(−)-(9S)-9-(3-Bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4H)-one 1,1-Dioxide (A-278637): A Novel ATP-Sensitive Potassium Channel Opener Efficacious in Suppressing Urinary Bladder Contractions. I. In Vitro Characterization

MURALI GOPALAKRISHNAN, STEVEN A. BUCKNER, KRISTI L. WHITEAKER, CHAR-CHANG SHIEH, EDUARDO J. MOLINARI, IVAN MILICIC, ANTHONY V. DAZA, RACHEL DAVIS-TABER, VICTORIA E. SCOTT, DONNA SELLERS, RUSS CHESS-WILLIAMS, CHRISTOPHER R. CHAPPLE, YI LIU, DONG LIU, JORGE D. BRIONI, JAMES P. SULLIVAN, MICHAEL WILLIAMS, WILLIAM A. CARROLL, and MICHAEL J. COGHLAN


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ABSTRACT

Alterations in the myogenic activity of the bladder smooth muscle are thought to serve as a basis for the involuntary detrusor contractions associated with the overactive bladder. Activation of ATP-sensitive K⁺ (KATP) channels has been recognized as a potentially viable mechanism to modulate membrane excitability in bladder smooth muscle. In this study, we describe the preclinical characterization of (−)-(9S)-9-(3-bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4H)-one 1,1-dioxide (A-278637), a novel 1,4-dihydropyridine KATP channel opener (KCO) that demonstrates enhanced bladder selectivity compared with other KCOs, WAY-133537 [(trimethyl-propylamino)cyclobut-1-enylamino]-3-ethyl-benzonitrile and ZD6169 [(trimethyl-propylamino)cyclobut-1-enylamino]-3-ethyl-benzonitrile. A-278637 did not interact with other ion channels, including L-type calcium channels or other neurotransmitter receptor systems. The pharmacological profile of A-278637 represents an attractive basis for further investigations of selective KATP channel openers for the treatment of overactive bladder via myogenic etiology.

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Alterations in the myogenic activity of the bladder smooth muscle are thought to serve as a basis for the involuntary detrusor contractions associated with the overactive bladder. Activation of ATP-sensitive K⁺ (KATP) channels has been recognized as a potentially viable mechanism to modulate membrane excitability in bladder smooth muscle. In this study, we describe the preclinical characterization of (−)-(9S)-9-(3-bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4H)-one 1,1-dioxide (A-278637), a novel 1,4-dihydropyridine KATP channel opener (KCO) that demonstrates enhanced bladder selectivity compared with other KCOs, WAY-133537 [(trimethyl-propylamino)cyclobut-1-enylamino]-3-ethyl-benzonitrile and ZD6169 [(trimethyl-propylamino)cyclobut-1-enylamino]-3-ethyl-benzonitrile. A-278637 did not interact with other ion channels, including L-type calcium channels or other neurotransmitter receptor systems. The pharmacological profile of A-278637 represents an attractive basis for further investigations of selective KATP channel openers for the treatment of overactive bladder via myogenic etiology.

Overactive bladder, a condition characterized by increased urinary urgency and frequency with or without urge incontinence, continues to be a chronic, highly prevalent condition affecting more than 15 million people in the United States alone (Payne, 1998; McGhan, 2001). Currently available options for the management of this condition, the muscarinic receptor antagonists such as oxybutynin and tolterodine, suffer from limited efficacy, in part, related to poor tolerability as a result of side effects such as dry mouth, blurred vision, and constipation (Andersson et al., 1999; Sullivan and Abrams, 1999; Chapple, 2000). Accordingly, overactive bladder continues to be an unmet medical need with efforts di-
rected to validate mechanisms and identify novel agents with superior efficacy and/or improved side effect profile. Alterations in the myogenic activity of the bladder smooth muscle have been proposed as a basis for the generation of involuntary detrusor contractions associated with the overactive bladder (Brading, 1997; Elbadawi et al., 1998). Although central nervous system and/or afferent signaling pathways may also participate (de Groat, 1997), overactive bladder may be viewed as a disorder of bladder smooth muscle tone and the underlying changes in spontaneous action potentials and phasic contractions. Tissue reactivity studies have shown that detrusor strips from unstable bladders exhibit spontaneous tetanic activity with enhanced electrical coupling between cells. Studies have shown agonist supersensitivity and altered spontaneous contractile activity in idiopathic detrusor instability, a common cause of lower urinary tract storage symptoms. This is consistent with enhanced electrical coupling of bladder smooth muscle cells (Elbadawi et al., 1993; Mills et al., 2000).

