.

Mean and standard deviations are shown throughout. Where applicable, simple paired Student's *t*-tests were used to assess significance in paired data ($P < 0.05$ was considered to indicate statistical significance). One-way ANOVA was used in all other cases ($P < 0.05$ was considered to indicate statistical significance).

*FIG. 4. Demonstration of the effects of different concentrations of mirabegron on a single fill cycle in different rats with partial BOO. **A**, The control cycle; **B**, the corresponding cycle in the same animal in the presence of the concentration of tolterodine (tolt) used. The two columns on the right illustrate the final 300 s of the control records and the tolterodine records in (**A**) and (**B**) on an expanded timescale.*

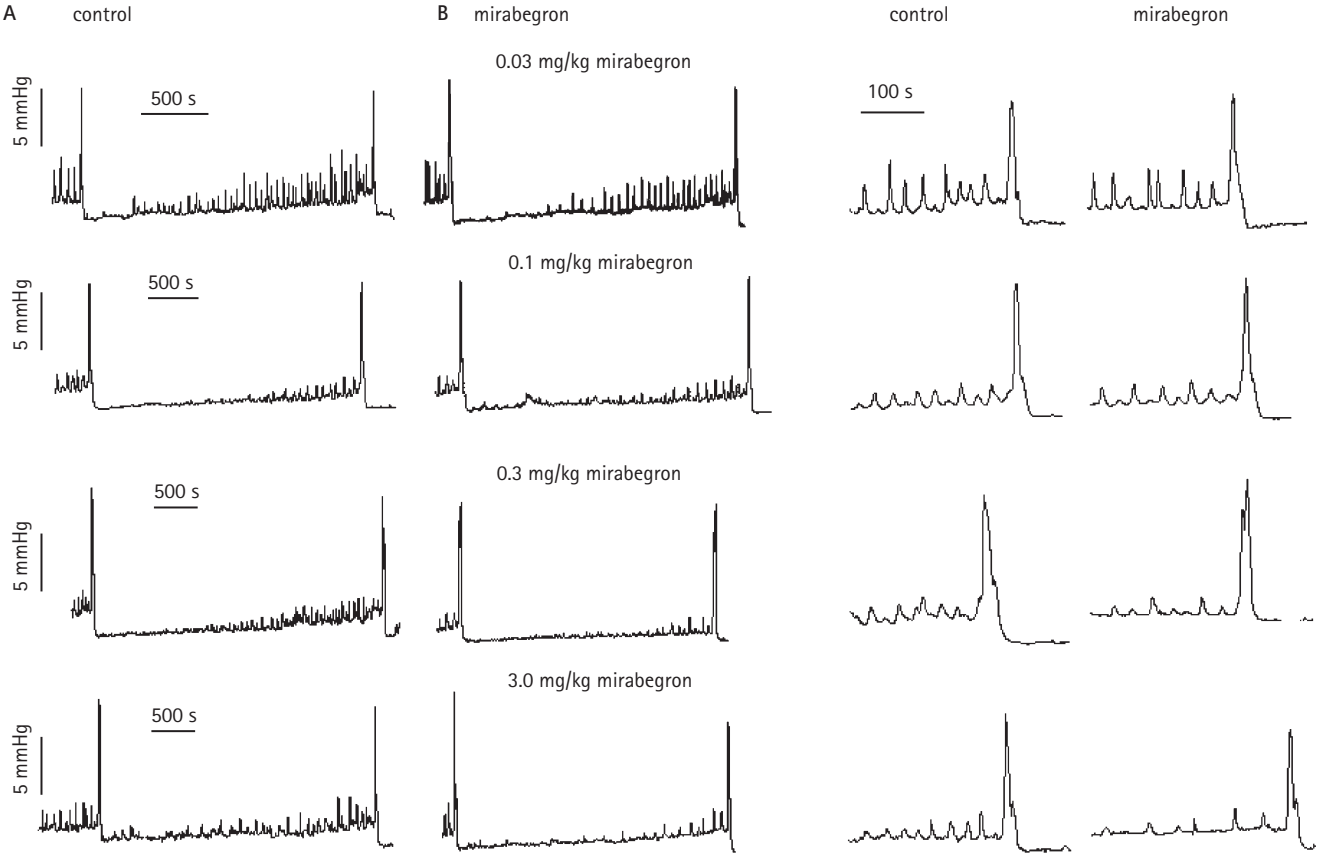
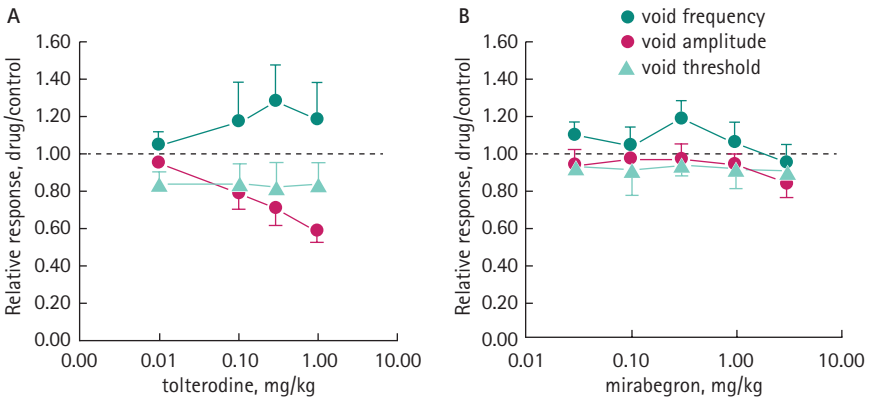


