British Journal of Pharmacology (2004) 143, 81–90
www.nature.com/bjp

In vitro and *in vivo* characterization of a novel naphthylamide ATP-sensitive K^+ channel opener, A-151892

*,[1]Murali Gopalakrishnan, [1]Steven A. Buckner, [1]Char-Chang Shieh, [1]Thomas Fey, [1]Adebola Fabiyi, [1]Kristi L. Whiteaker, [1]Rachel Davis-Taber, [1]Ivan Milicic, [1]Anthony V. Daza, [1]Victoria E.S. Scott, [2]Neil A. Castle, [2]David Printzenhoff, [2]Brecht London, [1]Sean C. Turner, [1]William A. Carroll, [1]James P. Sullivan, [1]Michael J. Coghlan & [1]Michael E. Brune

[1]Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA and [2]Icagen Inc., 4222 Emperor Boulevard, Suite 460, Durham, NC 27703, USA

1 Openers of ATP-sensitive K^+ channels are of interest in several therapeutic indications including overactive bladder and other lower urinary tract disorders. This study reports on the *in vitro* and *in vivo* characterization of a structurally novel naphthylamide *N*-[2-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-naphthalen-1-yl]-acetamide (A-151892), as an opener of the ATP-sensitive potassium channels.

2 A-151892 was found to be a potent and efficacious potassium channel opener (KCO) as assessed by glibenclamide-sensitive whole-cell current and fluorescence-based membrane potential responses ($-\log EC_{50} = 7.63$) in guinea-pig bladder smooth muscle cells.

3 Evidence for direct interaction with KCO binding sites was derived from displacement of binding of the 1,4-dihydropyridine opener [^{125}I]A-312110. A-151892 displaced [^{125}I]A-312110 binding to bladder membranes with a $-\log Ki$ value of 7.45, but lacked affinity against over 70 neurotransmitter receptor and ion channel binding sites.

4 In pig bladder strips, A-151892 suppressed phasic, carbachol-evoked and electrical field stimulus-evoked contractility in a glibenclamide-reversible manner with $-\log IC_{50}$ values of 8.07, 7.33 and 7.02 respectively, comparable to that of the potencies of the prototypical cyanoguanidine KCO, P1075. The potencies to suppress contractions in thoracic aorta ($-\log IC_{50} = 7.81$) and portal vein ($-\log IC_{50} = 7.98$) were not substantially different from those observed for suppression of phasic contractility of the bladder smooth muscle.

5 *In vivo*, A-151892 was found to potently suppress unstable bladder contractions in obstructed models of unstable contractions in both pigs and rats with $pED_{35\%}$ values of 8.05 and 7.43, respectively.

6 These results demonstrate that naphthylamide analogs exemplified by A-151892 are novel K_{ATP} channel openers and may serve as chemotypes to exploit additional analogs with potential for the treatment of overactive bladder and lower urinary tract symptoms.
British Journal of Pharmacology (2004) **143,** 81–90. doi:10.1038/sj.bjp.0705908

Keywords: ATP-sensitive K^+ channel; naphthylamides; A-151892; potassium channel opener; urinary bladder; overactive bladder; glibenclamide

Abbreviations: $DIBAC_4(3)$, bis-(1,3-dibutylbarbituric acid) trimethine oxonol; DMEM, Dulbecco's modified Eagle's medium; EC_{50}, molar concentration of test compound for 50% activation of response; FLIPR, fluorescent imaging plate reader; K_{ATP}, ATP-sensitive K^+ channel; KCO, potassium channel opener

Introduction

K^+ channels play fundamental roles in regulating smooth muscle excitability (Quayle *et al*., 1997). In urinary bladder smooth muscle, the roles of various K^+ channels including delayed rectifiers, large- and small-conductance Ca^{2+}-activated K^+ channels and ATP-sensitive K^+ (K_{ATP}) channels have been widely studied (Herrera *et al*., 2000; Petkov *et al*., 2001; Shieh *et al*., 2001; Buckner *et al*., 2002). K_{ATP} channels together with large- and small-conductance Ca^{2+}-activated K^+ channels are critical for the control of myogenic (smooth muscle) tone and excitability (Herrera *et al*., 2000; Buckner *et al*., 2002). Compounds that selectively open these K^+ channels decrease cellular (hyper)excitability, diminish smooth muscle cell activity and could suppress unstable bladder contractions, which may serve as one of the potential etiologies underlying bladder instability (Brading, 1997).

Of the K^+ channel classes, ATP-sensitive K^+ channels have received most attention over the years with openers identified from various structural classes including benzopyrans,

*Author for correspondence;
E-mail: murali.gopalakrishnan@abbott.com
Advance online publication: 9 August 2004

cyanoguanidines, tertiary carbinols and dihydropyridines, investigated as cardioprotectants and as relaxants of vascular and nonvascular smooth muscle (Coghlan *et al*., 2001; Gopalakrishnan & Shieh, 2004). More recent molecular and heterologous expression studies have revealed that the K_{ATP} channels are hetero-octameric complexes composed of four inward rectifying $K+$ channel subunits belonging to Kir6.1 or Kir6.2 that are responsible for ion permeation and four regulatory sulfonylurea receptor (SUR) subunits belonging to the ATP binding cassette superfamily that hosts binding sites for K_{ATP} channel openers including benzopyrans and cyanoguanidines (Babenko *et al*., 1998; Seino, 1999; Moreau *et al*., 2000). Studies from several laboratories have established the relaxant profile of K_{ATP} channel openers as assessed by effects on spontaneous phasic (myogenic), electrical or pharmacologically evoked contractile activity of bladder smooth muscle from a variety of species including humans (Brading, 1997; Buckner *et al*., 2002). Initial evidence for the utility of potassium channel openers (KCOs) for bladder overactivity in humans stemmed from preliminary pilot clinical studies of the benzopyran analogs such as cromakalim where modest effects in symptoms of urinary frequency and mean voided volume were observed (Nurse *et al*., 1991). Subsequently, compounds such as the tertiary carbinol ZD6169 and aryl squarate WAY 133537 with modestly improved *in vivo* selectivity for the urinary bladder relative to benzopyrans such as cromakalim and celikalim have emerged (Howe *et al*., 1995; Wojdan *et al*., 1999). More recently, compounds belonging to the 1.4-dihydropyridine class including A-312110 and A-278637 have been synthesized and characterized (Gopalakrishnan *et al*., 2002; Davis-Taber *et al*., 2003, Felsch *et al*., 2004). In particular, A-278637 was demonstrated to possess enhanced potencies for suppression of unstable bladder contractions vs mean arterial blood pressure effects in an obstructed pig model relative to earlier generation compounds (Brune *et al*., 2002).

Although preclinical efforts continue in the exploitation of openers of various potassium channel classes including K_{ATP}, calcium-activated and delayed rectifier K^+ channels such as KCNQ heteromers for the potential treatment of overactive bladder, optimally desirable efficacy in preclinical models and degree of separation from undesirable side effects remains unclear (Coghlan *et al*., 2001; Sellers *et al*., 2001). In the case of K_{ATP} channel openers, efforts continue to identify novel chemotypes that may differentially activate K_{ATP} channels in bladder, albeit with the challenging goal of overcoming associated vascular liabilities. In this study, we report the identification and characterization of a structurally novel K_{ATP} channel opener, A-151892 (*N*-[2-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-naphthalen-1-yl]-acetamide). A-151892 belongs to the naphthylamide series (Turner *et al*., 2003), a structure distinct from the chemotypes known thus far. The compound suppressed contractility of the bladder smooth muscle strips with nanomolar affinities comparable to the prototypical cyanoguanidine opener P1075 and showed *in vivo* efficacy in suppressing unstable bladder contractions. Our studies show that A-151892 may serve as a useful tool to characterize K_{ATP} channels and as a novel chemotype to exploit related analogs with improved bladder vs vascular selectivity.

Methods

Materials

Studies were carried out in accordance with guidelines outlined by the Animal Welfare Act, the Association for Assessment and Accreditation of Laboratory Animals (AAALAC) and the Institutional Animal Care and Use Committee of Abbott Laboratories. Bis-(1,3-dibutylbarbituric acid) trimethine oxonol ($DiBAC_4(3)$) was purchased from Molecular Probes (Eugene, OR, U.S.A.). Compounds were prepared in DMSO (Sigma) as a 5 or 10 mM stock, kept protected from light and serial dilutions were prepared in appropriate assay buffer just prior to use.

