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Effects of ATP-Sensitive K^+ Channel Openers and Tolterodine on Involuntary Bladder Contractions in a Pig Model of Partial Bladder Outlet Obstruction

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Aims: To compare in vivo the efficacy, potency, and bladder–vascular selectivity of ATP-sensitive potassium channel openers (KCOs), YM934 and (-)-cromakalim to a muscarinic antagonist, tolterodine in a novel partial outlet obstructed pig model. **Methods:** Partially obstructed female Landrace pigs were implanted with telemetry transmitters to allow the continuous measurement of intravesical, abdominal and arterial pressures. A subcutaneous port catheter was used to adjust bladder volume. Bladder and arterial pressure were simultaneously monitored under isoflurane anesthesia before and after increasing i.v. doses of test compounds. **Results:** Under anesthesia, voiding was completely inhibited, but spontaneous, nonvoiding bladder contractions were observed with mean amplitude of 16 ± 1 cm H_2O, duration of 35 ± 2 seconds, and intercontraction interval of 43 ± 4 seconds ($n = 25$). YM934 and (-)-cromakalim both caused dose-dependent decreases in bladder contraction area under the curve (AUC) with effective doses to inhibit AUC by 35% of 3.6 and 14.9 nmol/kg, i.v., respectively. However, concomitant reductions in mean arterial pressure of 12 and 13% were also observed. Tolterodine did not inhibit spontaneous bladder contractions at doses up to 100 nmol/kg, i.v. corresponding to plasma concentrations up to 41 ng/mL. **Conclusions:** The superior efficacy of KCOs to inhibit spontaneous bladder contractions relative to tolterodine support the hypothesis that KCOs may provide an alternate therapeutic mechanism to treat symptoms of overactive bladder if bladder-vascular selectivity can be sufficiently improved. The minimally invasive model described herein appears useful in the preclinical evaluation of potential therapeutics targeted to treat the overactive bladder. *Neurourol. Urodynam. 22:147–155, 2003.*

Key words: Potassium channel opener; YM934; (-)-cromakalim; tolterodine; involuntary detrusor contraction; in vivo model; overactive bladder

INTRODUCTION

Overactive bladder (OAB) is a condition characterized by symptoms of urinary frequency and urgency, with or without urge incontinence [Weber et al., 2001]. OAB is highly prevalent, affecting 17% of the population over 40 years of age [Milsom et al., 2001], and an estimated 15 million individuals in the United States alone suffer from this condition [McGhan, 2001]. Although not life-threatening, the symptoms can profoundly interfere with essentially all aspects of life, including work, sleep, and social and recreational activities.

One class of agents that continue to be explored as a viable option for the potential management of OAB are openers of ATP-sensitive potassium channels (K_{ATP}) [Cook, 1988; Andersson, 1997; Coghlan et al., 2001]. Openers of K_{ATP} channels decrease smooth muscle (hyper)excitability by hyperpolarizing the cell membrane, thereby limiting the entry of extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels [Quayle et al., 1997]. Progressive hyperpolarization could theoretically inhibit involuntary contractions preferentially leaving larger coordinated reflex voiding contractions relatively intact. Indeed, cromakalim has been shown to inhibit spontaneous nonvoiding contractions in obstructed pigs while the ability to void was maintained [Foster et al., 1989]. A therapy that could blunt involuntary bladder contractions has the potential to reduce symptoms of urge because symptoms in many patients correspond to the urodynamic finding of involuntary detrusor contractions during filling cystometry (detrusor instability).

The benzopyran (-)-cromakalim and the benzoxazin YM934, known to relax human bladder smooth muscle

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in vitro by activation of K_{ATP} channels [Martin et al., 1997] have been evaluated in clinical trials [Nurse et al., 1991; Burggraaf et al., 1998]. Although considerable interest exists in the potential utility of potassium channel openers (KCOs) for the treatment of overactive bladder, clinically relevant selectivity for effects on the bladder relative to other smooth muscle has yet to be demonstrated.

Detrusor instability has been modeled by partial bladder outlet obstruction in several animal species, including rat [Malmgren et al., 1989], guinea pig [Mostwin et al., 1991], and rabbit [Kitada et al., 1989]. The partial obstruction of the pig urethra with a silver ring, initially described by Sibley [1985], resulted in hypertrophy of the bladder wall, partial denervation of the bladder, and spontaneous contractile activity [Sibley, 1987; Speakman et al., 1987; Dixon et al., 1989]. An additional benefit of the pig model is that baseline urodynamic values are similar to humans [Melick et al., 1961] and innervation is similar, although not identical [Crowe and Burnstock, 1989]. More recently, the use of fully implantable, multichannel radiotelemetry for cystometrographic measurements has been validated in female pigs [Mills et al., 2000].