*FIG. 5. Combined data illustrating the effects of different doses of tolterodine (**A**) and mirabegron (**B**) on voiding parameters in rats with partial BOO. In each panel data illustrating the frequency of voiding (green circles), the amplitude of the voiding contraction (red circles) and the pressure at which the void contraction begins (threshold: green triangle). For the different doses of tolterodine (0.01, 0.1, 0.3 or 1 mg/kg) and mirabegron (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg) the number of animals used in each group were seven, seven, seven, five and six, five, five, six, seven respectively. The data are presented as ratio of drug/control for each variable (mean ± S.D.) (e.g. see Figs 1,2).*

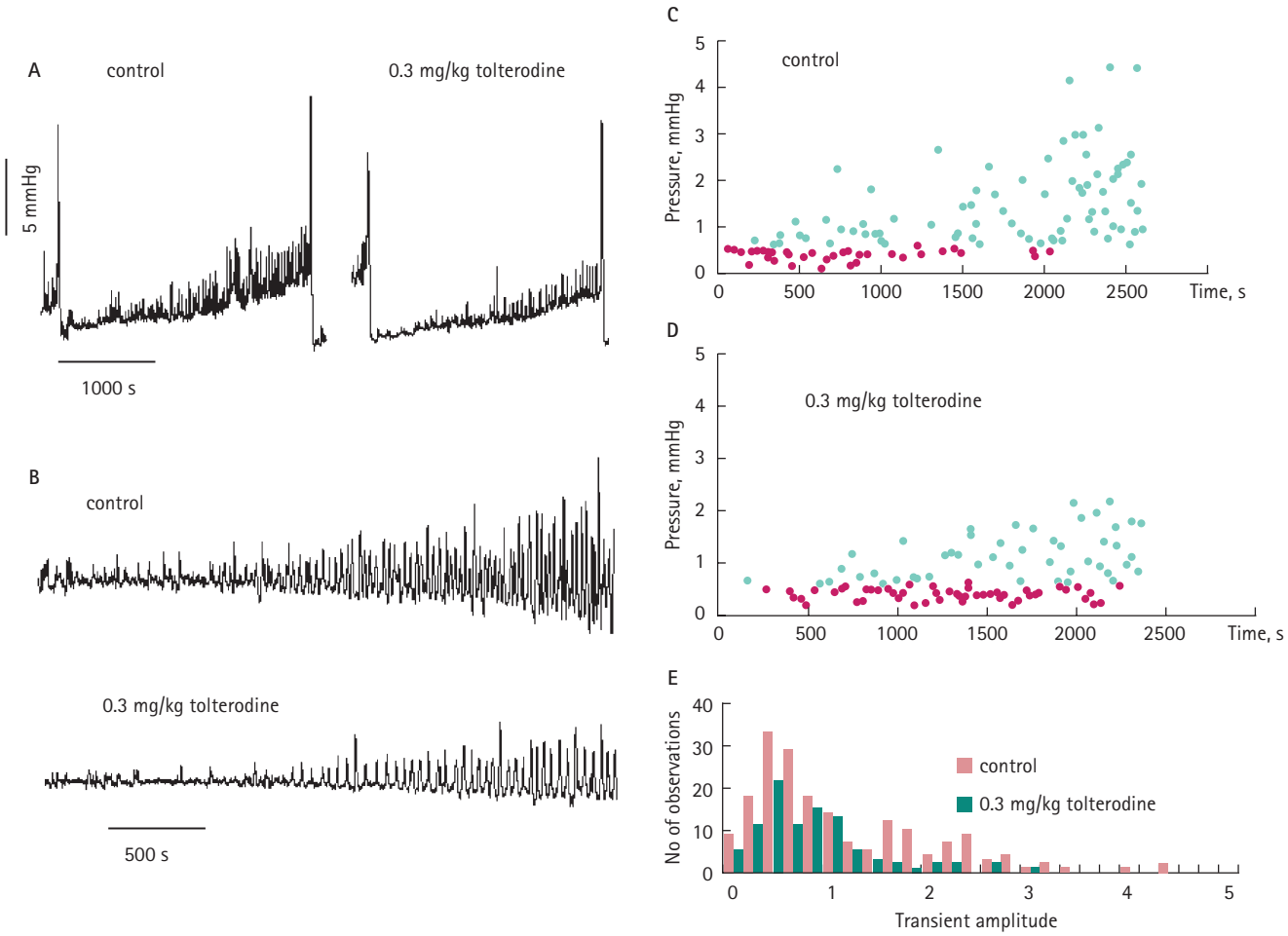


RESULTS

The mean bladder weight of rats with BOO was 620 ± 250 mg ($N = 55$). By comparison the bladder weights of control animals and animals undergoing a sham operation were 116 ± 11 mg ($N = 6$) and 122 ± 33 mg ($N = 6$), respectively.

Figures 1A and 2A illustrate, respectively, the effects of tolterodine (0.3 mg/kg i.v.) and mirabegron (0.3 mg/kg i.v.) on NVA in conscious rats with BOO. There were clear changes in the presence of tolterodine or mirabegron. Tolterodine reduced the amplitude of the voiding contraction, whereas mirabegron had little effect on voiding contractions. Both drugs altered the frequency of the NVA. Figures 1B and 2B show the NVA isolated from the slow increase in baseline pressure. Figures 1C and 2C show the integral of the activity using 60 s time windows. The integral is a first

FIG. 6. Example of detailed analysis of the effects of tolterodine (0.3 mg/kg i.v.) on the amplitude of the NVA in a conscious rat with partial BOO. ***A****, The original records showing the control and in the presence of tolterodine.* ***B****, The isolated phasic activity on an expanded timescale. Individual contractions are clearly seen.* ***C, D****, Analysis of the phasic activity. The amplitude of each transient pressure rise is plotted against the time the rise occurred. Small transients (<0.6 mmHg, this value was identified in this particular bladder; see panel* ***E****) are isolated from the population and plotted separately (red circles) from the larger transients (green circles).* ***E****, Aamplitude distribution histogram for the data in (****C****) and (****D****). The control data are shown by the red bars while the transients in the presence of tolterodine are shown by the green bars. Bin widths for the amplitude analysis were set at 0.2 mmHg.*