Membrane potential assays

Functional activity of K_{ATP} channels in guinea-pig bladder smooth muscle cells were assessed as previously described (Gopalakrishnan *et al*., 1999) by evaluating changes in the membrane potential using the bis-oxonol dye, $DiBAC_4(3)$, in a 96-well fluorescent imaging plate reader (FLIPR). Briefly, urinary bladders were removed from anesthetized male guinea-pigs (Hartley, Charles River, Wilmington, MA, U.S.A.) weighing 250–300 g and cells were isolated by enzymatic dissociation. The bladder was chopped into small sections and incubated in Dulbecco's phosphate-buffered saline (Life Technologies, Gaithersburg, MD, U.S.A.) containing 1 mg ml^{-1} collagenase (type VIII, Sigma, St Louis, MO, U.S.A.) and 0.2 mg ml^{-1} pronase (Calbiochem, La Jolla, CA, U.S.A.) with continuous stirring at 37°C in a cell incubator for 30 min. The mixture was then centrifuged at $1300 \times g$ for 5 min and the pellet was resuspended in Dulbecco's phosphate-buffered saline and recentrifuged to remove residual enzyme. The cell pellet was resuspended in 5 ml growth media (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U ml^{-1} penicillin, 100 U ml^{-1} streptomycin and 0.25 mg ml^{-1} amphotericin B) and dissociated further by pipetting repeatedly through a flame-polished Pasteur pipette and passing it through a polypropylene mesh membrane (Spectrum, Houston, TX, U.S.A.). The cell density was adjusted to 100,000 cells ml^{-1} by resuspension in growth media and plated in clear-bottomed black 96-well plates (Packard ViewPlate-96) for fluorescence studies at a density of 20,000 cells well^{-1}. Cells were maintained in a cell incubator with 90% air : 10% CO_2 for 5–7 days. Confluent cells, cultured in black clear-bottomed 96-well plates, were rinsed twice with 200 μl assay buffer (composition, mM: HEPES, 20; NaCl, 120; KCl, 2; $CaCl_2$, 2; $MgCl_2$, 1; glucose 5; pH 7.4 at 25°C) containing 5 μM $DiBAC_4(3)$ 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 and were initiated by the addition of various concentrations of the compound. Changes in $DiBAC_4(3)$ fluorescence 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 bladder smooth muscle cells as previously described (Shieh *et al*., 2001). Urinary bladders

were transferred directly into preoxygenated physiological saline solution containing the following (in mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 2; $MgCl_2$, 2; KH_2PO_4, 0.42; $NaHCO_3$, 4.17; HEPES, 10; glucose, 10 (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 the following (in mM): KCl, 107; $MgCl_2$, 1.2; $CaCl_2$, 1; EGTA, 10; HEPES, 5; ATP, 0.1 (pH 7.2 with KOH; total $K \sim 140$ mM). The bath solution contained the following (in mM): KCl, 60; NaCl, 80; $CaCl_2$, 2.6; $MgCl_2$, 1.2; HEPES, 5 (pH 7.4 with NaOH). Whole-cell currents were recorded at room temperature and were amplified using Axopatch-200B amplifier and low-pass filtered at 5 kHz (−3 dB, four pole Bessel filter) before digitization (Digidata 1200B) at a sampling rate of 10 kHz.

Bladder smooth muscle relaxation studies

Bladder strip relaxation studies were performed as previously described (Buckner *et al*., 2000). Briefly, female Landrace pigs (Wilson's Prairie View Farm, Burlington, WI, U.S.A.) weighing 9–25 kg were euthanized with an intraperitoneal injection of pentobarbital (150–200 mg kg^{-1}; Somlethol®, J.A. Webster Inc., Sterling, MA, U.S.A.). The entire urinary bladder was removed and placed in Krebs Ringer bicarbonate solution (composition, mM: NaCl, 120; $NaHCO_3$, 20; dextrose, 11; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.5; KH_2PO_4, 1.2 (equilibrated with 5% CO_2 : 95% O_2, pH 7.4 at 37°C)). The bladder was sectioned after discarding the top dome portion and the lower trigonal area. Approximately 3–5 × 20-mm strips were prepared from the remaining tissue adjacent to the trigonal area and cut in a circular fashion. The mucosal layer was removed and strips were mounted in 10 ml tissue baths maintained at 37°C with one end fixed to a stationary rod and the other to a Grass FT03 transducer at a basal preload of 1.0 g. Tissues were rinsed at 10 min intervals and allowed to equilibrate for at least 70 min.

Spontaneous myogenic phasic activity manifested as transient spikes that varied in frequency, duration and amplitude was observed in many tissues. Tissue strips were exposed to varying concentrations of the test agents for 15 min and changes in contractility were assessed. For electrical field stimulation studies, two parallel platinum electrodes were included and tissues were stimulated using a frequency of 0.05 Hz, 0.5 ms at 20 V. With carbachol-stimulated tissues, the protocol was noncumulative with rinse cycles between each concentration of test compound since the contractile response tended to wane over time. Tissues were pretreated with test compounds for 15 min, exposed to a fixed concentration of carbachol (300 nM, which corresponds to approximately an EC_{75} concentration of carbachol) and changes in tension assessed. The tissue was then rinsed for 15 min, and the cycle repeated with another concentration of test compound. Glibenclamide (10 μM) was added at the conclusion of each concentration response curve to assess reversibility of effects.

Vascular tissue relaxation studies

The entire thoracic aorta from male Sprague–Dawley rats (200–350 g) was removed and immediately placed into Krebs Ringer bicarbonate solution. The aorta was cleaned of extraneous tissue, endothelium removed, cut into 3–4 mm rings and mounted in 10 ml isolated tissue baths at 37°C. One end was fixed to a stationary glass rod and the other to a Grass FT03 transducer at a basal preload of 1.0 g. Tissues were rinsed every 10 min for a total of 45–60 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.

The effects of compounds on myogenic activity of the rat portal vein were assessed as previously described (Gopalakrishnan *et al*., 2002). The portal vein was cleansed of extraneous tissue and mounted in a 10 ml isolated tissue bath at 37°C. The portal vein contracted spontaneously with transient spikes that varied in frequency, duration and amplitude. Cumulative concentration response curves were obtained for the test compound with a 15 min exposure time after each addition. Data were recorded on a PowerLab/800 data acquisition system and analyzed as the area under the contractile curve (AUC) for 15 min intervals.

[^{125}I]A-312110 binding

Binding of the KCO ligand [^{125}I]A-312110 ($K_D \sim 5$ nM) was carried out as described previously (Davis-Taber *et al*., 2003). Briefly, urinary membranes were prepared from male guinea-pigs (300–400 g; Charles River, Wilmington, MA, U.S.A.) by homogenization using a Tekmar polytron followed by centrifugation initially at 500 × *g* for 10 min and the supernatant subsequently at 40,000 × *g* for 30 min at 4°C. The membrane pellet was resuspended in ice-cold assay buffer (composition: 139 mM NaCl, 5 mM KCl, 25 mM $MgCl_2$, 1.25 mM $CaCl_2$ and 20 mM HEPES; pH 7.4) for radioligand binding studies. Protein concentrations were determined using bovine serum albumin as a standard (Lowry *et al*., 1951).

Membranes (300 μg $tube^{-1}$) were incubated in a total assay volume of 0.25 ml in assay buffer containing an ATP regeneration system (20 mM creatine phosphate, 50 U creatine phosphokinase and 1 mM Na_2ATP) at 37°C for 90 min. In competition experiments, membranes were incubated with varying concentrations of test compounds and with approximately 1 nM of radioligand. Specific radioligand binding was defined by subtracting the nonspecific binding defined by the inclusion of 10 μM unlabeled A-312110 from total binding. Binding was terminated by rapid vacuum filtration through GF/B filters. Filters were washed twice with ice-cold 50 mM Tris-HCl (pH 7.2) and radioactivity bound to the filters was assessed by γ-counting at an efficiency of 80% (EG & G Wallac, Gaithersburg, MA, U.S.A.).

