Comparison of the Potassium Channel Openers, WAY-133537, ZD6169, and Celikalim on Isolated Bladder Tissue and In Vivo Bladder Instability in Rat

ALEXANDRA WOJDAN, CHRIS FREEDEN, MORGAN WOODS, GEORGE OSHIRO, WALTER SPINELLI, THOMAS J. COLATSKY, JEFFREY H. SHELDON, N. WESLEY NORTON, DAWN WARGA, MADELENE M. ANTANE, SCHUYLER A. ANTANE, JOHN A. BUTERA, and THOMAS M. ARGENTIERI


Accepted for publication January 29, 1999

ABSTRACT

The effects of the ATP-dependent potassium channel agonists ZD6169, celikalim, and WAY-133537 on bladder contractile function were examined in vitro on isolated bladder strips and in vivo on spontaneous bladder contractions. All three compounds produced a concentration-dependent relaxation of isolated rat detrusor strips (IC50 values = 0.93, 0.03, and 0.09 μM, respectively for ZD6169, celikalim, and WAY-133537. Contractile inhibition by all three compounds was fully reversed by 6 μM glyburide. These compounds also effectively inhibited spontaneous bladder contractions in the rat hypertrophied bladder model of detrusor instability. We also examined the electrophysiological properties of WAY-133537 on isolated rat bladder detrusor myocytes. Myocytes had an average resting membrane potential of −40 mV. Under patch current-clamp conditions, WAY-133537 (0.3 and 1.0 μM, n = 4–5) produced a significant hyperpolarization of 21 and 26 mV, respectively.

Hyperpolarization was reversed by the addition of 5 μM glyburide. In patch voltage-clamp studies, WAY-133537 (0.3 μM, n = 3) significantly increased outward current in response to both voltage step and ramp protocols consistent with activation of the ATP-dependent potassium channel. In the detrusor instability model, WAY-133537 and celikalim had similar oral potencies (ED50 = 0.13 and 0.3 mg/kg, respectively), whereas ZD6169 was less potent (ED50 = 2.4 mg/kg). The antihypertensive agent celikalim exerted effects on the bladder at doses that significantly reduced systemic blood pressure. In contrast, both WAY-133537 and ZD6169 inhibited bladder hyperactivity at doses that produced minimal changes in both mean arterial blood pressure and heart rate. These data suggest that both WAY-133537 and ZD6169 may be useful in the treatment of bladder instability at doses associated with minimal hemodynamic side effects.

Urge urinary incontinence (UUI), also known as unstable, hyperreflexive, or hyperactive bladder, is associated with abnormal spontaneous detrusor contractions that can be unrelated to bladder urine volume. These spontaneous contractions can produce chronic sensory urgency, as well as involuntary urine loss. Of the three major categories—stress, urge, and overflow, UUI accounts for 35 to 65% of cases depending upon the age of the patient (Resnick, 1995; American Health Care Policy Research Guideline, 1996). The etiology of UUI is known to be multivariant. The syndrome is often associated with spinal cord (upper motor neuron) injury, resulting in an augmentation of the spinal micturition reflex. Other etiologies may include bladder hypertrophy secondary to outlet obstruction and urinary tract infections. Treating bladder instability with antimuscarinics is a rational approach in the setting of hyperreflexia. However, with other etiologies that do not involve the presence of excessive autonomic efferent output, the rationale for anticholinergic therapy is less clear. Antimuscarinics such as oxybutynin are very effective at inhibiting bladder hyperreflexia; however, there are two major limitations with compounds of this class. First, although they are effective at inhibiting abnormal contractions, they can also inhibit the normal contractility needed for adequate bladder emptying, leading to significant residual urine volumes and possibly to exacerbation of the disease. Second, inhibition of muscarinic receptors outside the bladder can result in unacceptable side effects including dry mouth, reduced gastrointestinal motility, blurred vision and confusion. Nevertheless, to date, antimuscarinics are the mainstay of pharmacological therapy for UUI despite the fact that long-term compliance with these compounds is only about 30%.