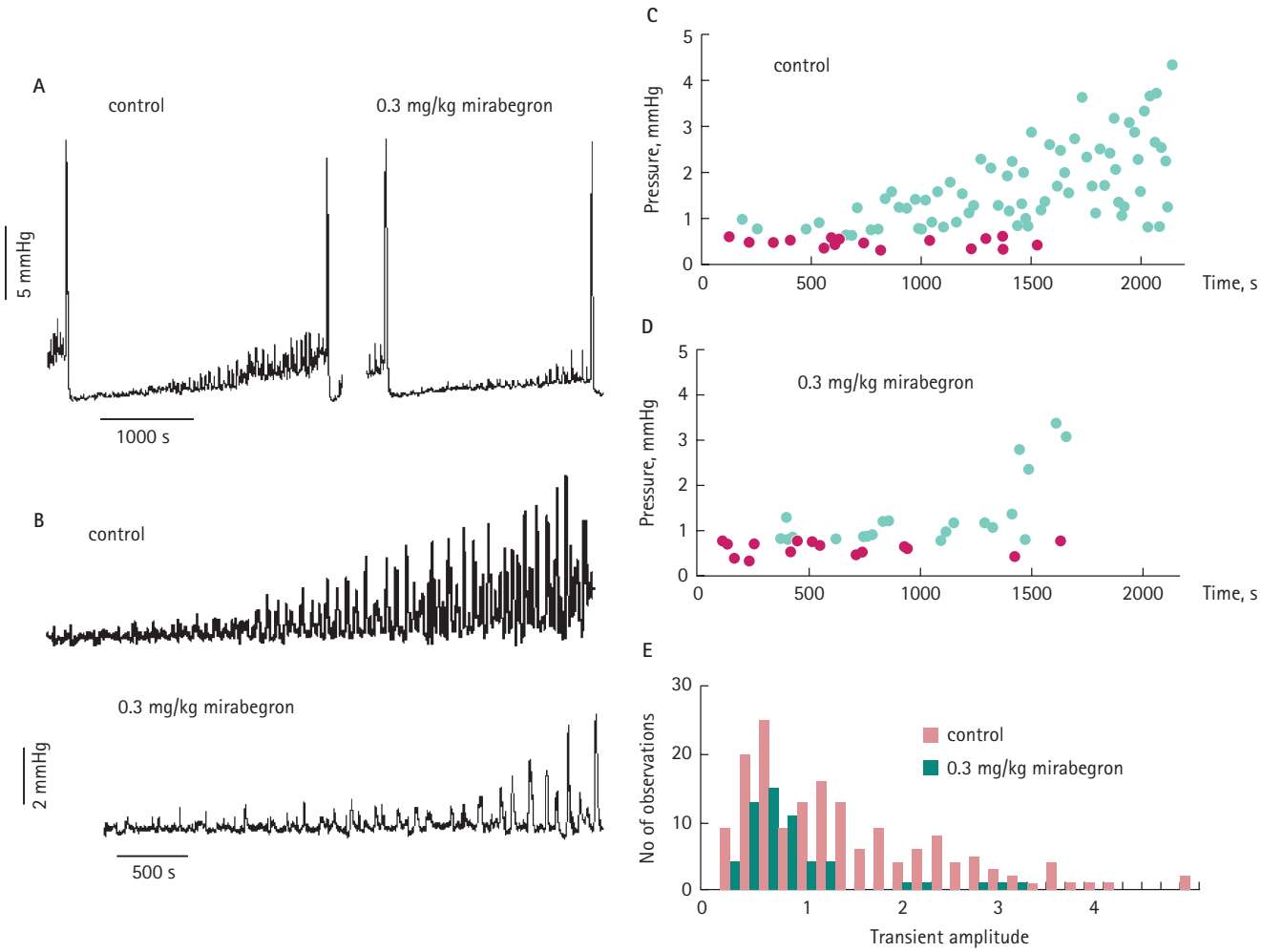


approximation to quantify the amount of NVA. It obviates the need to break down the activity into individual frequency and amplitude components. The integral of the NVA increases progressively during the filling phase in control conditions. In the presence of tolterodine and mirabegron, the activity is decreased. Figures 1D and 2D show superimposed plots of the time integral from a control period (green symbols) and in the presence of drug (red symbols).

Figures 3 and 4 show examples of different doses of tolterodine and mirabegron, respectively. Four drug doses are shown, each dose being tested on a different animal. Panels A and B show examples of control filling cycles and, in the same animal, with drug. Panels C and D show the final periods of a fill on an expanded timescale. At the higher doses, tolterodine reduced the amplitude of the voiding contractions, whereas mirabegron did not. Both drugs reduced the frequency of the NVA. Tolterodine reduced the amplitude of the NVA, whereas mirabegron had little effect. Figure 5 summarizes the data from 57 animals. The effects of tolterodine (Fig. 5A) and mirabegron (Fig. 5B) were explored on the voiding rate (green circles), voiding threshold (pressure at which voiding contraction is initiated; green triangles) and the amplitude of the voiding contractions (red circles). The data confirm that mirabegron has no effect on the void contraction, while tolterodine reduces it dose-dependently. Values for the voiding pressures before and after administration of mirabegron (3.0 mg/kg) were 22 ± 6 mmHg (control) and 19 ± 5 mmHg, respectively (not significantly different: $N = 7$). For tolterodine (1.0 mg/kg) the voiding pressures were 22 ± 2 and 12 ± 1 mmHg, respectively (significant difference, $P < 0.001$, $N = 6$).