Other selectivity studies

A-151892 was evaluated in a binding screen that contained representatives of most G-protein-coupled receptors, and a set of ligand and voltage-gated ion channel binding sites at CEREP (Receptor binding and Enzyme Profile; CEREP, Celle l'Evescault, France).

Cystometry in obstructed pigs

The method for producing partial outlet obstruction in pigs has been described elsewhere in more detail (Fey *et al*., 2003). Briefly, female Landrace/Yorkshire pigs (~12 weeks old; 14–20 kg) were obstructed with a 7.5-mm silver omega ring placed around the proximal urethra using an inguinal approach. Ring placement was confirmed at necropsy in all animals. At 17–20 weeks after placement of the ring, the pigs were instrumented with telemetry transducer-transmitters (Data Sciences, St Paul, MN, U.S.A.) for the measurement of carotid arterial pressure (unit 1: TA11PA-C40) and intravesical/abdominal pressures (unit 2: TL11M3-D70-PCP). A port catheter (TI-9, Access Technologies) was placed subcutaneously in the side of the abdomen and its distal catheter was secured in the bladder lumen. Animals were treated with amoxicillin and buprenorphine for 3–5 days postsurgery and allowed to recover for 10–14 days before testing. For urodynamic testing, pigs were anesthetized with a mixture of telazol (4.4 mg kg^{-1} i.m.) and xylazine (2.2 mg kg^{-1} i.m.), intubated and maintained on isoflurane/oxygen in the supine position. Anesthesia level and bladder volume (*via* the port catheter) were adjusted to establish a regular unstable contraction pattern and stable mean arterial pressure (MAP). After a 30 min baseline period, up to two increasing doses of A-151982 were administered intravenously at 30 min intervals. Each dose was administered over a 5 min period.

Cystometry in obstructed rats

Female Sprague–Dawley rats (190–210 g) were anesthetized with halothane and the proximal urethra was ligated using a monofilament ligature as previously described from our laboratory (Fabiyi *et al*., 2003). At 4 weeks after obstruction, rats were anesthetized with urethane and the femoral artery and vein were catheterized to measure arterial pressure and to administer test compounds, respectively. Intravesicular pressure was measured using a polyethylene catheter inserted into the apex of the bladder dome. Saline was infused at the rate of 0.1 ml min^{-1} until a volume was reached that was sufficient to trigger spontaneous nonvoiding contractions but below the threshold for voiding. These spontaneous contractions were allowed to stabilize, after which changes in bladder pressure and MAP were monitored simultaneously for 20 min before and after cumulative intravenous doses of test compound or vehicle.

Data analysis

The concentration dependence of maximal steady-state changes in fluorescence or changes in tension responses was fitted by nonlinear regression analysis (GraphPad Prism, San Diego, CA, U.S.A.) to obtain EC_{50} or IC_{50} values as appropriate. Spontaneous phasic activity of bladder strips was analyzed for changes in the area under the curve (AUC) of the contractile response during a 15 min interval. For carbachol-stimulated responses, values were expressed as percentage of the precontraction responses produced by carbachol. In electrical field-stimulated tissues, the concentration-dependent reduction in the peak amplitude (measured in grams) was used for calculating the EC_{50} values. In *in vivo* studies, data were acquired and analyzed using the Life Science Suite/Po-ne-mah Physiology Platform (Gould Instrument Systems, Valley View, OH, U.S.A.). Bladder contraction amplitude, frequency, duration and area under the bladder pressure curve (AUC) were determined using the Ponemah CYS analysis module. Data from obstructed pigs were averaged over the entire 30 min postdosing period and expressed as percent change from baseline values. In obstructed rats, an increase in bladder pressure over 1 cm H_2O is considered a contraction and data were averaged over the last 10 min of each period after dosing where bladder effects were maximal. Estimated intravenous doses of each compound required to reduce unstable contraction AUC by 35% values were estimated from the dose–response graphs using GraphPad Prism (GraphPad Software Inc., San Diego, CA, U.S.A.). Data are expressed as mean ± s.e.m. When comparing group means, a P-value <0.05 was considered statistically significant.

Results

$DiBAC_4(3)$ fluorescence responses

A-151892 evoked concentration-dependent decreases in membrane potential as assessed by $DiBAC_4(3)$ fluorescence changes in guinea-pig bladder smooth muscle cells with a $-\log EC_{50}$ value of 7.63 ± 0.10 ($EC_{50} = 20.3$ nM; slope $= 1.14 \pm 0.08$; $n = 6$). When normalized to a prototypical K_{ATP} channel opener, P1075 (for structures, see Figure 1), the maximal efficacy, $103 \pm 3.8\%$, attained at 1 μM was comparable to that evoked by P1075. As shown in Figure 2, the responses were attenuated by subsequent addition of glibenclamide (5 μM).

Whole-cell patch clamp measurements

Direct interaction of A-151892 with K_{ATP} channels was studied by whole-cell patch clamp in guinea-pig bladder smooth muscle cells. As shown in Figure 3, upon addition of A-151892 (10 μM), increases in membrane currents were observed under conditions where the cell was 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. In the presence of A-151892 (10 μM), the membrane current increased from control values of -11.5 ± 3.3 to -34.5 ± 3.4 pA. Addition of P1075 (10 μM) did not further enhance K_{ATP} currents. A-151892-evoked current was sensitive to the reversal by 5 μM glibenclamide, suggesting that the compound increases the membrane current by opening K_{ATP} channels in smooth muscle cells. As shown in Figure 2b, A-151892 also hyperpolarized membrane potential in bladder smooth muscle cells, measured under current clamp conditions, in a glibenclamide-sensitive manner.

Displacement of KCO binding

To assess the interaction of A-152892 with K_{ATP} channel opener binding sites, displacement of the dihydropyridine KCO ligand, [^{125}I]A-312110, to guinea-pig bladder smooth muscle membranes was carried out. In this preparation, A-151892 displaced binding of [^{125}I]A-312110 with a $-\log Ki$ value of 7.34 ± 0.05 ($Ki = 39.50$ nM) and with pseudo-Hill coefficient close to unity ($nH = 0.75 \pm 0.11$; $n = 4$; Figure 4).

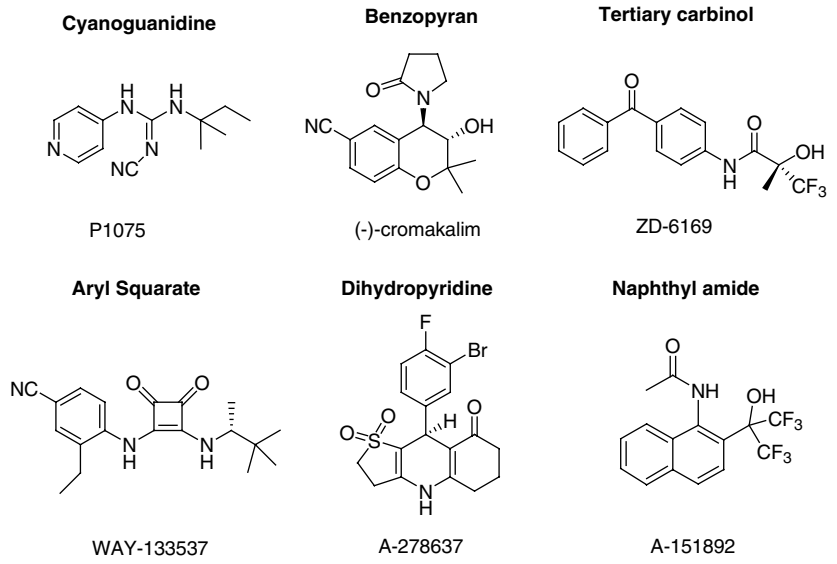

Figure 1 Structural diversity of K_{ATP} channel openers. Shown are chemical structures of the naphthylamide A-151892 and compounds belonging to diverse classes including cyanoguanidine (P1075), benzopyran (cromakalim), carbinol (ZD6169), aryl squarate (WAY-133537) and dihydropyridine (A-278637).