It has been suggested (Foster et al., 1989) that a potassium

ABBREVIATIONS: UUI, urge urinary incontinence; RMP, resting membrane potential; KCO, potassium channel opener; PSS, physiological salt solution; SBC, spontaneous bladder contraction; BP, mean arterial blood pressure; HR, heart rate; PE, polyethylene; PEG200, polyethylene glycol 200; BKca, calcium-dependent potassium channel.
channel opener (KCO) may be useful in the treatment of detrusor hyperactivity. An increase in potassium channel permeability would hyperpolarize the cell, bring the membrane potential further from the threshold for activation of calcium channels, and reduce excitability (Brading, 1992). Bladder smooth muscle contains a number of potassium channels (Klockner and Isenberg, 1985; Zografos et al., 1992) including ATP-dependent potassium channels (Bonev and Nelson, 1993) that can be activated by a variety of KCOs. A number of these compounds have shown activity in isolated tissues (Fuji et al., 1990; Malmgren et al., 1990; Grant and Zuzack, 1991) and efficacy in both experimental (Poster and Brading, 1987; Malmgren et al., 1989) and clinical bladder instability (Nurse et al., 1991). However, because these compounds also activate vascular smooth muscle channels causing vasodilation, the clinical efficacy has been severely limited by hemodynamic side effects including hypotension and tachycardia.

Recently, Trivedi et al. (1995) and Howe et al. (1995) described a new KCO, ZD6169, which was shown to open bladder potassium channels and selectively increase bladder compliance in the rat and dog without significant hemodynamic effects. Another group (Pandita et al., 1997) demonstrated that ZD6169 was similarly effective at inhibiting prostaglandin E2-induced spontaneous contractions in rat bladder.

The present study evaluates the electrophysiological activity of WAY-133537, a novel substituted 1,2-diaminocyclobutene-3,4-dione (4-(3,4-dioxo-2-(1,2,2-trimethyl-propylamino)cyclobut-1-enylamino)-3-ethyl-benzonitrile, Fig. 1) on isolated rat bladder detrusor myocytes, and compares the activity of WAY-133537 with two established KCOs (ZD6169 and celikalim) on isolated rat detrusor strips, and their efficacy in a rat pathophysiological model of bladder instability described previously by Malmgren et al. (1987). In addition to the effects on the bladder, the hemodynamic activity of these compounds was assayed in conscious, restrained rats by monitoring mean arterial blood pressure (BP) and heart rate (HR).

Materials and Methods

All animal studies were approved by the Wyeth-Ayerst Institutional Animal Care and Use Committee, and were performed in accordance with the guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care.

Isolation of Rat Detrusor Cells

Rat detrusor cells were isolated in a manner described previously for guinea pig detrusor (Sheldon and Argentieri, 1995). Male Sprague-Dawley rats (Charles River, Wilmington, MA; 200–400 g) were euthanized by CO2 inhalation and exsanguination. Their urinary bladders were rapidly removed and placed in 37°C physiological solution with the following composition (mM): Na glutamate (80.0), NaCl (54.7), KCl (126.0), MgCl2 2 H2O (2.5), CaCl2 (1.8), and NaHCO3 (25.0). In addition, CO2 saturated with 95% O2/5% CO2 for a final pH of 7.4. The dome of the bladder was isolated from the trigone region and the mucosa was removed. The dome was then cut into 2- to 3-mm-wide strips and placed into fresh buffer for 1 h. Tissues were then transferred into 10 ml of an isolation buffer containing the above composition plus collagenase type VIII (1.0 mg/ml) and pronase (0.25 mg/ml). After 10 min the isolation buffer was replaced with fresh isolation buffer for an additional 10 min. The tissue was then washed three times in fresh collagenase and pronase free solution and stored at room temperature until studied. Cells for study were prepared by triturating one to two pieces of detrusor tissue in 2 ml of fresh isolation buffer for 5 min with a polished Pasteur pipette, (tip diameter ~1.5 mm) attached to a modified Harvard Respirator pump (Harvard Apparatus, South Natick, MA) at a rate of 20–50/min., with an approximate volume of 5 ml. Cells were then placed on a microscope stage in a temperature-regulated tissue bath at 32.5°C and continually superfused with physiological salt solution (PSS).

Cell Electrophysiology

Single cell recordings were performed with a List-Medical EPC-7 patch clamp amplifier (Adams & List Assoc., Westbury, NY). Pipette electrodes had tip resistances of 2 to 4 MΩ and were filled with the following composition (mM): KCl (126.0), MgCl2 · 6H2O (4.5), ATP Mg salt (4.0), GTP Tris salt (0.3), creatine PO4 (14.0), 6-glucose (9.0), EGTA (9.0), HEPES (9.0). The pH was adjusted to 7.4 with KOH. Signals were acquired (3 kHz high frequency cut-off, 12-bit resolution) using a 586-based personal computer.