Over the past decade, several openers of ATP-sensitive K⁺ (KATP) channels have been evaluated for their effects on bladder function. First generation agents such as (−)-cromakalim, YM934, and ZM244085, as well as more recent compounds such as ZD6169 and WAY-133537 have been shown, in vitro, to evoke relaxation of isolated bladder smooth muscle strips from various species precontracted by a variety of stimuli, including electrical field, carbachol, or low external K⁺ (Foster et al., 1989; Fujii et al., 1990; Grant and Zuzack, 1991; Wojdan et al., 1999). These agents selectively open glyburide (glibenclamide)-sensitive KATP channels, critical to the control of membrane potential, leading to membrane hyperpolarization, attenuated Ca²⁺ influx through L-type voltage-gated Ca²⁺ channels and decrease in bladder smooth muscle excitability (Bonev and Nelson, 1993; Quayle et al., 1997). In particular, activation of a few KATP channels in bladder smooth muscle by low concentrations of KCOs far below that required for substantial KATP current activation has been shown to suppress spike action potentials and spontaneous myogenic activity (Petkov et al., 2001; Shieh et al., 2001). In unstable bladders with heightened spontaneous contractility, it is likely that activation by low concentrations of KCOs would serve to inhibit these contractions and dampen smooth muscle (hyper)excitability.

In a preliminary clinical study of patients with detrusor instability or detrusor hyperflexia, 6 of 17 patients responded to cromakalim with modest improvements in symptoms of urinary frequency and increase in mean voided volume (Nurse et al., 1991). Although this observation is consistent with the preclinical efficacy results in animal models (Malagren et al., 1989), in the absence of placebo-controlled and definitive clinical data, it is thought that cromakalim and subsequent analogs lack sufficient bladder selectivity relative to the vascular effects. Consequently, further clinical proof of principle for KCOs for bladder overactivity mandates a compound with improved selectivity. Improvements in bladder selectivity have been reported for certain chemotypes represented by ZD6169 and WAY-133537 in preclinical models where in vivo efficacies for inhibition of bladder overactivity were demonstrated at doses that do not substantially affect arterial pressure or heart rate (Howe et al., 1995; Pandita et al., 1997; Wojdan et al., 1999; Yu and de Groat, 1999). However, a clear need exists for the identification of agents efficacious in suppressing unstable bladder contractions with superior selectivity versus cardiovascular liabilities.

(9S)-9-(3-Bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4H)-one 1,1-dioxide (A-278637) is a novel KATP channel opener (Fig. 1) from a series of 1,4-dihydropyridine analogs (Carroll et al., 2001). In the present and accompanying article (Brune et al., 2002), the initial pharmacological characterization of this compound is described. Herein, we show that A-278637 potently and selectively interacts with KATP channels and suppresses contractility of bladder smooth muscle strips, including those from human hyperreflexic bladder. In the accompanying article (Brune et al., 2002), A-278637 is shown to display enhanced bladder selectivity for the suppression of unstable bladder contractions in vivo relative to other KCOs, ZD6169 and WAY-133537.

Materials and Methods

Materials

All studies were carried out in accordance with guidelines outlined by the Animal Welfare Act, the Association for Assessment and Accreditation of Laboratory Animals, and the Institutional Animal Care and Use Committee of Abbott Laboratories. DiBAC₄(3) was purchased from Molecular Probes (Eugene, OR). Compounds were prepared in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) as a 5 or 10 mM stock, protected from light, and serial dilutions prepared in appropriate assay buffer just before use.