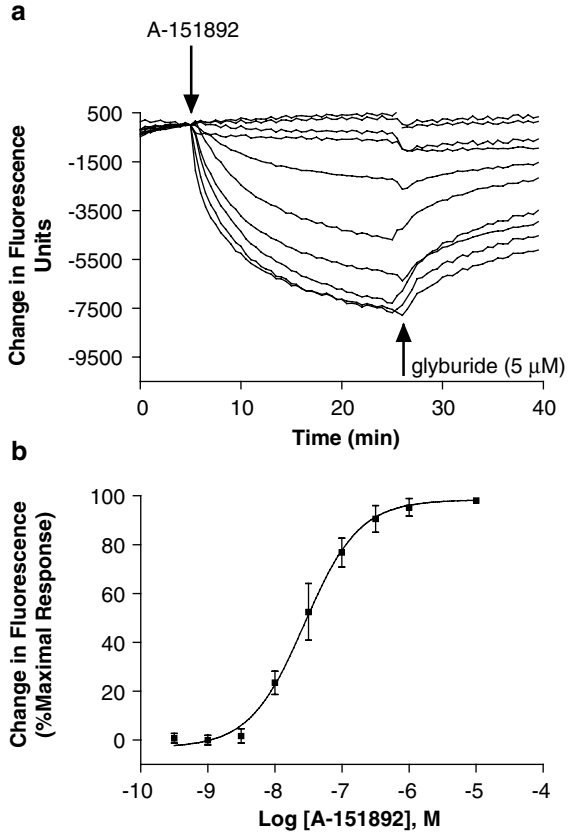

Figure 2 Effect of A-151892 on membrane potential responses in guinea-pig bladder smooth muscle cells assessed by the bis-oxonol dye $DiBAC_4(3)$. (a) Representative experiment showing typical changes in fluorescence upon addition (indicated by down arrows) of varying concentrations of A-151892 (3, 10, 30, 100, 300, 1000 and 3000 nM) and its attenuation (indicated by up arrows) by the addition of glibenclamide (5 μM). (b) Concentration response curves for fluorescence changes normalized to the maximal response. Depicted are mean $\pm$ s.e.m. of five separate determinations each carried out in duplicate.

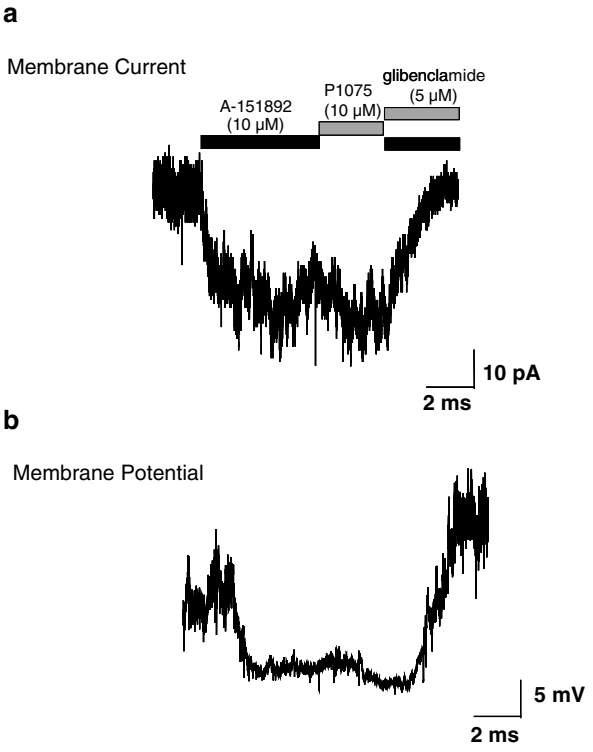

Figure 3 Effect of A-151892 on K_{ATP} currents and membrane potential in guinea-pig bladder smooth muscle cells. (a) Application of 10 μM A-151892 evoked an increase in inward whole-cell current in guinea-pig bladder smooth muscle cells that was not substantially increased with 10 μM P1075 and was attenuated upon the addition of 5 μM glibenclamide. Cells were voltage-clamped at -80 mV and changes in membrane currents were measured in bath solution containing 60 mM K^+ with pipette solution containing 140 mM K^+ and 0.1 mM ATP. (b) Membrane potential effects. Shown is a representative experiment where 10 μM A-151892 hyper-polarized membrane potential from the baseline value of -3 to -12.8 mV, which was reversed to control values in the presence of 5 μM glibenclamide.

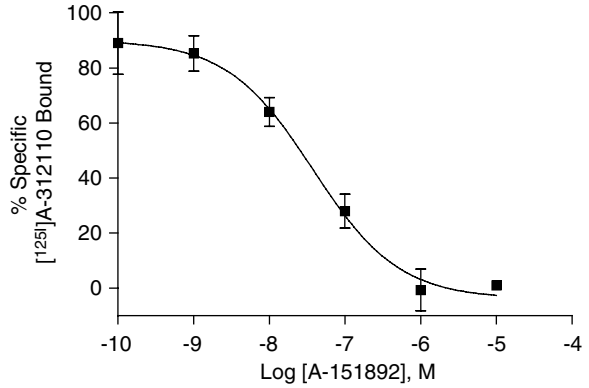

Figure 4 Displacement of [^{125}I]A-312110 binding by A-151892 in guinea-pig bladder smooth muscle membranes. Membranes were incubated with varying concentrations of A-151892 (indicated along the *X*-axis) with 1 nM [^{125}I]A-312110 as described in Methods. The Ki value for displacement of [^{125}I]A-312110 binding was 39.5 nM.

Under similar conditions, P1075 inhibited binding with a Ki value of 10.5 nM (nH = 0.95 ± 0.07). A-151892, when evaluated in a binding screen that contained representatives of most G-protein-coupled receptors, as well as ligand and voltage-gated ion channel binding sites (Receptor binding and Enzyme Profile; CEREP Inc., Redmond, WA, U.S.A.) did not show significant displacement of binding including [^{3}H]glibenclamide that binds with high affinity to SUR1.

Bladder strip relaxation

A-151892 evoked a concentration-dependent suppression of spontaneous phasic contractility of pig bladder smooth muscle strips. Addition of 5 μM glibenclamide restored contractile activity (Figure 5). The $-\log IC_{50}$ value of A-151892 calculated by measuring changes in the area under the force integral curve (AUC) was 8.07 ± 0.15 ($n = 6$). Under similar conditions, the $-\log IC_{50}$ value for P1075 was 7.58, which is similar to the value (7.6) previously reported (Buckner *et al*., 2002). Contractions evoked by low-frequency electrical stimulation (0.05 Hz, 0.5 ms, 20 V) that reflects presynaptic release principally of both acetylcholine and ATP were also inhibited by A-151892 with $-\log IC_{50} = 7.02 \pm 0.13$ and a maximal inhibition of 87 ± 6% ($n = 6$). Likewise, contractions evoked by carbachol were suppressed in a concentration-dependent manner by A-151892 with a $-\log IC_{50}$ value of 7.33 ± 0.1 and with a maximal inhibition of 100%. In every case, the inhibition of contractions was reversed by the addition of glibenclamide (5 μM).

Vascular tissue relaxation

To examine interactions with vascular K_{ATP} channels and to contrast this profile with effects on the bladder, the interaction of A-151892 was examined in rat thoracic aorta and portal vein. As depicted in Figure 6, the compound completely suppressed contractions evoked by 25 mM K^+ in aorta ($-\log IC_{50} = 7.81 \pm 0.09$; $n = 3$), whereas those evoked by 80 mM K^+ depolarization were relatively insensitive ($-\log IC_{50} \sim 4$) indicating that the relaxation is mediated *via* K^+ channel-dependent mechanisms. Myogenic contractions in rat portal vein were also suppressed in a concentration-

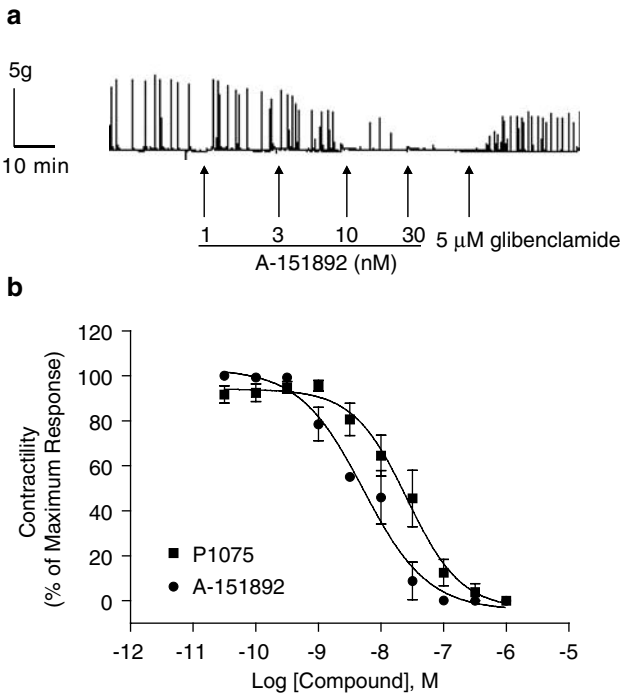

Figure 5 Inhibition of spontaneous phasic activity of isolated pig bladder strips by A-151892. (a) Representative experiment showing baseline spontaneous phasic activity and its suppression following the addition of increasing concentrations of A-151892. Glibenclamide (5 μM) reversed these effects. (b) Concentration response curves derived from the area under the curve (AUC) for the suppression of myogenic contractions by A-151892 and the cyanoguandine analog P1075. Depicted are mean ± s.e.m. of six separate determinations, each carried out using duplicate tissue strips. Mean potency values are summarized in Table 1.

