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Effect of the ATP-sensitive potassium channel opener ZM226600 on cystometric parameters in rats with ligature-intact, partial urethral obstruction

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Abstract

The activity of a recent K_{ATP} channel opener, the *N*-(4-Phenylsulfonylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZM226600) was investigated on a female rat model of overactive bladder with outlet obstruction. Both ZM226600 and pinacidil instilled into the bladder (10^{-7} M, 30 min) or following systemic administration (10, 100 nmol/kg e.v.) almost completely abolished bladder overactivity and improved residual volume and frequency of micturition. However, pinacidil affected arterial pressure. Oxybutynin instilled into the bladder (10^{-7}, 10^{-6}, 10^{-5} M, 30 min) decreased detrusor overactivity by about 16%, 25% and 46% respectively, but also blocked micturition reflexes at highest doses tested. Oxybutynin reduced detrusor overactivity by about 50% and 80%, after systemic administration (10, 100 nmol/kg e.v.), but also blocked micturition reflexes at the highest dose tested. In conclusion, ZM226600 is more active than oxybutynin in reducing bladder overactivity, and it is devoid of vascular side effects observed with pinacidil. Its short duration of action (about 1 h) is probably the main problem to solve, in order to consider this compound a valid alternative to antimuscarinics in the therapy of bladder overactivity.

Keywords: Bladder overactivity; K_{ATP} channel opener; (Rat)

1. Introduction

Detrusor overactivity, which is characterized by involuntary bladder contractions and loss of urine, is a major cause of urinary incontinence, a common health problem with great social impact and negative effect on quality of life for both sexes. Currently, muscarinic antagonists such as oxybutynin and tolterodine are still considered the first-line therapy for treating overactive bladder. Oral anticholinergics are effective for many patients with urinary incontinence: their antimuscarinic properties result in beneficial urodynamic effects, such as increase in bladder capacity and micturition threshold pressure, prolongation of micturition interval and reduction in maximal intravesical pressure during the collecting phase (Siddiqui et al., 2004). However, some patients require such high doses of anticholinergics that systemic side effects such as dry mouth, blurred vision, constipation and tachycardia, limit their clinical use. In other patients, the drugs appear to be ineffective. The treatment of bladder overactivity involves the selective inhibition of involuntary bladder contraction without altering the normal micturition reflexes. This issue seems difficult to be obtained with anticholinergics. Among drugs with a peripheral side of action, which may offer some attractive rationale in treating urge urinary incontinence, the ATP-sensitive K^+-channel openers represent a possible alternative to the anticholinergic drugs. It has been hypothesized that ATP-sensitive K^+-channel openers can inhibit unstable bladder contractions without affecting the normal voiding reflex. For instance, pinacidil in isolated human bladder

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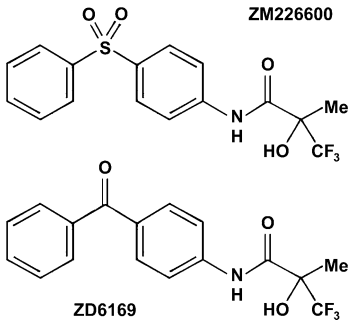

Fig. 1. Chemical structures for the compound *N*-(4-Phenylsulfonylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZM226600) and its analogue (*S*)-*N*-(4-benzoylphenil)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZD6169).

(Fovaeus et al., 1989) and cromakalim in pig and human bladder (Foster et al., 1989) have been shown to inhibit bladder overactivity. However, their clinical utility is dampened by an unacceptable level of hypotension at effective doses. Recently, an increasing number of new ATP-sensitive K^+-channel openers with improved bladder selectivity has been developed and tested (Howe et al., 1995; Petkov et al., 2001; Shieh et al., 2001; Brune et al., 2002; Gopalakrishnan et al., 2002; Lynch et al., 2003). The aim of the present work was to investigate the in vivo and in vitro activity of a recent K_{ATP} channel opener, the *N*-(4-Phenylsulfonylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZM226600) that is the analogue of the more studied (*S*)-*N*-(4-benzoylphenil)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZD6169) (Fig. 1) in female rats with ligature-intact, partial urethral obstruction. The efficacy of ZM226600 on bladder overactivity was compared with that of a prototypical K_{ATP} channel opener, pinacidil and with the antimuscarinic drug, oxybutynin.