DiBAC₄(3) Fluorescence Studies

Functional activity of KATP channels in guinea pig bladder smooth muscle cells was assessed as described previously (Gopalakrishnan et al., 1999) by evaluating changes in membrane potential using the bis-oxonol dye DiBAC₄(3) in a 96-well fluorescent imaging plate reader (FLIPR). Briefly, urinary bladders were removed from anesthetized male guinea pigs (Hartley; Charles River Laboratories, Inc., Wilmington, MA), weighing 250 to 300 g, and cells isolated by enzymatic dissociation using collagenase and pronase. Confluent cells, cultured in black clear-bottomed 96-well plates, were rinsed twice with 200 μl of assay buffer (20 mM HEPES, 120 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 5 mM glucose; pH 7.4 at 25°C) containing 5 μM DiBAC₄(3) and incubated with 180 μl of buffer in a cell incubator for 30 min to ensure dye distribution across the membrane. Assays were carried out at 37°C. After addition of various concentrations of the test compound, changes in DiBAC₄(3) fluo-
ence were measured at excitation and emission wavelengths of 488 and 520 nm, respectively.

**Whole-Cell Patch-Clamp Studies**

Whole-cell patch-clamp technique was used to measure changes in ionic currents from guinea pig bladder smooth muscle cells as described previously (Shieh et al., 2001). Urinary bladders were transferred directly into preoxygenated physiological saline solution containing 137 mM NaCl, 5.4 mM KCl, 2.0 mM CaCl₂, 2.0 mM MgCl₂, 0.42 mM KH₂PO₄, 4.17 mM NaHCO₃, 10 mM HEPES, and 10 mM glucose; pH 7.4 with NaOH. Pieces of bladder smooth muscle were incubated with collagenase, and single smooth muscle cells were obtained by triturating using a fire-polished large-bore Pasteur pipette. The intracellular pipette solution contained 107 mM KCl, 1.2 mM MgCl₂, 1 mM CaCl₂, 10 mM EGTA, 5 mM HEPES, and 0.1 mM ATP; pH 7.2 with KOH; total K+ ~140 mM. The bath solution contained 60 mM KCl, 80 mM NaCl, 1.2 mM MgCl₂, and 5 mM HEPES; pH 7.4 with NaOH. Whole-cell currents were recorded at room temperature and were amplified using Axopatch-200B amplifier (Axon Instruments, Union City, CA) and low pass filtered at 5 kHz (~3 dB; four-pole Bessel filter) before digitization (Digidata 1200B; Axon Instruments) at a sampling rate of 10 kHz.

**Bladder Relaxation Studies**

Bladder strip relaxation studies were performed as described previously (Buckner et al., 2000). Briefly, female Landrace pigs (Wilson’s Prairie View Farm, Burlington, WI), weighing 9 to 25 kg, were euthanized with an intraperitoneal injection of pentobarbital (150–200 mg/kg, Somlethol; J.A. Webster Inc., Sterling, MA). The entire urinary bladder was removed and placed in Krebs-Ringer bicarbonate solution. The portal vein was cleaned of extraneous tissue and cut into 3- to 4-mm rings, and mounted in 10-ml isolated tissue baths at 37°C. Tissues were rinsed every 10 min for a total of 45 to 60 min. The aorta was primed once with 80 mM KCl rinsed to basal tension and again with phenylephrine (10 μM). Absence of functional endothelium was confirmed by loss of the acetylcholine (10 μM)-induced relaxation. After an additional 60-min equilibration period, tension was established using 25 mM KCl solution, and cumulative concentration-relaxation-response curve was generated for the test compound.

**Radioligand Binding and Electrophysiology Selectivity Studies**

[³H]Glyburide and [³H]isradipine binding to membranes was performed as described previously (Wei et al., 1989; Gopalakrishnan et al., 1991). Whole-cell patch-clamp studies using ion channels expressed in clonal cell lines or native cell types (Table 3) were carried out using standard electrophysiological techniques (Hamill et al., 1981).