Current-Clamp Recordings. Cell resting membrane potential (RMP) was measured in current clamp using the above mentioned instrumentation and pipette solutions. For these experiments nystatin was also added to the pipette solution (100 µg/ml) to allow recording through utilization of the perforated patch technique (Korn et al., 1991). After stable access was achieved, RMP was recorded for a 5-min control period followed by 5 min of drug application (0.3 and 1.0 µM). After this time, glyburide (5 µM) was also added to the perfusate and RMP was recorded for an additional 5 min.

Voltage-Clamp Recordings. Whole cell recordings were made using broken patch access. Currents were evoked using either voltage steps [holding potential (Vh) = −50; test potential (Vt) = −60 mV to 40 mV] or voltage ramps (−60 mV to 40 mV at 3.3 mV/s). The exact voltage clamp protocols are described in the figure legends. After stability was achieved, control currents were recorded. Next, WAY-133537 (0.3 and 1 µM) was added to the superfusate. Currents were recorded for 5 to 10 min or until compound effects reached steady state. This was followed either by washout or addition of glyburide (5 µM) to the superfusate.

Fig. 1. Chemical structures of WAY-133537, ZD6169, and celikalim.
Isolated Bladder Strip Contraction Studies

Female Sprague-Dawley rats (Charles River, Wilmington, MA; 250–350 g) were rendered unconscious via CO2 inhalation and exsanguinated. The entire bladder was removed and placed in room temperature PSS of the following composition (mM): NaCl (118.4), KCl (4.7), CaCl2 (2.5), MgSO4 (1.2), KH2PO4 (1.2), NaHCO3 (24.9), and n-glucose (11.1) gassed with O2-CO2, 95%/5% to achieve a pH of 7.4. The dome of the bladder was isolated from the trigone region and the mucosa was removed. The dome was then cut into strips 4- to 5-mm wide by 10-mm long. One end was secured to the bottom of a 10-ml tissue bath and the other to a Grass isometric force transducer (Grass Instruments, Quincy, MA). Tissues were pretensioned (0.25–0.5 g), and after 30 min of equilibration were contracted with an additional 15 mM KCl and again allowed to equilibrate. Compounds were administered directly into the tissue baths as cumulative concentrations. Signals from the tissues were digitized (486-based personal computer, 12-bit resolution, 1-s sampling interval, custom software) for on-line analysis. Isolated bladder strips contract with irregular frequency and amplitude, therefore, a 5-min area-under-the-contraction curve was used to assess contractility at steady state for each concentration.

In Vivo Studies

Hypertrophied Bladders. The method for producing hypertrophied, unstable bladders was modified from that reported by Malmgren et al. (1987). Briefly, female Sprague-Dawley rats (Charles River, Wilmington, MA; 190–210 g) were anesthetized with isoflurane and through a midline incision the bladder and urethra (Charles River, Wilmington, MA; 190–210 g) were anesthetized with isoflurane and through a midline incision the bladder and urethra were exsanguinated. The entire bladder was removed and placed in room temperature PSS of the following composition (mM): NaCl (118.4), KCl (4.7), CaCl2 (2.5), MgSO4 (1.2), KH2PO4 (1.2), NaHCO3 (24.9), and n-glucose (11.1) gassed with O2-CO2 95%/5% to achieve a pH of 7.4. The dome of the bladder was isolated from the trigone region and the mucosa was removed. The dome was then cut into strips 4- to 5-mm wide by 10-mm long. One end was secured to the bottom of a 10-ml tissue bath and the other to a Grass isometric force transducer (Grass Instruments, Quincy, MA). Tissues were pretensioned (0.25–0.5 g), and after 30 min of equilibration were contracted with an additional 15 mM KCl and again allowed to equilibrate. Compounds were administered directly into the tissue baths as cumulative concentrations. Signals from the tissues were digitized (486-based personal computer, 12-bit resolution, 1-s sampling interval, custom software) for on-line analysis. Isolated bladder strips contract with irregular frequency and amplitude, therefore, a 5-min area-under-the-contraction curve was used to assess contractility at steady state for each concentration.

Catheter Implantation. After six weeks, the ligature was removed under isoflurane anesthesia, and a flared catheter (PE60) was placed in the dome of the bladder and secured with a purse-string suture. The catheter was tunneled under the skin and exteriorized through an opening in the back of the neck. The abdominal incision was sutured and the free end of the catheter was sealed. After surgery, animals were given bicillin C-R, i.m. (Wyeth Laboratories, Philadelphia, PA). During the following 6 weeks, bladder hypertrophy and instability developed as a result of the partial outlet obstruction.