2. Materials and methods

Female Sprague–Dawley rats (200–225 g, 2 months old) were used in this investigation. All experimental protocols were approved by the Review Committee of the Department of Pharmacological Sciences of Milan, and met the Italian guidelines for laboratory animals that conform to the European Communities Directive of November 1986 (86/609/EEC).

2.1. Partial urethra obstruction

Animals were anaesthetized with ketamine (40 mg/kg) and xylazine (20 mg/kg). The skin was shaved and disinfected with an iodine/alcohol mixture. The urethra was exposed by a low midline abdominal incision and was freed from surrounding connective tissue. According to Steers and De Groat (1988), a double silk (4.0) ligature was placed around the urethra and tied in the presence of an extraluminally placed 1 mm diameter polyethylene tubing. The polyethylene tubing was then removed and the abdominal incision was closed using a double layer continue silk (4.0) suture. Sham operated rats underwent urethral manipulation with no double silk urethral ligature.

2.2. In vivo cystometry

After 6–8 weeks from partial urethral ligature, rat weights were not different from those of sham operated (control) rats, being 293±8 and 295±13 g, respectively. Animals were anaesthetized with urethane (1.1 g/kg i.p.) and the appropriate level of anesthesia for testing was determined by the lack of response to a toe pinch. When rats were fully anaesthetized, they underwent anaesthetized cystometry. The rats were placed supine and the bladder was exposed by a low midline abdominal incision, the intravesical urine was removed and its volume was measured by puncturing the dome of the bladder with a 10 ml syringe. Then, a double-lumen catheter was inserted into the punctured site. The urethers were not ligated and the urethral ligature was not removed during cystometry. The outside catheter (G21, 0.8 mm o.d.) was connected to a pressure transducer (Gould P23 ID), and the intravesical pressure was recorded continuously on an ink-write two-channel Gemini 7070 (Basile, Italy). The cystometry was performed with the abdomen open, so that the recorded pressure should be the same as the detrusor pressure. The inner catheter (G25, 0.5 mm o.d.) was connected to a peristaltic pump Peri-Star (World Precision Instruments, USA), and saline at 37 °C was infused. The infusion rate was set to 0.11 ml/min for control rats and for operated animals. Distal urethra was catheterized with 1 mm diameter polyethylene tube. The volume of fluid during each voiding phase was collected and measured by means of an isometric transducer (Basile, Italy) coupled to the two-channel Gemini7070. The carotid artery was exposed by a midline incision of the neck, and an i.v. catheter of PUR (G22, 0.9 mm-Terumo) was inserted into the lumen of the artery and was connected to a pressure transducer (Gould P23 ID) coupled to an eight-channels digital recorder PowerLab 8SP (ADInstruments-Basile, Italy) to monitor and record the arterial pressure and the heart rate. Bladder outflow obstruction was successfully produced 6–8 weeks from the surgical operation in about 70% of the animals, and about 40% of these animals also showed detrusor overactivity. In the event three to five filling/voiding cycles were reproducible, the animal was included in the experimental protocol and the effects of drugs on cystometric parameters and on spontaneous bladder overactivity, if present, were studied. Acute retention was probably the cause of death in 10% of animals during the first week from operation. The residual 20% of animals showed absence of micturition reflexes, absence of detrusor overactivity, large bladder capacity (from 8 to 16 ml), and were not included in the experimental protocol. The effects of ZM226600, pinacidil and oxybutynin on cystometric parameters were studied after bladder instillation for 30 min. Adverse side-effects for ZM226600 and pinacidil were observed and recorded during 120 min from bladder instillation, or from i.v. administration in the tail vein. In preliminary experiments, the effect of the compound ZM226600 on bladder functionality was also studied after intravenous administration.

2.3. Organ bath experiments

Animals that presented spontaneous bladder overactivity during the first filling/voiding cycles, were used for either in vivo or in vitro experiments. Rats were killed by asphyxiation with CO_2 followed by cervical dislocation. The abdomen was opened and the bladder was quickly removed and put in cold, modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.4, $MgSO_4$ 0.6, $CaCl_2$ 2.5,

$NaHCO_3$ 16.4 and glucose 7.7; pH 7.3. Four longitudinal strips of detrusor smooth muscle (10 × 5 mm) were prepared from each bladder and suspended in 5-ml organ baths containing modified Krebs solution gassed with 95% O_2 and 5% CO_2 at 37±0.5 °C. Tissues were equilibrated for 1 h under a resting tension of 1.0 g (9.8 mN). Tension was recorded by means of a Fort 10 isometric force transducer (World Precision Instruments, USA) coupled to the digital recorder PowerLab 8SP.