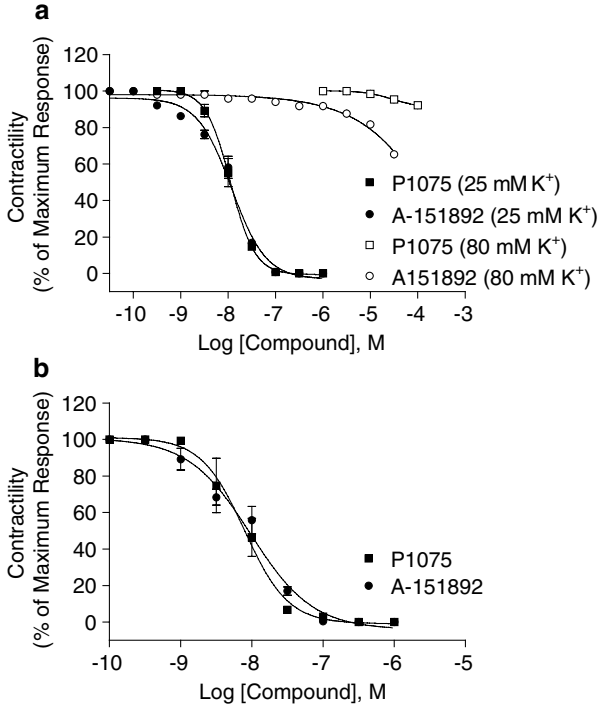

Figure 6 Inhibition of contractility of thoracic aorta and portal vein in the rat. (a) Inhibition of contractility evoked by 25 mM K^+ and 80 mM K^+ by A-151892 and P1075 in thoracic aorta strips. (b) Inhibition of spontaneous myogenic contractions of the portal vein by A-151892 and P1075.

dependent manner by A-151892 ($-\log IC_{50} = 7.98 \pm 0.10$; $n = 4$), with potencies comparable to that of P1075 (see Table 1).

Effect of A-151892 on unstable bladder contractions in obstructed pigs

A-151892 caused dose-dependent inhibition of unstable contractions in anesthetized obstructed pigs. Mean reductions in total contraction AUC of 9.6 ± 13, 48.4 ± 17.6 and $96 \pm 1.2\%$ were observed after doses of 3, 10 and 30 nmol kg^{-1} i.v., respectively. As depicted in Figure 7, the observed decrease in total area was largely derived by decreases in the contraction frequency. As previously reported in this model with other KCOs (Brune *et al*., 2002), contraction duration did not change despite marked decreases in other contraction parameters. For comparative purposes with other KCOs from our laboratory (Brune *et al*., 2002; Fey *et al*., 2003), the dose of compound that suppressed contraction AUC by 35% (AUC $pED_{35\%}$) was chosen. The estimated $-\log$ mol kg^{-1} dose of A-151892 to inhibit contraction AUC by 35% (AUC $pED_{35\%}$) was 8.05 (corresponding to 8.9 nmol kg^{-1} dose). Under similar conditions, the $pED_{35\%}$ value for P1075 is 8.67 (corresponding to 2.1 nmol kg^{-1} dose, data not shown).

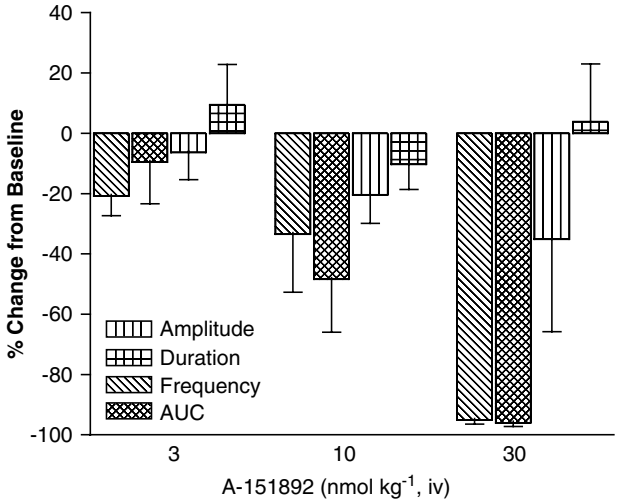

Figure 7 Suppression of unstable bladder contractions in the obstructed pig model by A-151892. Mean changes in amplitude, duration, frequency and AUC of bladder contractions after A-151892. Values depicted are mean $\pm$ s.e.m. of changes observed in three separate animals.

Effect of A-151892 on unstable bladder contractions in obstructed rats

A-151892 also evoked inhibition of unstable contractions in anesthetized obstructed rats. As shown in Figure 8a, A-151892 (100 nmol kg^{-1}) evoked maximal changes in both AUC and MAP at 10–20 min postdosing. The return of the effects to near basal levels at 30 min and beyond may be attributable to the relatively short half-life of the compound. For dose–response evaluations, data were collected at 10–20 min postdosing. Mean reductions in total contraction AUC of 19.8 ± 4, 28.8 ± 8.5 and $75.3 \pm 8.3\%$ were observed after doses of 10, 30 and 100 nmol kg^{-1} i.v., respectively ($n = 6$). The estimated $-\log$ mol kg^{-1} dose of A-151892 to inhibit contraction AUC by 35% (AUC $pED_{35\%}$) was 7.44 (corresponding to 36 nmol kg^{-1} i.v.).

In vivo *selectivity for bladder vs hypotensive effects*

The effects on MAP were assessed concurrently with bladder effects in both pig and rat models. In obstructed pigs, A-151892 evoked dose-dependent reductions in MAP with 4.6 ± 2.1, 22.2 ± 1.8 and $48.7 \pm 4.2\%$ after doses of 3, 10 and 30 nmol kg^{-1} i.v., respectively. The MAP $ED_{10\%}$ was 4.6 nmol kg^{-1}. At these doses tested, no compensatory increases in heart rate were noted (-15 ± 3.9, -13 ± 5.8 and $-12.2 \pm 3.9\%$ at 3, 10 and 30 nmol kg^{-1}), which is consistent with effects of KCOs in this model under isoflurane anesthesia conditions (Fey *et al*., 2003). The estimated bladder selectivity, derived as the ratio of MAP $ED_{10\%}$ vs $ED_{35\%}$ is 0.51, which is in the range previously reported for ZD6169 (0.6) and WAY-133537 (0.5), and some six-fold lower than the dihydropyridine A-278637 in this model (ratio of 3; Brune *et al*., 2002). In the obstructed rat model, A-151892 also evoked dose-related decreases in MAP with 0.3 ± 1.5, 8.7 ± 2.6 and $28.9 \pm 3.5\%$ after doses of 10, 30 and 100 nmol kg^{-1} i.v., respectively, and with an estimated MAP $ED_{10\%}$ value of about 30 nmol kg^{-1}.