Further details of the effects of tolterodine and mirabegron are illustrated in Figs 6

*FIG. 7. A detailed analysis of the effects of mirabegron (0.3 mg/kg i.v.) on the amplitude of the NVA in a conscious rat with partial BOO. **A**, The original records showing the control and in the presence of mirabegron. **B**, The isolated phasic activity on an expanded timescale. Individual contractions are clearly seen. **C, D**, An analysis of the phasic activity. The amplitude of each transient pressure rise is plotted against the time the rise occurred. Small transients (< 0.6 mmHg) are isolated from the population and plotted separately (red circles) from the larger transients (green circles). **E**, Amplitude distribution histogram for the data in (**C**) and (**D**). The control data are shown by the red bars while the transients in the presence of mirabegron are shown by the green bars. Bin widths for the amplitude analysis were set at 0.2 mmHg.*



and 7. The control and drug traces are shown in Figs 6A and 7A. Figures 6B and 7B show the isolated phasic activity. The different effects of the study drugs on the amplitude and frequency of NVA activity can be seen: panels C and D in Figs 6 and 7 show the amplitudes of each pressure transient measured from the traces in Figs 6B and 7B, respectively. Figures 6E and 7E show the amplitude distribution histograms for control data (red bars) and in the presence of drug (green bars). Both show a maximum at ≈0.6 mmHg. Small (<0.6 mmHg) and larger (>0.6 mmHg) transients were isolated and are graphically represented in panels C and D of both figures.

Data from all treated animals are shown in Fig. 8 for tolterodine and mirabegron. In both drugs the mean amplitude of the NVA in the final phase (P3) is shown (green circles). Tolterodine and mirabegron have similar actions on the integrated activity. With tolterodine the reduced integral is the result of a decrease in both amplitude and frequency. By contrast, mirabegron has little effect on the amplitude of the large transients but has a marked effect on the frequency.

DISCUSSION

Non-voiding activity in the present cystometry data consists of small phasic contractions in the bladder as it fills. NVA is thought to represent the motor component of a motor/sensory system [23–27] and is accentuated in pathological situations such as BOO [4]. Under such conditions, an increased output of the motor/sensory system could contribute to increased bladder afferent outflow and sensation. NVA also occurs in asymptomatic human subjects and in patients with OAB [28], suggesting that a motor/sensory system operates in

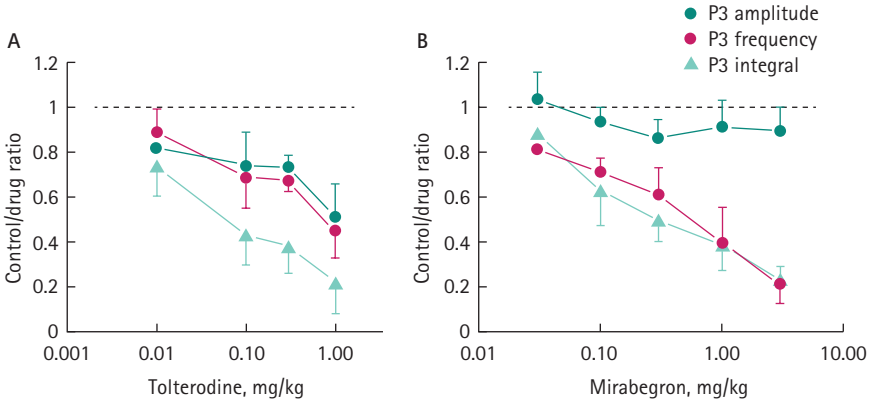
humans and that it is altered in pathology. Thus, increased activity in this system in pathological states might be associated with heightened bladder sensations and increased frequency.

The afferent outflow from the bladder is complex, involving different types of afferent fibre, both structurally and functionally. Fibres originate from different regions of the urinary tract and contribute to bladder reflexes; some of these fibres travel only to the spinal cord while others reach higher centres [29]. In an attempt to simplify this complexity, the idea that bladder afferent systems could be grouped into different systems has led to the concept of 'afferent noise' [27].