Cystometric Evaluation. Two days after catheter implantation, the animals were used for cystometric evaluations. The night before testing the animals were placed in the metabolic cages, the catheter was connected to a Harvard infusion pump, and bladders were perfused overnight with saline at 2 ml/h. The next morning the bladder catheter was attached (using a “T” connector) to a Statham pressure transducer (Model P23Db) and to a Harvard infusion pump. A plastic beaker attached to a force displacement transducer (Grass FTO3) was placed under the rat’s cage to collect and record urine volume. The cystometric evaluation of bladder function was started by infusing saline (20 ml/h) and after the first micturition the infusion was maintained for 20 min. Two hours after the first cystometry period, the rats were dosed orally with the test compound and a second cystometry was performed between 30 min and 4 h after administration of test compound. The appropriate vehicle [polyethylene glycol 200 (PEG200) or methylcellulose] was similarly administered to groups of rats that served as controls and the cystometry was performed at the respective time point.

Hemodynamic Assessment. Male Sprague-Dawley rats (Charles River, Wilmington, MA; 315–410 g) were anesthetized with isoflurane. A femoral artery and vein were cannulated with polyethylene tubing (PE50). The rats were placed in Bollman cages and the tail and two cannulae were extended through a hole at one end of the cage. The tail was taped securely to a firm sponge rubber board to prevent the rat from turning in its cage and dislodging the cannulae. BP was obtained from the cannulated femoral artery by means of a Statham pressure transducer (Model P23Db). The transducer was connected to a Grass (model 7) polygraph to record BP. HR was calculated manually from the BP traces.

Data Evaluation

In Vitro. Whole cell and steady-state currents were acquired and analyzed using a 586-based personal computer and pClamp (Axon Instruments, Foster City, CA) software. Changes in current and voltage were calculated as changes between control and treatment for each cell. The ED50 value for contractile inhibition was derived from a Michaelis-Menten fit to all the data points at each dose. This method takes into account the variability around the means and generates a confidence interval (95%) around the data. Other data were tabulated as mean ± S.E.M. Data were analyzed for statistical significance utilizing a paired Student’s t test at the p < .05 level of significance.

In Vivo. The most characteristic finding in the hypertrophied rat bladder model was spontaneous bladder contractions (SBCs), which developed during bladder filling. For each animal the maximum change in the total number of SBCs measured after treatment were compared to the values obtained before treatment and expressed as a percentage of change. The percent changes in SBC induced by each drug were, in turn, compared with the changes induced by the administration of the respective vehicle in the control group. ED50 values were derived from Michaelis-Menten fits to all the data points at each dose; 95% CI around the value were generated as above. The results were analyzed using a Dunnet’s t test for multiple comparisons at the p < .05 level of significance. Maximum changes in BP and HR were similarly analyzed. ED50 values were derived from linear fits through the data points.

Compounds and Dosing. The test compounds used in this study, WAY-133537, ZD6169, and celikalim were synthesized in house. For the in vitro studies, all compounds were dissolved in dimethyl sulfoxide (Aldrich Chemical Co., Inc., Milwaukee, WI) and further diluted in PSS. The maximum concentration of dimethyl sulfoxide that tissues or myocytes were exposed to was 0.01%. Glyburide and nystatin were obtained from Sigma Chemical Co. (St. Louis, MO). For in vivo studies, WAY-133537 and ZD6169 were dissolved in PEG200 (Aldrich Chemical Co., Inc.), whereas celikalim was suspended in methylcellulose (0.5%; Sigma). Compounds were administered by gastric gavage in a volume of 5 ml/kg.

Bladder Cystometry Studies. WAY-133537 was dosed at 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg. ZD6169 was dosed at 1, 3, and 10 mg/kg. Celikalim was dosed at 0.25, 0.3, 1.0, and 2.5 mg/kg.

Hemodynamic Studies. WAY-133537 was dosed at 1, 3, and 10 mg/kg. ZD6169 was dosed at 3, 10, and 30 mg/kg. Celikalim was dosed at 0.1, 0.25, 0.5, and 1 mg/kg.

Results

Cell Electrophysiology

Isolated cells were typically 5 to 15 μm wide and 100 to 150 μm long. The average resting potential (5 mM external potassium) recorded with pipettes containing 140 mM potassium was −39.6 ± 3.2 mV (n = 7–9). Cells had an average input resistance of 0.48 ± 0.13 GΩ (@ −50 mV) and a cell area of 3930.7 ± 908.9 μm2 (assuming 1 μF/cm2). Based on cell size, specific membrane resistance was 17.1 ± 5.1 KΩcm2.