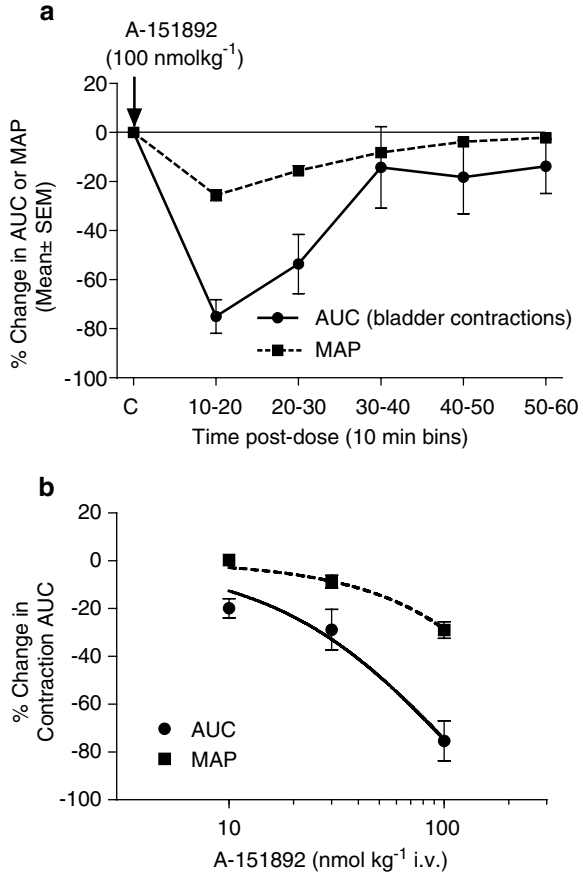

Figure 8 Inhibition of bladder contractions in the obstructed rat model by A-151892. (a) Changes in AUC of bladder contractions or MAP by A-151892 (100 nmol kg^{-1} i.v.) at various time intervals. Data points represent average changes over a 10 min duration. (b) Dose-dependent effects on AUC of bladder contractions and mean arterial blood pressure. Data represent mean $\pm$ s.e.m. of 5–6 animals.

Again, the estimated bladder selectivity ratio of A-151892 is 1.7, in the range previously observed with ZD6169 (1.2) and WAY-133537 (1.1) in this model (Fabiyi *et al*., 2003).

Discussion

Overactive bladder in humans is characterized by the symptoms of increased urinary frequency, urgency and/or incontinent episodes. Overactivity associated with the urodynamic finding of involuntary bladder contractions is referred to as bladder instability. Existing pharmacological therapies for overactive bladder include antimuscarinics, which, although widely prescribed, are limited by side effects such as dry mouth. In the light of the fundamental role(s) of K^+ channels in governing bladder smooth muscle excitability, openers of these channels serve as an attractive means of dampening smooth muscle excitability, provided such agents are able to selectively activate channels present in bladder smooth muscle vs other smooth muscle particularly in vascular tissues. In the search of chemotypes that activate K_{ATP} channels that may be further exploited, we have identified a structurally novel and potent naphthylamide analog, A-151892 (Turner *et al*., 2003). A-151892 evoked concentration-dependent decreases in membrane potential responses and suppressed spontaneous phasic activity of bladder smooth muscle in a glibenclamide-reversible manner. A-151892 also suppressed contractions evoked by muscarinic receptor activation and by electrical field stimulation of bladder smooth muscle strips. Additional evidence for interaction with native K_{ATP} channels was confirmed by whole-cell patch clamp studies, where the compound was efficacious in activating glibenclamide-sensitive currents in isolated bladder smooth muscle cells and by displacement of [^{125}I]A-312110 binding to bladder smooth muscle membranes. Comparison of pharmacological profile of A-151892 across membrane potential, radioligand binding and tissue relaxation assays showed that the compound exhibited potencies and efficacies quite comparable to the prototypical cyanoguanidine KCO P1075 (Table 1). *In vivo*, A-151892 effectively suppressed unstable bladder contractions in both pig and rat models of bladder outlet obstruction in a dose-dependent manner.

Membrane potential studies in guinea-pig bladder smooth muscle cells showed that A-151892 evoked concentration-dependent decreases in $DiBAC_4(3)$ fluorescence. Likewise, A-151892 evoked concentration-dependent suppression of spontaneous phasic activity of pig bladder smooth muscle strips in a glibenclamide-sensitive manner. Comparison of profiles of A-151892 with the prototypical cyanoguanidine opener by fluorescence and tissue relaxation assays showed that A-151892 is about three- to four-fold more potent than P1075, but equiefficacious by these measures. Whole-cell patch clamp studies in bladder smooth muscle cells showed that A-151892 is as efficacious as P1075, albeit at a single maximal concentration, in activating membrane currents or hyperpolarizing the membrane potential. A-151892 also displaced binding of a dihydropyridine K_{ATP} channel opener radioligand [^{125}I]A-312110 to guinea-pig bladder membranes with a Ki value of 39 nM. Previous molecular analysis have revealed the presence of predominantly SUR2B as a component of K_{ATP} channels in bladder smooth muscle from guinea-pig, pig and human (Buckner *et al*., 2000; Davis-Taber *et al*., 2000). Collectively, these observations provides direct evidence that membrane hyperpolarization assessed by $DiBAC_4(3)$ fluorescence and relaxation of bladder smooth muscle strips are mediated *via* interaction with the smooth muscle K_{ATP} channels. The lack of significant interaction with high-affinity [^{3}H]glibenclamide binding sites demonstrates selectivity of A-151892 to K_{ATP} channels derived from SUR2-containing subunits. Additionally, lack of significant interaction in radioligand binding and/or functional patch clamp across representatives of most G-protein-coupled receptors as well ligand- and voltage-gated channels further demonstrates the *in vitro* selectivity of the compound.

A-151892 was potent in suppressing phasic contractility of bladder smooth muscle ($IC_{50} = 10$ nM). In contrast, A-151892 was about 10-fold less potent in suppressing electrically evoked contractions, which is consistent with earlier observations that, in general, K_{ATP} channel openers are about 15-fold more potent in suppressing spontaneous phasic activity than those evoked by electrical field stimulation (Buckner *et al*., 2002). The potencies to suppress contractions in thoracic aorta ($-\log IC_{50} = 7.81$) and portal vein ($-\log IC_{50} = 7.98$) were not substantially different from those observed for suppression of phasic contractility of the bladder smooth muscle, suggesting

Table 1 *In vitro* characterization of A-151892: comparison to P1075

	Assay	*A-151892*	*P1075*
Membrane potential (FLIPR)	Guinea-pig bladder smooth muscle cells $-\log EC_{50}$	7.63±0.10 ($n=6$)	7.27[a]
[^{125}I]A-312110 binding	Guinea-pig bladder membranes $-\log Ki$	7.34±0.05 ($n=4$)	7.97[b]
Bladder contractility (pig)	Spontaneous phasic activity $-\log IC_{50}$	8.07± 0.15 (100%; $n=4$)	7.60 (100%)[c]
	Electrical field-evoked $-\log IC_{50}$	7.02±0.13 (87±6%; $n=6$)	7.24 (100%)[d]
Vascular contractility thoracic aorta (rat)	20 mM K^+-evoked $-\log IC_{50}$	7.81±0.09 (100%; $n=3$	7.81±0.15 (97± 3%; $n=7$)
	80 mM K^+-evoked $-\log IC_{50}$	4.33±0.01; $n=3$) (69±1.5%)	4.18 ±0.13 12±3 % ($n=4$)
Portal vein (rat)	Portal vein $-\log IC_{50}$	7.98±0.10 (100%; $n=4$)	8.18 ±0.23 (100%; $n=3$)

Values in parentheses represent maximal inhibition values and '*n*' represents the number of determinations, each performed in duplicate tissue strips or assay samples. Comparative data with P1075 are taken from previously published data from our laboratory conducted under similar assay conditions.
[a]Gopalakrishnan *et al*. (1999).
[b]Davis-Taber *et al*. (2003).
[c]Buckner *et al*. (2002).
[d]Buckner *et al*. (2000).

no *in vitro* tissue selectivity for A-151892. Interestingly, compared to P0175 that was equipotent in suppressing phasic contractility, low K^+-evoked contractions of aorta and myogenic activity of the portal vein, A-151982 showed about four-fold greater potency to suppress phasic contractility of bladder strips compared to the potencies in vascular tissues.