After the equilibration period, to confirm the action of ZM226600 on K_{ATP} channels, strips were precontracted with carbachol (0.1 μM), until a stable contraction was evoked. Then, tissues were challenged with ZM226600 (10 μM) and while this was still present in the organ bath, the K_{ATP} channel inhibitor glybenclamide (50 μM) was added.

In a separate set of experiments, bladder preparations were incubated with either ZM226600, pinacidil, indomethacin or oxybutynin to evaluate their effect on spontaneous overactivity of detrusor smooth muscle.

2.4. Solutions and drugs

Carbachol, glybenclamide, indomethacin and oxybutynin chloride were purchased from Sigma-Aldrich Company Ltd. (Milan, Italy), pinacidil and ZM226600 from Tocris Cookson Ltd. (Avonmouth, UK).

2.5. Data analysis

All data are the means±S.E.M. of 6 to 10 individual observations. Bar graphs showing changes on cystometric parameters and curves showing changes in mean arterial pressure were prepared using the computer program Prism (v.3.0; GraphPad). Inhibitory effect evoked by oxybutynin, pinacidil or ZM226600 on bladder overactivity in anaesthetized animals was calculated as the ratio between area under curve (AUC) measured from the trace of spontaneous contractions, before and after drug administration, using the computer program Scion Image (v.4.0; Scion Corporation). A one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparisons was used to compare differences between means. A value of $P<0.05$ was regarded as significant.

3. Results

Six to eight weeks from surgical operation, bladder mean weights were 102±9 vs 462±93 mg in sham operated (control) and in partial obstructed rats, respectively.

Table 1 shows a significant increase in resting pressure, pressure threshold, maximal amplitude of contraction, residual volume and frequency of micturition in obstructed rats, whereas the intercontraction interval was decreased. The micturition volume was not impaired.

3.1. Effect of ZM226600 on carbachol-precontracted preparations

The compound ZM226600 (10 μM) partially antagonized, and the K_{ATP} channel inhibitor glybenclamide (50 μM) completely restored to the initial value the contraction evoked by carbachol (0.1 μM) in control bladder preparations ($n=6$). Tension values were: 0.68±0.04, 0.31±0.03 and 0.67±0.03 g in carbachol-precontracted strips before, after ZM226600 and after glybenclamide in the presence of ZM226600, respectively.

3.2. Effect of ZM226600 on bladder spontaneous activity in vitro and in vivo

Bladder preparations from obstructed rats showed increased spontaneous activity at the resting tone as compared to strips from controls: 0.83±0.14 vs 0.08±0.02 g ($n=6$) (Fig. 2A,B). This activity was not impaired after incubation with indomethacin (10^{-6} M, for 30 min), being 0.80±0.03 g ($n=6$) (Fig. 2B). Incubation with ZM226600 (10^{-6} M, for 30 min) completely abolished spontaneous motility, being 0.05±0.02 ($n=6$) (Fig. 2C). In anaesthetized rats, bladder instillation of ZM226600 (10^{-7} M, for 30 min) as well as pinacidil, inhibited spontaneous bladder motility in obstructed rats by 90±5%, calculated as the AUC ratio before and after treatment (Fig. 3A). At a concentration of 10^{-6} M the inhibition was almost complete (97±2%). Oxybutynin at a concentration of 10^{-7} M was less effective than ZM226600, reducing bladder overactivity by 16±3% (Fig. 3B). At the highest doses tested (10^{-6} and 10^{-5} M), oxybutynin reduced bladder overactivity by about 25±5% and 46±8% ($n=6$), but also inhibited micturition reflexes. Following systemic administration (10, 100 nmol/kg e.v.), ZM226600 and pinacidil completely abolished bladder overactivity. Oxybutynin reduced bladder overactivity by 46±7% and by 80±6% ($n=6$) at 10 and 100 nmol/kg, respectively. However, at the highest dose tested, it inhibited micturition reflexes.