Current-Clamp Recordings. RMP was recorded in normal buffer for 5 min, then cells were exposed to WAY-133537 (0.3 and 1.0 μM) in the superfusate. Hyperpolarization of the membrane potential was evident within 2 min. The magnitude of this hyperpolarization was similar at the two concentra-
trations tested (20.8 ± 3.1 and 25.6 ± 5.1 mV, respectively). This hyperpolarization was reversed toward control in the presence of 5 μM glyburide. A representative experiment at a concentration of 1.0 μM is shown in Fig. 2. A summary of the effects of WAY-133537 on RMP at 0.3 and 1.0 μM is shown in the bottom panel (n = 4–5).

**Voltage-Clamp Recordings.** Outward currents were evaluated by holding the cells at −50 mV and pulsing from −60 mV to +40 mV in 10-mV steps for 1000 ms, or by ramping the voltage from −60 mV to 40 mV at 3.3 mV/s. After the cell had stabilized (5 min), the cell was exposed to WAY-133537 (0.3 or 1.0 μM in the superfusate). WAY-133537 increased the net outward current, with both protocols, (n = 4) at test potentials above −40 mV. The increase in outward current was partially reversed upon washout or when exposed to 5 μM glyburide. An illustration of the effect of 0.3 μM WAY-133537 and washout on outward currents is shown in Fig. 3. A summary of the increase in outward current produced by WAY-133537 and the reversal with glyburide at various test potentials is shown in Fig. 4.

**Isolated Bladder Strip**

WAY-133537, ZD6169, and celikalim inhibited KCl-induced contraction of detrusor tissue in a concentration-dependent manner. The mean IC₅₀ values (±95% CL) for ZD6169, celikalim, and WAY-133537 were 0.93 (0.83, 1.24; n = 8), 0.03 (0.02, 0.04; n = 11), and 0.09 (0.06, 0.16; n = 6) μM, respectively. Contractile inhibition with all three compounds was reversed by exposure to 6 μM glyburide. A typical trace of a rat bladder strip exposed to increasing concentrations of WAY-133537 is shown in Fig. 5A. The concentration-response curves for all three compounds are presented in Fig. 5B.

**Hypertrophied Bladders—Effects on Spontaneous Contractions**

Each of the KCOs produced a dose-dependent decrease in the frequency of SBCs that occurred during the bladder filling phase. A representative cystometric recording before and after administration of celikalim is shown in Fig. 6A. None of the KCOs produced a significant effect on bladder volume or the magnitude of the detrusor contraction peak.

---

**Fig. 2.** Top, representative current-clamp tracing from a single rat detrusor myocyte. Control RMP was approximately −40 mV after exposure to WAY-133537 (1 μM); the cell hyperpolarized by approximately 25 mV. This hyperpolarization was reversed with the addition of glyburide (5 μM) to the tissue bath. Bottom, summarizes the mean RMP for control, 0.3, and 1.0 μM WAY-133537 (n = 4–5).

**Fig. 3.** Whole cell voltage-clamp tracings from an isolated rat detrusor myocyte. Cells were held at −50 mV and stepped in +10 mV increments for 1000 ms (Figure shows currents elicited at −50, −20, 10, and 40 mV). WAY-133537 increased outward current at all test potentials over the voltage range associated with activation of the ATP-dependent potassium channel. The increases in outward current were reversed upon wash out.

**Fig. 4.** Summary of the effects of WAY-133537 (0.3 μM; n = 4) on the measured current at various test potentials. Current was significantly increased at all potentials and restored to control levels by the addition of glyburide.
the compounds tested had any significant effect on micturition pressure (see Fig. 6A). The dose-response for each compound is presented in Fig. 6B, and a summary of each compound’s effects on SBCs is presented in Table 1.

WAY-133537

The maximum effect of WAY-133537 was observed approximately 1 h after oral administration. WAY-133537 produced a dose-dependent decrease in the frequency of SBCs. At 0.3, 1, 3, and 10 mg/kg, the decreases in frequency of SBCs were significantly (p < .05) different from the control group and pretreatment values. The calculated ED$_{50}$ value was estimated to be 0.13 (0.04, 0.5; 95% CL) mg/kg. A separate group of rats was dosed with PEG200 alone and served as control. PEG200 had no effect on SBC frequency.

ZD6169

Thirty minutes after exposure to ZD6169 (3 and 10 mg/kg) there was a significant (p < .05) decrease in the frequency of SBC relative to vehicle control and pretreatment values. The calculated ED$_{50}$ value was 2.4 (0.25, 18.3; 95% CL) mg/kg. One hour after oral administration, there was no significant effect on SBCs. A separate group of rats was dosed with PEG200 alone and served as control. PEG200 had no effect on SBC frequency.