**Data Analysis**

The concentration dependence of maximal steady-state changes in fluorescence or changes in tension responses was fitted by nonlinear
versed by subsequent addition of glyburide (5 μM). As shown in Fig. 2A, A-278637-evoked responses were reversed these effects. B, concentration-response curves derived from the AUC of the contractile response during a 15-min interval. For carbachol-stimulated tissues, relaxations (measured in grams) were assessed by Student's t test, and a p value <.05 was considered statistically significant.

**Results**

**Effects on DiBAC4(3) Fluorescence Changes.** A-278637 evoked concentration-dependent decreases in membrane potential in guinea pig bladder smooth muscle cells as assessed by decreases in DiBAC4(3) fluorescence with an EC50 value of 102 nM (−log EC50 = 2.04 ± 0.11; slope = 1.76 ± 0.12; n = 5; Fig. 2). As shown in Fig. 2A, A-278637-evoked responses were reversed by subsequent addition of glyburide (5 μM). In comparison, A-278637 was 3-fold more potent compared with ZD6169 in this assay (−log EC50 = 6.56 ± 0.10; slope = 1.07) but about 5-fold less potent than WAY-133537 (−log EC50 = 7.72 ± 0.06; slope = 0.62; n = 6).

**Whole-Cell Patch Clamp.** Direct interaction of A-278637 with KATP channels in guinea pig bladder smooth muscle cells was studied by whole cell patch clamp. As shown in Fig. 3A, upon addition of 10 μM A-278637, membrane currents were increased by 50.6 ± 5.8 pA (n = 4) under conditions where cells were bathed in solution containing 60 mM K+ and voltage clamped at −80 mV with patch pipette containing 140 mM K+ and 0.1 mM ATP. The current-voltage relationship of A-278637-evoked responses is presented in Fig. 3B. Experiments performed under current-clamp conditions showed that A-278637 (100 nM) decreased smooth muscle membrane potential by 23.97 ± 4.4 mV (n = 3). A-278637-evoked current and membrane potential effects were both sensitive to inhibition by 5 μM glyburide, suggesting that the compound opens KATP channels in bladder smooth muscle cells.

**Bladder Strip Relaxation.** Isolated pig bladder strips exhibit spontaneous phasic activity that is myogenic in nature (Buckner et al., 2002). Addition of A-278637 resulted in a concentration-dependent suppression of the spontaneous activity that was restored by the addition of 5 μM glyburide (Fig. 4A). The IC50 value of A-278637 measured as changes in AUC of the contractile response was 22.7 nM (−log IC50 = 7.64 ± 0.06; n = 6). As shown in Fig. 4B, the reduction in AUC at low concentrations primarily involves a significant reduction in the contractile frequency (37 ± 8.4% at 10 nM), with no significant effect on the duration or the amplitude of the contractile response. A-278637 is about 6- to 10-fold more potent in suppressing phasic activity compared with ZD6169 (−log IC50 = 6.68 ± 0.11) and WAY-133537 (−log IC50 = 6.99 ± 0.06), respectively (Table 1).

A major component of the neurogenic stimulus for physiological bladder contractions is acetylcholine-induced stimulation of postjunctional muscarinic receptors on the bladder smooth muscle (Eglen et al., 1999; Hegde and Eglen, 1999). The effect of A-278637 on muscarinic receptor-mediated contractions was assessed in both pig and human bladder strips.
Contractions evoked by the muscarinic receptor agonist carbachol were effectively suppressed in a concentration-dependent manner by A-278637 in pig bladder strips with an IC\text{50} value of 164 nM (−log IC\text{50} = 5.22 ± 0.22; n = 4), which is ~400-fold higher than those observed at bladder K\text{ATP} channels (Table 3).