In the rat, the motor component of this system is seen *in vivo* as NVA and as 'micromotions' displaying autonomous activity in the isolated bladder [30]. In rats with BOO, NVA increases in both frequency and amplitude as the bladder fills, as found in the present and other studies [3,4], suggesting that the amount of afferent information sent to the CNS increases as the bladder fills. The work of Streng *et al.* [22] in normal rats has clearly shown that NVA consists of transients with different frequency/amplitude characteristics, and that large-amplitude NVA is increased near micturition. This appears to be similar in BOO control rats in the present study. Further, treatment of rats with BOO using the ganglionic blocker hexamethonium presented a further increase in amplitude, but a decrease in frequency [31], suggesting the opposite modulatory influence of the autonomic nervous system for amplitude and frequency of NVA. We assume that the large transients are associated with pathology and, as the present study shows, that it is these transients (in the P3 phase as shown in Figs 7,8) that are dose-responsively affected both by the anticholinergic drug tolterodine and the β_3-adrenoceptor agonist mirabegron. The question is how this difference can be interpreted in relation to the pathology created in BOO in rats, in other animals and finally in patients with OAB.

In cats, NVA is modulated by sympathetic activity [32,33]. This observation is important as it provides a wider physiological framework for sympathetic-adrenergic

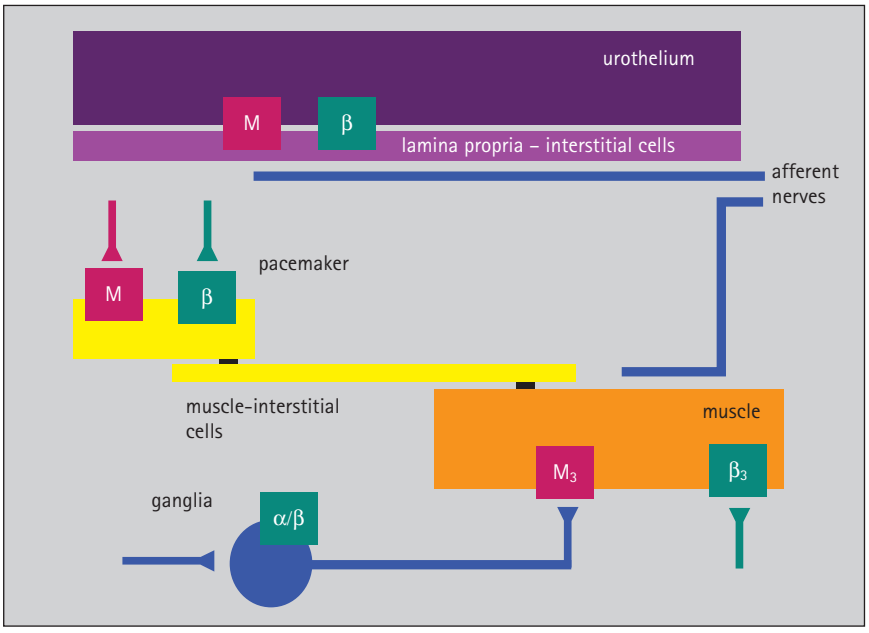
*FIG. 8. Data illustrating the effects of different concentrations of tolterodine (**A**) and mirabegron (**B**) on the components of the NVA in the final period of the filling phase (P3). Data show the ratio of the respective responses (drug/control) for each concentration of drug.The amplitude of the non-voiding contractions (green circles), the frequency of the contractions (red circles) and the integral of the activity (green triangle) are shown. Values are mean values ± SD (N = 5–8 rats).*



regulation of NVA and a potential rationale for the use of β_3-adrenoceptor agonists. Studies in guinea pigs of the correlate of NVA in the isolated bladder, autonomous activity, suggest the possibility of regulatory mechanisms controlling the amplitude and frequency of the NVA. Excitatory inputs include acetylcholine via an M_3-dependent mechanism in normal bladders [34] and those with BOO [35], while inhibition is associated with adrenergic activation in normal bladders [34] and those with BOO [35]. Taken together these observations suggest that NVA is highly regulated and that the afferent output associated with it might be under the control of the CNS [26]. There could be differences in mechanisms organizing NVA in the different animals discussed, but no side-by-side studies are available. However, the similar effects of hexamethonium and tetrodotoxin on NVA seen in rats [31] and guinea pigs with BOO [35], have been interpreted differently by the authors of these papers: myogenic in the rat and pacemaker-related in the guinea pig. By nature of the systems studied, entirely conclusive experiments for a myogenic mechanism in an *in vivo* experiment are not easily given, as the pharmacological tools used have the capability of interaction with their molecular target at different degrees of physiological functioning in the animals. We therefore suggest that NVA as measured in cystometric experiments is the motor component of a sensory system. Afferent activity has not been measured under non-anaesthetized conditions to our knowledge, so at present, an integrated view, supported by data in this respect, is missing. Studies by Iijima *et al.* [36] and Aizawa *et al.* [37] have investigated the effects of anticholinergics (oxybutynin and darifenacin) and a partial β_3-adrenoceptor agonist (CL316,243) on Aδ and C-fibre activity in anaesthetized rats, where only the β_3-adrenoceptor agonist was studied with and without sensitizing with PGE_2 instillation of the bladder. These studies show differential modulation of mechanico-sensitive and C-fibre activity on sensory function. This is a valuable observation, but the activities measured in these studies unfortunately cannot be aligned to our observations because of the difference in experimental conditions and variables studied.