Celikalim

The maximum effect of celikalim was observed approximately 4 h after dosing. There was a dose-dependent (0.25–2.5 mg/kg) decrease in the frequency of SBCs. The calculated ED$_{50}$ was 0.3 (0.06, 0.5; 95% CL) mg/kg. No significant changes were seen with vehicle administration.

Hemodynamic Assessment–Effects on Blood Pressure and HR

All three compounds decreased BP after oral administration (Fig. 10). Celikalim was the most potent (ED$_{20}$ ~ 0.2 mg/kg) followed by WAY-133537 (ED$_{20}$ ~ 2.3 mg/kg) and ZD6169 (ED$_{20}$ ~ 6.9 mg/kg). In general, decreases in BP greater than 10% were also accompanied with significant increases in HR (approx. 15–30%).

WAY-133537

Administration of WAY-133537 produced a dose-dependent decrease in BP. Maximum effects were observed approximately 15 min after dosing. Statistically significant decreases were observed at 1 mg/kg and above. Significant increases in HR were also observed at the same doses. The hemodynamic effects lasted approximately 2 h. The results are presented in Fig. 7 and summarized in Table 1.
ZD6169 produced decreases in BP over the dose range studied, and the effect was statistically significant at 3 mg/kg and above. Significant increases in HR were also observed at these doses. The onset of action was rapid with decreases in BP noted by 5 min and the maximum effect was achieved within 15 to 30 min after dosing. The duration of action was dose-dependent and varied from 30 to 120 min. The results are presented in Fig. 8 and summarized in Table 1.

**Celikalim**

Celikalim produced a dose-dependent decrease in BP. The decrease was statistically significant at 0.1 mg/kg and above. The decreases in BP were accompanied by concomitant increases in HR. The onset of action was slow and the maximum effect was achieved within 2 to 4 h after dosing. The duration of action was greater than 6 h. The results are presented in Fig. 9 and summarized in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Dose (mg/kg)</th>
<th>%ΔSBC</th>
<th>Hemodynamics</th>
<th>BP %Δ from Vehicle</th>
<th>HR %Δ from Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY-133537</td>
<td>6</td>
<td>0.03</td>
<td>−11 ± 13</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.1</td>
<td>−45 ± 14</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.3</td>
<td>−61 ± 8*</td>
<td>NT</td>
<td>−5 ± 1*</td>
<td>−30 ± 2*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>−76 ± 8*</td>
<td>6</td>
<td>0.5 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>−72 ± 10*</td>
<td>5</td>
<td>−30 ± 1*</td>
<td>40 ± 2*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>−70 ± 13*</td>
<td>5</td>
<td>−36 ± 3*</td>
<td>36 ± 3*</td>
</tr>
<tr>
<td>ZD6169</td>
<td>8</td>
<td>1</td>
<td>−32 ± 8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3</td>
<td>−55 ± 7*</td>
<td>5</td>
<td>−12 ± 3*</td>
<td>13 ± 4*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>−89 ± 3*</td>
<td>6</td>
<td>−21 ± 3*</td>
<td>25 ± 4*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>NT</td>
<td>NT</td>
<td>6</td>
<td>−38 ± 3*</td>
<td>37 ± 2*</td>
</tr>
<tr>
<td>Celikalim</td>
<td>0.1</td>
<td>NT</td>
<td>NT</td>
<td>4</td>
<td>−15 ± 5*</td>
<td>5 ± 5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.25</td>
<td>−42 ± 10</td>
<td>4</td>
<td>−21 ± 2*</td>
<td>18 ± 3</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.5</td>
<td>−72 ± 6*</td>
<td>4</td>
<td>−22 ± 4*</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>−63 ± 7*</td>
<td>4</td>
<td>−39 ± 1*</td>
<td>20 ± 2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.5</td>
<td>88 ± 3*</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* = p < .05 from vehicle; Dunnett’s t-test.
BP %Δ = maximum % change in mean arterial pressure over course of study
HR %Δ = heart rate % change at time of maximum blood pressure change
NT = not tested

Baseline blood pressure values were 114 ± 3 mmHg and 122 ± 2 mmHg in the PEG200 and methylcellulose vehicle groups, respectively. Baseline heart rate values were 390 ± 12 BPM and 399 ± 23 in the PEG200 and methylcellulose vehicle groups, respectively.
Comparing the ratios of the ED_{20} value for blood pressure to the ED_{50} value for inhibition of SBCs, celikalim had a ratio of 0.7, indicating no selectivity for the bladder. The ratio for ZD6169 was 2.9 in favor of bladder selectivity. WAY-133537 was the most selective with a ratio of 17.7 (Table 2).