The *in vivo* efficacy of A-151892 was examined in both obstructed swine and rat models by evaluating effects on unstable bladder contractions and MAP as described previously (Brune *et al*., 2002; Fabiyi *et al*., 2003; Fey *et al*., 2003). Comparison of the $-\log ED_{35\%}$ values to lower AUC of unstable contractions in the pig model showed that A-151892 is substantially more potent than ZD6169 and WAY-133537, but comparable to the potency of the dihydropyridine A-278637 (8.38; Brune *et al*., 2002) and to the cyanoguanidine P1075 (8.67, this study). Further, the *in vitro* and *in vivo* potencies of A-151892 correlate well, which is consistent with the previous observations (Brune *et al*., 2002) where a good correlation to inhibit myogenic contractions in isolated pig bladder smooth muscle strips vs suppression of unstable bladder contractions in the obstructed pig model was observed.

Although quite generally KCOs cause dose-dependent reductions in MAP, apparent differences as to the extent of hypotension at a given level of bladder contraction inhibition have been previously noted, suggesting differences in bladder selectivity for certain compounds. For example, previous studies using the obstructed pig model have shown that (−)-cromakalim at doses that suppress unstable contractions also substantially decrease MAP, consistent with the lack of selectivity for the first-generation KCOs (Fey *et al*., 2003). The dihydropyridine analog A-278637 has been shown to be five- to six-fold more bladder selective (comparing doses required to decrease contraction AUC by 35% (AUC $ED_{35\%}$) vs decrease MAP by 10%, values that enable comparisons across multiple compounds) compared to KCOs such as ZD6169 and WAY-133537 in the obstructed pig model. By the same comparison, the relative bladder selectivity of A-151892 was found to be similar to that previously reported with ZD6169 and WAY-155537, suggesting that this compound does not exhibit superior bladder selectivity. Similar conclusions were derived in the obstructed rat model, where A-151892 effectively suppressed contractions in a dose-dependent manner, but again with bladder vs vascular selectivity comparable to that of ZD6169 and WAY-133537.

Targeting specific K^+ channels expressed in bladder smooth muscle cells and/or afferent nerve fibers that control bladder smooth muscle function, or selective partitioning or delivery of drugs into the bladder are some of the approaches that, in principle, offer opportunities for selective modulation of ion channel function. Recent molecular cloning and expression studies have revealed considerable diversity of various K^+ channels including calcium-activated K^+ channels, KCNQ-related K^+ channels (M channels) and ATP-sensitive K^+ channels, thereby offering opportunities for selectively targeting subunits present that modulate bladder function. For example, retigabine that opens KCNQ heteromers has been shown to hyperpolarize bladder smooth muscle and inhibit cystometric contractions in rats with experimental bladder instability (Sheldon *et al*., 2002). More recently, KW-7158, an opener of transient K^+ channels, was shown to depress vesicovascular reflexes as well as xylene-induced bladder hyperactivity, suggesting that this drug affects reflex bladder activity at least in part by depressing afferent pathways (Lu *et al*., 2002; Sculptoreanu *et al*., 2004). With K_{ATP} channels, splice variants of the SUR2 gene – SUR2A vs SUR2B – in cardiac and smooth muscle, respectively, exhibit pharmacological differences. Additional splice variants of both SUR1 and SUR2 genes at the level of exon 17 have been reported (Chutkow *et al*., 1999; Sakura *et al*., 1999; Davis-Taber *et al*., 2000; Hambrock *et al*., 2002). For example, a splice variant of SUR1 that lacks exon 17, in combination with Kir6.2, has been shown to form functional K_{ATP} channels with glibenclamide binding and ATP sensitivity similar to the wild-type SUR1 subunit (Hambrock *et al*., 2002). With the SUR2 gene, in addition to the C-terminal variants that generate SUR2A and SUR2B, splicing at the level of exon 17, a region close to the nucleotide binding fold NBF-1, has been reported with channels showing modest reductions in ATP sensitivity (Chutkow *et al*., 1999). Molecular analyses of K_{ATP} channels in bladder smooth muscle cells have shown that SUR2BΔ17 variant is the predominant isoform, which interestingly differs from other smooth muscle tissues including the vasculature where SUR2B is the predominant species. Our previous pharmacological analysis showed that K_{ATP} openers from diverse chemically distinct series including the naphthylamide A-151892 were modestly (~4-fold) more potent in opening K_{ATP} channels in cell lines expressing the SUR2BΔ17/Kir6.2 ($-\log EC_{50} = 7.7$) compared to the SURB/Kir6.2 ($-\log EC_{50} = 7.3$) combination (Scott *et al*., 2004). Although openers that more substantially differentiate among the two splice forms have not been reported thus far, an agent that preferentially modulates the SUR2BΔ17 splice variant should, in principle, show enhanced selectivity toward suppressing spontaneous myogenic activity of bladder smooth muscle.

In summary, our present studies show that the naphthylamide analog A-151892 is a novel and potent opener that activates bladder K_{ATP} channels, relaxes bladder smooth muscle and suppresses unstable contractions in obstructed bladders *in vivo*. A-151892 may serve as a tool to study K_{ATP} channel interactions and serve as an attractive chemotype to further optimize analogs with enhanced bladder vs vascular selectivity with potential for treatment of lower urinary tract disorders including overactive bladder.

References

BABENKO, A.P., AGUILAR-BRYAN, L. & BRYAN, J. (1998). A view of SUR/KIR6.X, KATP channels. *Annu. Rev. Physiol.,* **60,** 667–687.

BRADING, A.F. (1997). A myogenic basis for the overactive bladder. *Urology,* **50,** 57–67 discussion 68–73.

BRUNE, M.E., FEY, T.A., BRIONI, J.D., SULLIVAN, J.P., WILLIAMS, M., CARROLL, W.A., COGHLAN, M.J. & GOPALAKRISHNAN, M. (2002). (-)-(9*S*)-9-(3-bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-*b*]quinolin-8(4*H*)-one 1,1-dioxide (A-278637): a novel ATP-sensitive potassium channel opener efficacious in suppressing urinary bladder contractions II. *In vivo* characterization. *J. Pharmacol. Exp. Ther.,* **303,** 387–394.

BUCKNER, S., MILICIC, I., DAZA, A., COGHLAN, M. & GOPALAKRISHNAN, M. (2002). Spontaneous phasic contractions of the pig urinary bladder smooth muscle: characteristics and sensitivity to potassium channel modulators. *Br. J. Pharmacol.,* **135,** 639–648.

BUCKNER, S.A., MILICIC, I., DAZA, A., DAVIS-TABER, R., SCOTT, V.E.S., SULLIVAN, J.P. & BRIONI, J.D. (2000). Pharmacological and molecular analysis of ATP-sensitive K+ channels in the pig and human detrusor. *Eur. J. Pharmacol.,* **400,** 287–295.

CHUTKOW, W.A., MAKIELSKI, J.C., NELSON, D.J., BURANT, C.F. & FAN, Z. (1999). Alternative splicing of sur2 exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. *J. Biol. Chem.,* **274,** 13656–13665.

COGHLAN, M.J., CARROLL, W.A. & GOPALAKRISHNAN, M. (2001). Recent developments in the biology and medicinal chemistry of potassium channel modulators: update from a decade of progress. *J. Med. Chem.,* **44,** 1627–1653.

DAVIS-TABER, R., CHOI, W., FENG, J., HOOGENBOOM, L., MCNALLY, T., KROEGER, P., SHIEH, C.C., SIMMER, R., BRIONI, J.D., SULLIVAN, J.P., GOPALAKRISHNAN, M. & SCOTT, V.E. (2000). Molecular characterization of human SUR2-containing KATP channels. *Gene,* **256,** 261–270.

DAVIS-TABER, R., MOLINARI, E., ALTENBACH, R.J., WHITEAKER, K., SHIEH, C.C., ROTERT, G., BUCKNER, S.A., MALYSZ, J., MILICIC, I., MCDERMOTT, J.S., GINTANT, G., COGHLAN, M.J., CARROLL, W.A., SCOTT, V.E.S. & GOPALAKRISHNAN, M. (2003). [^{125}I]A-312110, A novel high affinity 1,4-dihydropyridine ATP-sensitive K+ channel opener: characterization and pharmacology of binding. *Mol. Pharmacol.,* **64,** 143–153.