**Cardiac K\text{ATP}**. The potential interaction of A-278637 on cardiac K\text{ATP} channels was examined by assessing decreases in DiBAC\text{4}(3) fluorescence in cultured neonatal rat cardiac myocytes as described previously (Whiteaker et al., 2002). A-278637 evoked responses in cardiac myocytes with an EC\text{50} value of 40.2 μM (−log EC\text{50} = 6.52 ± 0.22; n = 4), which is ~400-fold higher than those observed at bladder K\text{ATP} channels (Table 3).

**Pancreatic K\text{ATP}**. A-278637 was also examined for interaction with sulfonylurea receptor SUR1-containing K\text{ATP} channels that is critical to metabolic regulation in the pancreas (Babenko et al., 1998; Seino, 1999). In rat insulinoma RINm5F cell line, A-278637 did not evoke a decrease in DiBAC\text{4}(3) fluorescence or activate \text{86}Rb\text{"} efflux up to concentrations of 10 μM. To further examine potential interactions with the SUR1, A-278637 was tested for displacement of \text{3H}glyburide binding to RINm5F cell membranes. A-278637 weakly displaced \text{3H}glyburide binding with a K\text{d} value of 12.49 ± 3.66 μM (n = 3), which is 120-fold higher than the potency to activate bladder K\text{ATP} channels as measured by FLIPR DiBAC\text{4}(3) assays.

**Ion Channel/Receptor Selectivity**. Although A-278637 is structurally related to 1,4-dihydropyridine cardiac channel ligands, the compound did not inhibit \text{3H}isradipine binding to bladder smooth muscle membranes at the highest concentration (30 μM) tested, consistent with its lack of effect in suppressing 80 mM K\text{+}-depolarized smooth muscle strips. Furthermore, A-278637 had no significant effect on native Ca\text{2+} currents expressed in GH3 cells or other representative K\text{+} channels (K\text{v}1.5, hERG, Kir2.3, and BK\text{ca}) expressed in various clonal cell lines (n ≥ 3).

A-278637 was evaluated in a binding screen that contained representatives of most G protein-coupled receptors, as well as some ligand- and voltage-gated ion channel binding sites at CEREP (receptor binding and enzyme profile; CEREP, Inc., Redmond, WA). The compound was tested at a single concentration (10 μM) in duplicate. A-278637 did not show significant displacement of binding with the exception of vasopressin V\text{1A} receptors where it displaced the binding of \text{3H}arginine-vasopressin to human recombinant V\text{1A} receptors expressed in mammalian cells (82% at 10 μM). Further examination of the concentration response for binding inhibition revealed a K\text{d} value 0.92 μM (n = 3). A-278637 was tested for agonist and antagonist activities at V\text{1A} receptors in the rat caudal artery. The compound was ineffective when tested as an antagonist, it shows modest bladder selectivity versus thoracic aorta compared with ZD6169 and WAY-133537, which were somewhat more potent in relaxing aortic strips.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Membrane Potential (FLIPR; −log EC\text{50})</th>
<th>Spontaneous Phasic Activity</th>
<th>Carbachol-Evoked Contractions</th>
<th>Electrical Field-Stimulated Contractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-278637</td>
<td>7.04 ± 0.11 (n = 5)</td>
<td>7.64 ± 0.06 (n = 6)</td>
<td>6.78 ± 0.08 (n = 6)</td>
<td>6.51 ± 0.09 (n = 6)</td>
</tr>
<tr>
<td>WAY-133537</td>
<td>7.72 ± 0.06 (n = 6)</td>
<td>6.99 ± 0.06 (n = 5)</td>
<td>6.82 ± 0.01 (n = 6)</td>
<td>6.17 ± 0.13 (n = 5)</td>
</tr>
<tr>
<td>ZD6169</td>
<td>6.56 ± 0.10 (n = 12)</td>
<td>6.68 ± 0.11 (n = 3)</td>
<td>6.87 ± 0.05 (n = 4)</td>
<td>5.59 ± 0.09 (n = 6)</td>
</tr>
</tbody>
</table>