### Discussion

Bladder instability associated with UUI is often reported as the most common form of urinary incontinence (Resnick, 1995). At present, the most accepted treatment for UUI is with anticholinergic-spasmolytic agents that block the action of the parasympathetic neurotransmitter acetylcholine in the bladder, and also block voltage-activated calcium channels in the muscle membrane (see Turner and Brading, 1997). Efficacy with these agents is reasonably good; however, patient compliance is only around 30% due to the side effects associated with muscarinic receptor blockade (dry mouth, blurred vision, increased HR, etc.). In addition, a significant portion of the patient population with UUI, despite bladder hyperactivity, also have an impaired ability to generate a sustained voiding contraction (Resnick and Yalla, 1985). Weakening normal contractility in these patients can often times lead to overflow incontinence (Blavas et al., 1980). It has been suggested that an agent that hyperpolarizes bladder smooth muscle would move the membrane potential further from the threshold for activation of calcium channels and thereby inhibit spontaneous contractions (Quast, 1993). The sustained micturition contraction would be less affected because normally more neurotransmitter is released from the motor nerve terminals than is necessary for maximal contraction.

Several investigators have evaluated the efficacy of antihypertensive KCOs in isolated detrusor tissues (Foster et al., 1989; Fovaeus et al., 1989). In addition, Nurse et al. (1991) reported the results of a small clinical trial with the KCO, cromakalim. In their study, 35% of patients with detrusor instability showed an improvement in the symptoms of urinary frequency and demonstrated an objective increase in mean voided volume. The authors concluded that although efficacious, the utility of cromakalim was severely limited by the accompanied decrease in BP. Hedlund et al. (1991) and Komersova et al. (1995) evaluated the efficacy of the antihypertensive KCOs pinacidil and levcromakalim in patients with outlet obstruction and high spinal cord lesions, respectively. Neither compound showed any activity at doses that did not decrease blood pressure to unacceptable levels, and it was concluded that the use of KCOs for the treatment of bladder hyperactivity must await drugs with greater selectivity for bladder smooth muscle.

In this study the ability of a novel compound (WAY-133537) to activate ATP-dependent potassium channels and hyperpolarize bladder smooth muscle cells in vitro, and to block KCl-induced contraction of rat isolated detrusor strips was evaluated. The in vivo ability of WAY-133535 to inhibit abnormal spontaneous contractions in a pathophysiological model of bladder instability was also evaluated. Comparisons were made to ZD6169, a compound recently shown to hyperpolarize bladder smooth muscle via activation of the ATP-dependent potassium channel (Trivedi et al., 1995, Heppner et al., 1996) and to increase bladder capacity (Howe et al., 1995) with minimal hemodynamic side-effects, and to the antihypertensive ATP-dependent KCO celikalim.

It is believed that hyperpolarization of the cell is the mechanism underlying the efficacy of KCOs. Bladder smooth muscle contraction is a calcium-dependent event (Nakayama and Brading, 1993). Activation of calcium channels in the bladder is voltage-dependent. The further the cell’s membrane poten-
tial resides from the threshold potential for activation of calcium channels, the less excitabile it is. Normal cells enzymatically dissociated from rat bladders had RMPs near −40 mV. Exposure to WAY-133537 (0.3 and 1.0 μM) produced a maximal hyperpolarization of approximately 25 mV. In the isolated tissue contraction studies, exposure to 1 μM produced nearly complete inhibition of spontaneous contractions, suggesting that a 25 mV hyperpolarization is sufficient to adequately inhibit contractility.

WAY-133537 produced an increase in outward current over the range of test potentials. Similar results were seen with both step-clamp and ramp-clamp protocols. The effects of WAY-133537 were partially reversed upon washout, and completely reversed by glyburide. The observed activation of current over the voltage range used, and the reversal of this effect with glyburide is consistent with activation of the ATP-dependent potassium channel (Spruce et al., 1985; see Gopalakrishnan et al., 1993).

Isolated Bladder Strip. All three compounds produced a concentration-dependent inhibition of KCl-induced detrusor strip contractions. Celikalim was the most potent compound. The intrinsic activity of WAY-133537 appeared to be slightly less than either celikalim or ZD6169. The activity of each compound was reversed by exposure to glyburide. This finding confirms the previous reports that the underlying mechanism of action of ZD6169 (Trivedi et al., 1995) and celikalim (de Lorenzi, 1994) involves activation of the ATP-dependent potassium channel in the rat bladder.