3.3. Effect of ZM226600 on cystometric parameters

The activity of a bladder instillation (10^{-7} M, for 30 min) of ZM226600 or pinacidil on cystometric parameters in anaesthetized rats with ligature-intact partial urethral obstruction (6 to 8 weeks) is summarized in Fig. 4. Resting pressure, pressure threshold and maximal amplitude of contraction were significantly increased ($P<0.05$) in operated animals; treatments with either ZM226600 or pinacidil reduced without restoring them to control values (Fig. 4A). Intercontraction interval was markedly shorter ($P<0.05$) in operated animals; bladder instillation with either ZM226600 or pinacidil slightly increased this value,

Table 1
Cystometric parameters evaluated in anaesthetized female rats with ligature-intact partial urethral obstruction (6 to 8 weeks)

		Control	Obstructed
Resting pressure	(cm H_2O)	2.96±0.39	10.59±1.52[a]
Pressure threshold	(cm H_2O)	6.54±0.67	18.41±2.46[a]
Maximal contraction	(cm H_2O)	36.61±1.84	46.92±2.22[a]
Intercontraction interval	(s)	183.1±14.2	51.06±8.37[a]
Contraction time	(s)	62.6±2.99	54.94±3.87
Expulsion time	(s)	17.14±1.17	14.7±1.44
Micturition volume	(ml)	0.67±0.05	0.49±0.07
Residual volume	(ml)	0.23±0.04	3.95±0.48[a]
Bladder capacity	(ml)	0.91±0.05	4.45±0.39[a]
Frequency	(h^{-1})	15.3±0.68	30.7±3.23[a]

Data are the means±S.E.M. of 6 to 8 observations. Statistical significance ($P<0.05$): [a]obstructed vs control.

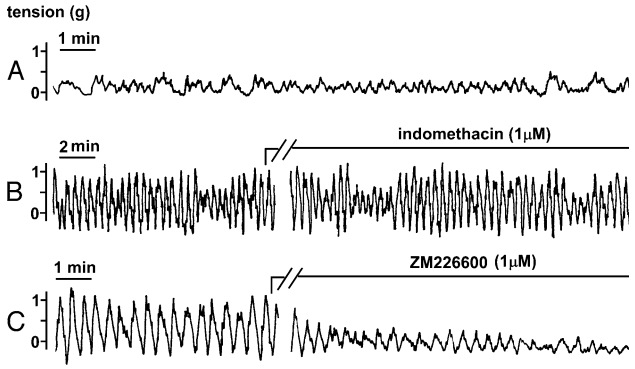

Fig. 2. Representative traces of spontaneous contractions in vitro of a bladder preparation from (A) control rat, (B) rat with partial urethral obstruction (8 weeks), before and after incubation with indomethacin (1 μM), and (C) rat with partial urethral obstruction (8 weeks), before and after incubation with ZM226600 (1 μM). Double diagonal lines indicate the incubation time (30 min) during which activity of strips was not recorded.

whereas contraction time and expulsion time were unchanged (Fig. 4B). Bladder capacity and frequency of micturition were also significantly increased ($P<0.05$) in obstructed rats, whereas micturition volume was decreased as compared to control (Fig. 4C and D). Bladder instillation with either ZM226600 or pinacidil slightly increased micturition volume but significantly decreased the frequency of micturition. Oxybutynin at a concentration of 10^{-7} M did not induce any beneficial effects on cystometric parameters, whereas at the higher concentration tested (10^{-6}, 10^{-5} M) it inhibited micturition reflexes (data not shown). The compound ZM226600 was also active in restoring some of cystometric parameters in anaesthetized rats with partial urethral obstruction, after intravenous injection (10–100 nmol/kg). In particular, frequency of micturition decreased by about 25%, whereas micturition volume increased by about 30%, 20 min after injection in the tail vein. Systemic administration of oxybutynin (10 nmol/kg) did not significantly affect cystometric parameters, whereas at a concentration of 100 nmol/kg, oxybutynin blocked micturition reflexes.