Data from pig detrusor strips except where indicated.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bladder Spontaneous Phasic Activity</th>
<th>Thoracic Aorta 25 mM K\text{+} Evoked Contractions</th>
<th>Portal Vein Spontaneous Phasic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-278637</td>
<td>7.64 ± 0.06</td>
<td>7.34 ± 0.07</td>
<td>7.74 ± 0.39</td>
</tr>
<tr>
<td>WAY-133537</td>
<td>6.99 ± 0.06</td>
<td>7.36 ± 0.07</td>
<td>7.30 ± 0.08</td>
</tr>
<tr>
<td>ZD6169</td>
<td>6.68 ± 0.11</td>
<td>7.49 ± 0.27</td>
<td>7.47 ± 0.22</td>
</tr>
</tbody>
</table>

Number of experiments was three to six each carried out in duplicate.
agonist but did weakly suppress contractions evoked by arginine-vasopressin (3 nM) with a 57% inhibition observed at 10 μM. The potency of A-278637 to inhibit arginine-vasopressin-mediated contractions in the vasculature is at least 100-fold higher than that required to activate bladder KATP channels in the membrane potential studies or to inhibit bladder contractions in vitro.

Discussion

The present study describes the in vitro pharmacological profile of a novel dihydropyridine KATP channel opener, A-278637. This KCO evoked concentration-dependent decreases in membrane potential in bladder smooth muscle cells and suppressed spontaneous phasic activity of bladder strips in a glyburide-reversible manner. A-278637 also suppressed contractions evoked by muscarinic receptor activation in pig and human bladder strips with comparable potencies. Direct interaction with native KATP channels was confirmed in bladder smooth muscle cells where the compound was efficacious in activating glyburide-sensitive currents.

KATP Channel Activity of A-278637. Previous studies from our laboratory have shown a good correlation between KCO potencies to evoke membrane potential changes as measured by evaluating fluorescence changes in bladder smooth muscle cells with relaxation of detrusor strips in vitro (Gopalakrishnan et al., 1999). A-278637 was about 60-fold more potent in evoking membrane potential effects through KATP channels compared with ZM244085 (6.1 μM; Gopalakrishnan et al., 1999), a structurally related 1,4-dihydropyridine KATP channel opener previously shown to inhibit bladder activity (Li et al., 1996). The reversal by glyburide of A-278637-evoked fluorescence responses in guinea pig bladder smooth muscle cells and its attenuation of spontaneous phasic activity in bladder strips are consistent with the notion that A-278637 interacts with the KATP channel. The observation that A-278637 also evoked glyburide-sensitive K+ currents in guinea pig bladder smooth muscle cells provides additional direct evidence that membrane hyperpolarization measured in DiBAC4(3) fluorescence assays and relaxation observed in the bladder smooth muscle are mediated by interactions with the KATP channel. Importantly, these effects do not involve interaction with L-type calcium channels because A-278637 did not inhibit [3H]isradipine binding, was ineffective in suppressing aortic strip contractions evoked by 80 mM K+, and had no significant effects on native L-type Ca2+ currents.

Effects on Bladder Smooth Muscle Function. Urinary bladder smooth muscle, unlike arterial smooth muscle, exhibits action potentials and phasic myogenic activity. Previous studies have shown that KCOs can effectively suppress spontaneous contractions in human urinary bladder (Wammack et al., 1994), guinea pig bladder (Fuji et al., 1990; Hashitani et al., 1996), or hypertrophied rat bladder with instability (Malmgren et al., 1989). In the present study, a comparison of the IC50 values to suppress spontaneous phasic activity showed that A-278637 is 5- to 10-fold more potent compared with both ZD6169 and WAY-133537. It should be noted that WAY-133537 was found to be about 7-fold more potent than A-278637 in the FLIPR assay, but less potent in various tissue reactivity assays; the reason for this discrepancy remains unclear. The suppression of spontaneous phasic activity of bladder strips is attributed to the inhibition of action potentials in smooth muscle cells at relatively low concentrations of KCOs. It has been demonstrated that low concentrations of KCOs could open a fraction of KATP channels evoking a small increase in K+ conductance sufficient to lower the membrane potential away from the threshold for action potential firing (Petkov et al., 2001; Shieh et al., 2001). In support of this notion, a significant inhibition of the integrated muscle force (AUC) and contraction frequency of isolated bladder strips by A-278637 was noted at concentrations as low as 10 nM. This value is comparable with the estimated plasma concentrations required to inhibit unstable contractions by 50% in the pig model in vivo (~8 nM corresponding to 3.3 ng/ml; Brune et al., 2002).