Hypertrophied Bladder. Oral administration of each compound produced a dose-dependent inhibition of SBCs. Both celikalim and WAY-133537 had similar potencies. ZD6169 was approximately 8 to 18 times less potent than WAY-133537 and celikalim. None of the compounds had any effect on micturition pressure, suggesting that the compounds should not impair normal voiding contractility. This is an important distinction between KCOs and the antimuscarinic/spasmodyltics used to treat this disease. Because acetylcholine is the primary neurotransmitter (in humans) underlying the bladder contraction, antimuscarinics such as oxybutynin tend to weaken the contractions necessary for bladder emptying (Peterson et al., 1990; Guarneri et al., 1991). This can often result in incomplete emptying and lead to exacerbation of symptoms (Andersson, 1988). Previous reports have shown that ZD6169 can increase bladder capacity and decrease micturition interval in normal rats (Howe et al., 1995) and rats exposed intravesically to prostaglandin E2 (Pandita et al., 1997). In this study, we have demonstrated that ZD6169 also prevents SBCs in an experimental model of bladder instability.

Hemodynamic Effects. All three compounds decreased BP after oral administration. Celikalim was the most potent, followed by WAY-133537 and ZD6169. Celikalim showed no selectivity for the bladder, whereas both ZD6169 and WAY-133537 demonstrated bladder selectivity, with WAY-133537 being the more selective of the two compounds.

The mechanism of action underlying the bladder selectivity for ZD6169 and WAY-133537 is unclear. Neither compound demonstrated selectivity for detrusor smooth muscle over isolated thoracic aorta rings. In fact, the isolated aorta was approximately 10 times more sensitive than was detrusor smooth muscle to WAY-133537 (N.W.N., D.W. and T.M.A., unpublished observation). Hu and Kim (1997) examined the effects of ZD6169 on ATP-dependent potassium channels and the large conductance calcium-dependent potassium (BKCa) channels over a range of concentrations in guinea pig bladder smooth muscle cells. They determined that the concentration-response for activation of the ATP-dependent potassium channel was bell shaped, indicating that at lower concentrations the channel was activated, but at higher concentrations (>20 μM) ZD6169 inhibited the channel. In addition, at these higher concentrations, the compound activated the BKCa channel. The authors speculate that this characteristic may underlie in part the improved cardiovascular side effect profile that ZD6169 demonstrates when compared with drugs like cromakalim. This does not entirely explain the bladder selectivity because although the heart is devoid of BKCa channels, the RMP of the aorta appears to depend upon an iberiotoxin-sensitive current (Suarez-Kurtz et al., 1991). Even though activating these channels would inhibit bladder contractions (Sheldon et al., 1997) it would also produce vasodilation, leading to a decrease in vascular resistance and a decrease in BP.

As previously mentioned, bladder selectivity was also reported by Howe et al. (1995) for ZD6169 although they reported a greater separation between cystometric and hemodynamic effects (ratio ≈ 187). There may be several reasons for the difference in selectivity between the two studies. First, the spontaneously contracting, hypertrophied bladder model may be a more severe test of a compound’s activity, requiring more compound to inhibit spontaneous contractions. Second, we reported the maximal hemodynamic change regardless of the duration. Howe et al. (1995) only considered those changes sustained in excess of 90 min after administration to be significant. Nevertheless, both ZD6169 and WAY-133537 demonstrate clear bladder selectivity compared with compounds like cromakalim and celikalim.

Summary. KCOs have been shown to effectively inhibit contractions in bladder smooth muscle. The ATP-dependent KCOs in this study decreased contractility in isolated rat detrusor strips depolarized with KCl. All three compounds were efficacious at inhibiting SBCs in the rat hypertrophied bladder model of detrusor instability. The antihypertensive agent celikalim showed no selectivity toward the bladder over hemodynamic effects. However, both WAY-133537 and ZD6169 demonstrated bladder efficacy at doses where there were minimal hemodynamic side effects. These data suggest that compounds like WAY-133537 and ZD6169 should be useful in the treatment of bladder instability at doses that produce minimal hemodynamic compromise.

References


Send reprint requests to: Dr. Thomas M. Argentieri, Ph.D., Wyeth-Ayerst Research, CN-8000, Princeton, NJ 08543-8000. E-mail: argentti@war.wyeth.com