3.4. Effect of ZM226600 on mean arterial pressure and heart rate

Bladder instillation (10^{-7}–10^{-5} M, for 30 min) and intravenous injection (10, 100, 300, 1000 nmol/kg) of ZM226600 or pinacidil differently affected the mean arterial pressure, as shown in Fig. 5. A bladder instillation of ZM226600 at effective dose (10^{-7} M) had no significant effects on mean arterial pressure, and at the higher dose used (10^{-5} M), it only decreased by about 5% during the first 10 min of recording, returning to the original value after 30 min (Fig. 5A). On the contrary, pinacidil decreased the mean arterial pressure by about 12% and by about 26% at 10^{-7} M and 10^{-5} M, respectively. Mean arterial pressure did not restore to the original value even after 120 min from treatment (Fig. 5B). Injection of ZM226600 in the tail vein decreased from 3% to 7% at 10 to 300 nmol/kg, 5–10 min after injection, and pressure recovered after 30 to 60 min. At the highest dose tested (1000 nmol/kg), pressure decreased by about 12%, but did not return to the original value even after 120 min (Fig. 5C).

Intravenous administration of pinacidil had more pronounced and persistent effects on mean arterial pressure than ZM226600. In fact, pinacidil lowered the mean arterial pressure by about 10%, 13%, 22% and 38% at 10, 100, 300 and 1000 nmol/kg, respectively. Furthermore at the last two doses tested, pressure did not return to the original value even after 120 min from the injection (Fig. 5D). Oxybutynin either after intravesical or intravenous administration did not alter significantly the mean arterial pressure. All compounds tested, either administered by bladder instillation or intravenous injection, did not alter significantly the heart beats rate in anaesthetized rats (Fig. 6).

4. Discussion

Our study showed that partial urethral obstruction, consistently with published results (Steers and De Groat, 1988; Lynch et al., 2003), led to a significant increase in bladder weight, capacity, frequency of micturition, and to the development of the detrusor overactivity in the majority of the animals. These effects were similar to those observed in humans (Groutz et al., 2000; Coyne et al., 2003). Although prostaglandins do not play a major role in controlling bladder contraction in physiological conditions, their synthesis is reported to be increased in the bladder smooth muscle and epithelium and may be involved in the enhancement of detrusor activity in pathological disorders such as diabetes (Tarcan et al., 2000), cystitis or overactive bladder (Takagi-Matsumoto et al., 2004). In our experimental conditions, spontaneous overactivity observed in detrusor preparations from obstructed rats does not seem to be associated to prostaglandins release, because indomethacin was unable to inhibit it. Since physiologic bladder contractions as well as bladder overactivity seem to be evoked by muscarinic receptor stimulation, anticholinergic drugs have been used in the therapy of bladder overactivity associated with outflow obstruction, though with limited success. Our study showed that oxybutynin instilled into the bladder (10^{-7} M) reduced detrusor overactivity by

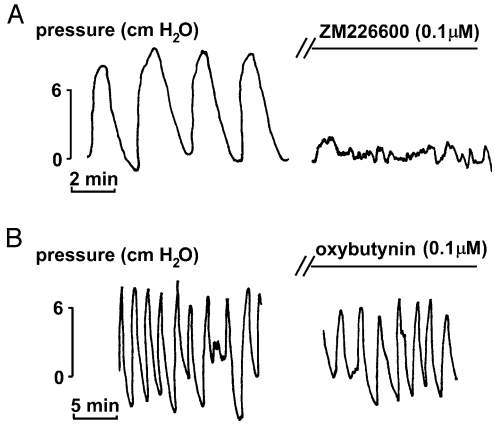

Fig. 3. Representative traces showing bladder overactivity in rats with partial urethral obstruction (8 weeks), before and after incubation with (A) ZM226600 (0.1 μM) or (B) oxybutynin (0.1 μM). Double diagonal lines indicate the incubation time (30 min) during which activity of strips was not recorded.