It is known that activation of the parasympathetic nerves releases acetylcholine from postganglionic nerves, which in turn activates muscarinic receptors present in bladder smooth muscles, resulting in bladder contractions and normal voiding function. Although the muscarinic M3 receptors seem to be the predominant subtype present in most species, it is the postsynaptic muscarinic M2 receptor present in the bladder smooth muscle that is responsible for contractions (Eglen et al., 1999). Although equiefficacious in tissue relaxation assays, A-278637 was 8- to 13-fold less potent in inhibiting contractions evoked by direct muscarinic receptor activation with carbachol or by stimulation of parasympathetic postganglionic nerves that triggers the release of both acetylcholine and ATP compared with the suppression of myo-

### Table 3

<table>
<thead>
<tr>
<th>Target Channel</th>
<th>Assay</th>
<th>Activity</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>Rat cardiac myocytes, −log EC&lt;sub&gt;50&lt;/sub&gt; [FLIPR-DiBAC&lt;sub&gt;4(3)&lt;/sub&gt;]</td>
<td>5.52 ± 0.22 (n = 4)</td>
<td>400</td>
</tr>
<tr>
<td>Pancreatic K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>[3H]Glyburide binding, −log K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>4.96 ± 0.11 (n = 3)</td>
<td>120</td>
</tr>
<tr>
<td>L-type Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Electrophysiology (Inhib)</td>
<td>14.3 ± 5.5% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>Electrophysiology (Inhib)</td>
<td>8.2 ± 3.9% (n = 4)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>hERG</td>
<td>Electrophysiology (Inhib)</td>
<td>4.6 ± 0.8% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>BK&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>Electrophysiology (Act)</td>
<td>3.2 ± 1.4% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Kv2.3</td>
<td>Cation efflux (Inhib)</td>
<td>10.5 ± 1.5% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Electrophysiology (Inhib)</td>
<td>5.0 ± 2.6% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>CEREP profile</td>
<td>Radioligand binding (Inhib)</td>
<td>2.9 ± 1.5% (n = 3)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>70 Receptors/enzymes</td>
<td>(various)</td>
<td>&lt;10% at 10 μM</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* Selectivity versus FLIPR EC<sub>50</sub> value measured in bladder smooth muscle cells.
genic phasic activity (Table 1). Previous studies have shown that KATP channel openers, in general, are some 15-fold more potent in suppressing spontaneous activities compared with electrical field-stimulated contractions, primarily via reduction in contraction frequency consistent with the idea that small increases in K⁺ conductance evoked by KCOs are sufficient to suppress spontaneous myogenic activity (Petkov et al., 2001; Buckner et al., 2002). The suppression of contractions induced by muscarinic receptor activation or nerve stimulation by KCOs, including A-278637, can be attributed to activation of KATP channels that lead to membrane hyperpolarization and attenuation of Ca²⁺ influx through L-type voltage-gated Ca²⁺ channels.