There are clear differences in the actions of tolterodine and mirabegron: tolterodine affects the amplitude of the voiding contraction and NVA, while mirabegron only works on the NVA. Tolterodine affects both the amplitude and frequency of NVA while mirabegron affects primarily the frequency. In addition, antimuscarinics are proposed to affect urothelial afferent signalling [38]. The fact that two completely different drugs, a muscarinic antagonist and a β_3-adrenoceptor agonist, have similar actions on the system generating NVA is interesting and invites speculation about the underlying mechanism. Using the conceptual framework described earlier, in which NVA is the motor component

FIG. 9. Diagram suggesting the possible mechanism that might be involved in the generation and modulation of non-voiding motor activity in the bladder wall. The detrusor muscle is shown with muscarinic receptors (type 3: M_3) and β_3-adrenoceptors. The muscarinic receptors will be associated with the post-ganglionic parasympathetic nerves, which are classically involved in generating the coordinated contraction of a void. An adrenergic innervation of the detrusor may be involved in activating these receptors. A second system is proposed which involves the network of interstitial cells [26,27]. Activity in this system is generated by a mechanism, which has properties of a 'pacemaker' (autonomous activity). The intrinsic activity of this system can be modulated by excitatory inputs involving acetylcholine and muscarine (most likely M_3) receptors and inhibited by inputs involving noradrenaline and β (most likely β_3-adrenoceptors. Local contractions of the bladder and associated stretches activate afferent fibres, resulting in phasic afferent discharge. Structurally, the network of interstitial cells is now well established in the bladder wall but the anatomical location and functional basis of the pacemaker are not known at the present time. A third location for muscarinic and adrenergic receptors is within the urothelium lamina propria complex. The role of these receptors and how they are activated are currently unknown. Also, it is not clear how interactions between urothelial-derived signals impact on afferent nerve activity or non-voiding activity.



of a modulated motor/sensory system, an explanation can be put forward (Fig. 9). NVA is possibly generated by a 'pacemaker-like' mechanism in the bladder wall. Its intrinsic activity is modulated by excitatory and inhibitory inputs, acetylcholine and noradrenaline [27]. The actions of tolterodine (M antagonist) and mirabegron (β_3-adrenoceptor agonist) therefore represent, respectively, an inhibition of the excitatory mechanism and an activation of the inhibitory mechanism.

The decrease of the voiding contraction with tolterodine implies that, in addition to acting on the proposed 'pacemaker', tolterodine is also acting at the parasympathetic neuromuscular junction. Mirabegron does not have this effect. This suggests that, at the concentrations used, mirabegron does not cause a decrease of the voiding contractions. This difference could be highly significant therapeutically. There is the potential with tolterodine to weaken the bladder and induce urine retention. Mirabegron, by not having this action, is less likely to produce retention at therapeutic does and therefore has, at least theoretically, advantages over the anticholinergic drugs.

The present study highlights the importance of NVA as a key element in the generation of OAB and, possibly, sensory urge. The data demonstrate that both anticholinergic drugs and β_3-adrenergic sympathico-mimetics modulate NVA. As these drugs are effective therapeutically in OAB, this suggests that their site of action could be on the system modulating non-voiding motor/sensory activity. Further studies are planned to study this concept in more detail.

CONFLICT OF INTEREST

James I. Gillespie is a Consultant to Sponsor and Cees Korstanje is an Employee of Sponsor. Stefano Palea, Veronique Guilloteau, Marc Guerard and Philippe Lluel are Study Investigators on project paid by Sponsor. Source of funding: Astellas Pharma Europe B. V.

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Abbreviations: **DO**, detrusor overactivity; **NVA**, non-voiding activity; **OAB**, overactive bladder.