FABIYI, A., GOPALAKRISHNAN, M., LYNCH, J.J., BRIONI, J.D., COGHLAN, M.J. & BRUNE, M.E. (2003). *In vivo* evaluation of the potency and bladder-vascular selectivity of the ATP-sensitive potassium channel openers, (-)-cromakalim, ZD6169 and WAY-133537 in rats. *BJU Int,* **91,** 284–290.

FELSCH, H., LANGE, U., HAMBROCK, A., LOFFLER-WALZ, C., RUSS, U., CARROLL, W.A., GOPALAKRISHNAN, M. & QUAST, U. (2004). Interaction of a novel dihydropyridine K+ channel opener, A-312110, with recombinant sulphonylurea receptors and K_{ATP} channels: comparison with the cyanoguanidine P1075. *Br. J. Pharmacol.,* **141,** 1098–1105.

FEY, T.A., GOPALAKRISHNAN, M., STRAKE, J.G., KING, L.L., BRIONI, J.D., SULLIVAN, J.P., COGHLAN, M.J. & BRUNE, M.E. (2003). Effects of ATP-sensitive K^+ channel openers and tolterodine on involuntary bladder contractions in a pig model of partial outlet obstruction. *Neurourol. Urodyn.,* **22,** 147–155.

GOPALAKRISHNAN, M., BUCKNER, S.A., WHITEAKER, K.L., SHIEH, C.C., MOLINARI, E.J., MILICIC, I., DAZA, A.V., DAVIS-TABER, R., SCOTT, V.E., SELLERS, D., CHESS-WILLIAMS, R., CHAPPLE, C.R., LIU, Y., LIU, D., BRIONI, J.D., SULLIVAN, J.P., WILLIAMS, M., CARROLL, W.A. & COGHLAN, M.J. (2002). (−)-(9*S*)-9-(3-bromo-4-fluorophenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4*H*)-one 1,1-dioxide (A-278637): a novel ATP-sensitive potassium channel opener efficacious in suppressing urinary bladder contractions. I. *In vitro* characterization. *J. Pharmacol. Exp. Ther.,* **303,** 379–386.

GOPALAKRISHNAN, M. & SHIEH, C.C. (2004). Potassium channel subtypes as molecular targets for overactive bladder and other urological disorders. *Exp. Opin. Ther. Targets,* (in press).

GOPALAKRISHNAN, M., WHITEAKER, K.L., MOLINARI, E.J., DAVIS-TABER, R., SCOTT, V.E.S., SHIEH, C.-C., BUCKNER, S.A., MILICIC, I., CAIN, J.C., POSTL, S., SULLIVAN, J.P. & BRIONI, J.D. (1999). Characterization of the ATP-sensitive potassium channels (KATP) expressed in guinea pig bladder smooth muscle cells. *J. Pharmacol. Exp. Ther.,* **289,** 551–558.

HAMBROCK, A., PREISIG-MULLER, R., RUSS, U., PIEHL, A., HANLEY, P.J., RAY, J., DAUT, J., QUAST, U. & DERST, C. (2002). Four novel splice variants of sulfonylurea receptor 1. *Am. J. Physiol.,* **283,** C587–C598.

HERRERA, G.M., HEPPNER, T.J. & NELSON, M.T. (2000). Regulation of urinary bladder smooth muscle contractions by ryanodine receptors and BK and SK channels. *Am. J. Physiol.,* **279,** R60–R68.

HOWE, B.B., HALTERMAN, T.J., YOCHIM, C.L., DO, M.L., PETTINGER, S.J., STOW, R.B., OHNMACHT, C.J., RUSSELL, K., EMPFIELD, J.R., TRAINOR, D.A., BROWN, F.J. & KAU, S.T. (1995). ZENECA ZD6169: a novel KATP channel opener with *in vivo* selectivity for urinary bladder. *J. Pharmacol. Exp. Ther.,* **274,** 884–890.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* **193,** 265–275.

LU, S.H., YAMAGATA, T., ATSUKI, K., SUN, L., SMITH, C.P., YOSHIMURA, N., CHANCELLOR, M.B. & DE GROAT, W.C. (2002). Effect of KW-7158, a putative afferent nerve inhibitor, on bladder and vesico-vascular reflexes in rats. *Brain Res.,* **946,** 72–78.

MOREAU, C., JACQUET, H., PROST, A.-L., D'HAHAN, N. & VIVAUDOU, M. (2000). The molecular basis of the specificity of action of KATP channel openers. *EMBO J.,* **19,** 6644–6651.

NURSE, D.E., RESTORICK, J.M. & MUNDY, A.R. (1991). The effect of cromakalim on the normal and hyperreflexic human detrusor muscle. *Br. J. Urol.,* **68,** 27–31.

PETKOV, G.V., HEPPNER, T.J., BONEV A. D, ., HERRERA, G.M. & NELSON, M.T. (2001). Low levels of KATP channel activation decrease excitability and contractility of urinary bladder. *Am. J. Physiol.,* **280,** R1427–R1433.

QUAYLE, J.M., NELSON, M.T. & STANDEN, N.B. (1997). ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol. Rev.,* **77,** 1165–1232.

SAKURA, H., TRAPP, S., LISS, B. & ASHCROFT, F.M. (1999). Altered functional properties of KATP channel conferred by a novel splice variant of SUR1. *J. Physiol. (Cambridge, U.K.),* **521,** 337–350.

SCOTT, V.E., DAVIS-TABER, R.A., SILVIA, C., HOOGENBOOM, L., CHOI, W., KROEGER, P., WHITEAKER, K.L. & GOPALAKRISHNAN, M. (2004). Characterization of human urinary bladder K_{ATP} channels containing SUR2B splice variants expressed in L-cells. *Eur. J. Pharmacol.,* **483,** 195–205.

SCULPTOREANU, A., YOSHIMURA, N. & DE GROAT, W.C. (2004). KW-7158 enhances A-type K^+ currents in neurons of the dorsal root ganglion of the adult rat. *J. Pharmacol. Exp. Ther.,* **310,** 159–168.

SEINO, S. (1999). ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu. Rev. Physiol.,* **61,** 337–362.

SELLERS, D.J., CHAPPLE, C.R. & CHESS-WILLIAMS, R. (2001). Potential therapeutic targets for treatment of the overactive bladder. *World J. Urol.,* **19,** 307–311.

SHELDON, J.H., DAVIS, A., WESLEY NORTON, N., WOODS, M., CARSON, N., WANG, Q., MITCHELL, R., LITTRELL, J. & ARGENTIERI, T.M. (2002). Evidence for KCNQ gene expression and M-current activity in rat and human urinary bladder smooth muscle. ASPET-Ray Fuller Symposium Series, Lower Urinary Tract Disorders: Physiology, Pharmacology and Therapeutic Approaches, Abstract. *J. Pharmacol. Exp. Ther.*

SHIEH, C.-C., FENG, J., BUCKNER, S.A., BRIONI, J.D., COGHLAN, M.J., SULLIVAN, J.P. & GOPALAKRISHNAN, M. (2001). Functional implication of spare ATP-sensitive K^+ channels in bladder smooth muscle cells. *J. Pharmacol. Exp. Ther.,* **296,** 669–675.

TURNER, S.C., CARROLL, W.A., WHITE, T.K., BRUNE, M.E., BUCKNER, S.A., GOPALAKRISHNAN, M., FEY, T., COGHLAN, M.J., CASTLE, N.A., SCOTT, V.E., WHITEAKER, K.L., DAZA, A.V., MILICIC, I. & SULLIVAN, J.P. (2003). Structure activity relationship study of a novel class of naphthyl amide K_{ATP} channel openers. *Bioorg. Med. Chem. Lett.,* **13,** 1741–1744.

WOJDAN, A., FREEDEN, C., WOODS, M., OSHIRO, G., SPINELLI, W., COLATSKY, T.J., SHELDON, J.H., NORTON, N.W., WARGA, D., ANTANE, M.M., ANTANE, S.A., BUTERA, J.A. & ARGENTIERI, T.M. (1999). Comparison of the potassium channel openers, WAY-133537, ZD6169, and celikalim on isolated bladder tissue and *in vivo* bladder instability. *J. Pharmacol. Exp. Ther.,* **289,** 1410–1418.

(Received March 29, 2004
Revised June 3, 2004
Accepted June 9, 2004)