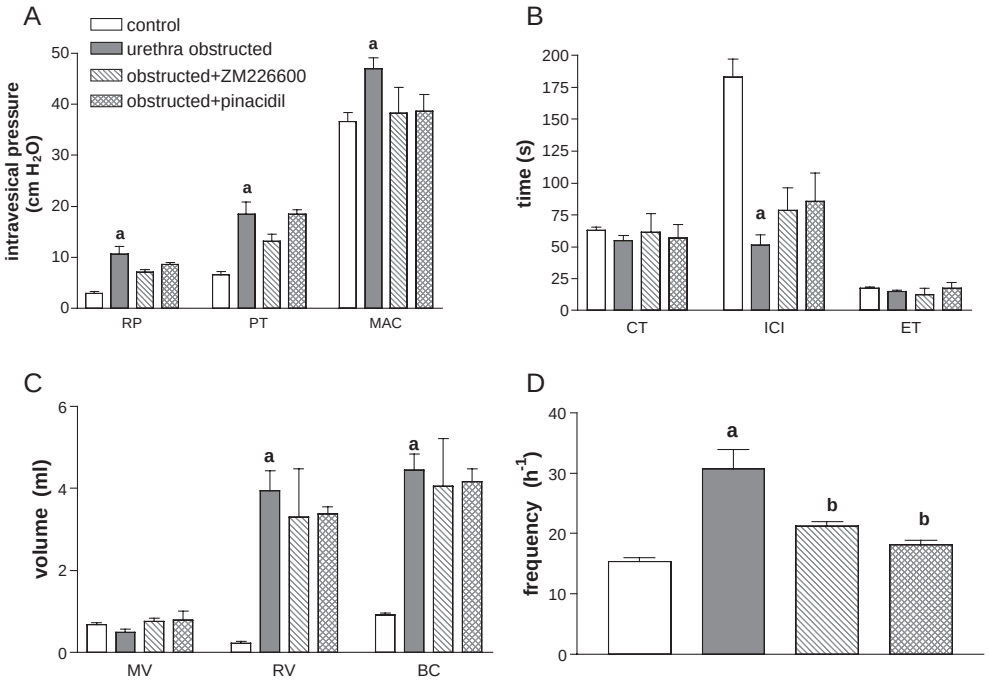

Fig. 4. Collected data, obtained in anaesthetized controls ($n=10$), obstructed ($n=10$), obstructed after bladder instillation of ZM226600 (10^{-7} M) ($n=6$), or pinacidil (10^{-7} M) ($n=6$). The cystometric parameters evaluated in anaesthetized rats were: A) resting pressure (RP), pressure threshold (PT), maximal amplitude of contraction (MAC), B) contraction time (CT), intercontraction interval (ICI), expulsion time (ET), micturition volume (MV), C) residual volume (RV), bladder capacity (BC) and D) frequency of micturition. Bars show mean and vertical lines S.E.M. of 6 to 8 experiments. Statistical significance ($P<0.05$): [a]vs control; [b] vs obstructed.