**In Vitro Selectivity of A-278637.** The cloning and expression of cDNAs for KATP channel subunits indicates a diversity of KATP channel types arising by heteromorphic assembly of SURs and pore-forming inwardly rectifying K⁺ channels. The SUR2B subunit in conjunction with Kir6.2 or Kir6.1 is thought to constitute diverse smooth muscle type KATP channels (Babenko et al., 1998; Seino, 1999) although more recently, functional coassembly of both pore-forming subunits, Kir6.1 and Kir6.2, with SUR2B has also been reported (Cui et al., 2001). SUR1–Kir6.2 combination generates KATP channels with properties typical of those expressed in pancreas and neurons, whereas the components of plasmalemmal cardiac KATP channel are derived from SUR2A–Kir6.2. A-278637 was found to display selectivity toward activation of KATP channels in bladder smooth muscle (EC₅₀ = 102 nM) relative to KATP channels in cardiomycocytes (EC₅₀ = 40 μM) or pancreatic KATP channels expressed in RINm5F cells (EC₅₀ > 10 μM). In radioligand binding studies, A-278637 weakly displaced [³H]glyburide binding to high-affinity sulfonylurea receptors with a Kᵢ value that was at least 100-fold higher than the concentrations required to activate bladder smooth muscle KATP channels. Thus, at concentrations effective in suppressing bladder contractility, A-278637 displays substantial selectivity versus other predominant KATP channel subtypes.

Because a key goal in the development of KATP channel openers is to identify bladder-selective openers with minimal hemodynamic effects, the interactions of A-278637 were assessed in two vascular tissues. Although no absolute in vitro bladder versus vascular selectivity could be demonstrated, our studies show that A-278637 is about 2-fold less potent in relaxing 25 mM K⁺-stimulated thoracic aorta, whereas both WAY-133537 and ZD6169 are 2- and 6.5-fold more potent in relaxing aortic smooth muscle compared with inhibition of bladder contractions. Similarly, WAY-133537 and ZD6169 are 2- and 6-fold, respectively, more potent in inhibiting portal vein than bladder contractility, whereas A-278637 showed comparable potencies in these two assays. Thus, although no absolute selectivity is apparent, A-278637 seems to be somewhat more selective toward suppression of bladder versus vascular contractility compared with ZD6169 and WAY-133537.

As shown in the accompanying article (Brune et al., 2002), A-278637 showed potent inhibition of bladder contractions in a myogenic model of bladder instability with in vivo selectivity superior to both ZD6169 and WAY-133537. Although A-278637 exhibits a modest degree of relative selectivity at the in vivo level, whether this contributes to the selectivity noted in vivo remains to be established. Considerable diversity exists for the nucleotide-regulated K⁺ channels in smooth muscle tissues with varying conductance and bio-physical properties. These have been confirmed by coexpression of the SUR2B subunit with various combinations of Kir6.2 and Kir6.1 subunits in heterologous expression systems (Babenko et al., 1998; Seino, 1999; Yamada et al., 1999). The selectivity of A-278637 could, in principle, arise from distinctions in interactions with KATP channel combinations at the level of SUR-Kr complex or via differential couplings of nucleotide and KCO binding to regulate KATP channel activity in the obstructed bladder. Although SUR2B RNA expression is widespread, splicing of the SUR2 gene has been shown to occur at exon 17 located in the first nucleotide-binding motif with differential expression patterns and nucleotide sensitivities (Chutkow et al., 1999; Davis-Taber et al., 2000). There is also some evidence suggesting that the KATP channel complex functions not only solely as a K⁺ conductance but also as an enzyme regulating nucleotide-dependent channel gating through an intrinsic ATPase activity of the SUR subunit (Bienengraeber et al., 2000). Whether any of these mechanisms modulate bladder KATP channel function and differentially influence interactions of A-278637 remains to be investigated.

In conclusion, the findings of the present study show that A-278637 is a novel and potent KCO with efficacy in suppressing myogenic activity in vitro in both pig and human detrusor. A-278637 is more selective in suppressing bladder myogenic activity versus inhibition of vascular contractility relative to reference KCOs, and as noted in the accompanying article (Brune et al., 2002), suppresses unstable myogenic contractions in vivo with enhanced bladder selectivity. Such a profile presents an attractive basis for further investigations of selective KATP channel openers with potential for the treatment of overactive bladder.

**References**


Molecular characterization of human SUR2-containing K<sub>ATP</sub> channels. Gene 256: 261–270.