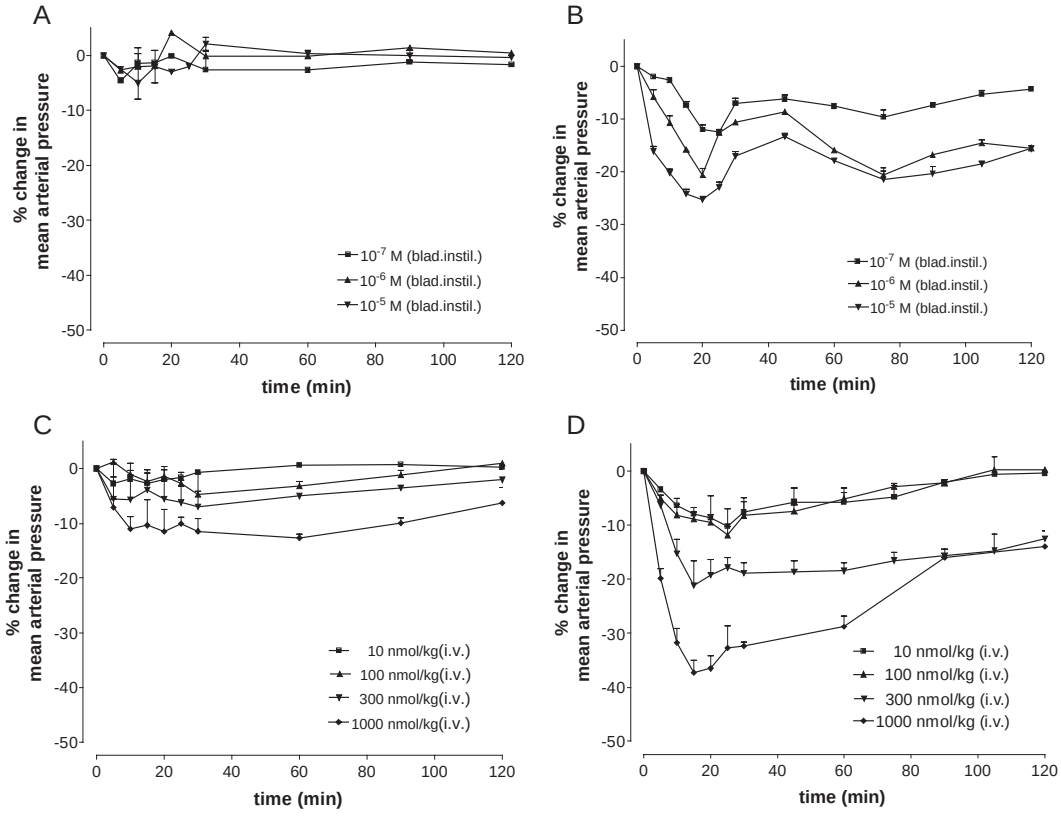

Fig. 5. Effects of ZM226600 (left panel) and pinacidil (right panel) on mean arterial pressure after bladder instillation (A, B) or intravenous injection (C, D) in the tail vein. Points show mean and vertical lines S.E.M. of 6 experiments.

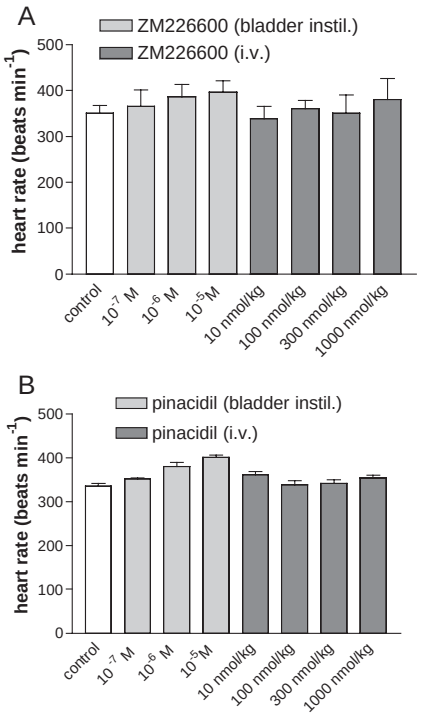

Fig. 6. Effects of ZM226600 (upper panel) and pinacidil (lower panel) on heart rate, after bladder instillation or intravenous injection. Bars show mean and vertical lines S.E.M. of 6 experiments.

only 16%, without blocking micturition reflexes or altering mean arterial pressure. The potential therapeutic use of ZM226600 appeared to be greater than that of pinacidil or oxybutynin. In fact, the former almost totally inhibited spontaneous rat bladder contractions, following bladder instillation (10^{-7} M) or intravenous injection (10–100 nmol/kg), without affecting the mean arterial pressure. Oxybutynin at the same concentration (10^{-7} M) slightly reduced bladder overactivity, but at increased doses also blocked micturition reflexes. Pinacidil inhibited spontaneous bladder contractions, but at effective doses also affected significantly the mean arterial pressure. These data are in agreement with that of Hedlund et al. (1991) who observed that pinacidil, at doses devoid of cardiovascular side-effects, was ineffective for treatment of detrusor overactivity in human beings.

Our results also showed that bladder instillation with either ZM226600 or pinacidil increased micturition volume and decreased significantly the frequency of micturition. It has been found that damage of the mucosal barrier of the urinary bladder is often associated to cystitis and to diseases that provoke bladder overdistension, such as urinary incontinence or diabetes (Levin et al., 1994; Leppilahti et al., 1999; Barlas, 2002). The lack of bladder epithelium integrity should be taken into consideration when bladder instillation is chosen as therapeutic approach for bladder incontinence. Bladder epithelium damage may be responsible of drug leakage into the circulation, and may explain the significant reduction of arterial pressure observed in our experiments after treatment with pinacidil. No significant changes in heart rates have been observed after ZM226600 or pinacidil administration, even at doses that caused significant reduction of mean arterial pressure. This observation has already been described in dogs (Sakai et al., 2000) and pigs (Fey et al., 2003), and has been attributed to anesthesia. The duration of action (about 1 h) for ZM226600 was short, similarly to that observed for its analogue ZD6169, which is currently undergoing Phase II clinical trials for the treatment of urge urinary incontinence (Fabiyi et al., 2003). This is at present the main disadvantage for these compounds, together with the way of administration (bladder instillation), which may reduce their therapeutic usefulness against detrusor overactivity and bladder incontinence.

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