In this study, we describe a model that extends previous application of implantable telemetry technology to allow simultaneous measurement of both bladder and arterial pressure. In addition, implantable port catheter technology is used to (1) adjust bladder volume to optimize the consistency of the involuntary contraction pattern, and (2) allow routine bladder draining to avoid problems due to retention. The use of anesthesia allows evaluation of treatment effects on involuntary contractions in isolation without confounding effects due to movement artifacts or reflex contractions. We then evaluated the efficacy and bladder vs. cardiovascular selectivity of two K_{ATP} channel openers, (-)-cromakalim and YM934, and the muscarinic receptor antagonist tolterodine. Preliminary results of this study have been reported previously in abstract form [Fey et al., 2001].

MATERIALS AND METHODS

Obstruction and Instrumentation of Pigs

Female Landrace/Yorkshire crossbred pigs (Wilson Prairie View Farms, Burlington, WI), each weighing 18–22 kg, were obstructed with 7.5 mm internal diameter sterling silver omega ring placed around the proximal urethra by using an inguinal approach. Seventeen to 20 weeks after obstruction, pigs were anesthetized with a mixture of telazol (4.4 mg/kg, i.m.) and xylazine (2.2 mg/kg, i.m.) and maintained on isoflurane/oxygen during surgery. A TA11PA-C40 implant (Data Sciences, St. Paul, MN) was inserted into the external carotid artery, and a TL11M3-D70-PCP implant (Data Sciences) was secured inside the peritoneal cavity. One pressure catheter of the PCP implant was introduced into the bladder vesica at the mid-body level and secured with dual purse string sutures and silk Chinese fingers. The other pressure catheter (P_{abd}) was left unsecured inside the peritoneal cavity. The body of the port catheter (TI-9, Access Technologies, Skokie, IL) was secured subcutaneously on the side of the animal caudal to the rib cage, and the 9-French catheter was tunneled subcutaneously to the ventral midline then secured into the ventral bladder with Chinese fingers and dual purse string sutures. The port catheter tip was beveled at 45 degrees, and two small scallops were cut from the indwelling portion of the catheter. Approximately 3 cm of the catheter resided inside the bladder.

Animals were treated with amoxicillin and buprenorphine for 3–5 days after obstruction surgery and enrofloxacin and buprenorphine after telemetry implantation surgery; they were allowed to recover for 10–14 days before pharmacologic testing. Complete blood counts and blood chemistries were done at least biweekly. Urine was drained completely by means of the port catheter at least once a week for animals retaining approximately 4 L or less, and up to three times per week for animals retaining higher volumes, and this greatly improved general animal health and prolonged the experimental lifespan of the obstructed animals. Animals were group housed, and protocols were approved by the Institutional Animal Care and Use Committee of Abbott Laboratories.

For cystometry, pigs were anesthetized with telazol and xylazine as above, intubated, and maintained on isoflurane/oxygen in the supine position with water blankets to maintain body temperature. Bladder volume was adjusted by means of the port catheter to establish a regular involuntary contraction pattern.

Data Acquisition and Analysis

Radiotelemetry signals were obtained using a RLA3000 receiver placed in close proximity to the carotid implant and a RMC-1 receiver (Data Sciences) placed under the pelvis of the animal to acquire the abdominal signals. Data were acquired using the Data Sciences Advanced Research Technology system, and a two-point calibration was performed for each pressure channel before every experiment. Data acquisition and semiautomated analysis were performed using the cystometry analysis software in the Ponemah Physiology Platform (Gould Instrument Systems, Valley View, OH). Discriminator algorithms for determining the start and end of contractions were set by means of a user-friendly graphic interface. The derived data were transferred to Microsoft Excel for analysis. Software discriminators were set to accept only contraction amplitudes $\geq$4 cm H_2O. Data were analyzed for changes in frequency, amplitude, duration, intercontraction interval (ICI), and area under the curve (AUC). AUC, which incorporates frequency, amplitude, and duration, was found to be a useful composite measure of involuntary bladder activity for comparison of dose–response relationships. The effective dose to inhibit bladder contraction AUC by 35% (AUC $ED_{35\%}$) and the effective dose to decrease mean arterial pressure by 10% (MAP $ED_{10\%}$) were estimated

from the dose–response curves using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, U.S.A.). Data are expressed as means ± SEM or as percentage of mean baseline values ± SEM. One sample *t*-test was used to assess significance from baseline values at the $P < 0.05$ level.

Compounds and Dosing

Tolterodine was purified from the marketed product and was dissolved in saline. (-)-Cromakalim and YM934 were synthesized in house and were dissolved in a solution containing equal parts of hydroxypropyl-beta-cyclodextrin (100g/200 mL water; Sigma, St. Louis, MO) and sterile water. Test compounds were administered at 0.1 mL/kg, i.v, over 3 minutes, by means of an 18-gauge catheter placed in an ear vein. After an initial 30-minute baseline data acquisition period, two increasing doses were administered i.v. sequentially at 30-minute intervals. Venous blood samples were taken from the contralateral ear vein 15 and 28 minutes after each dose. Data for the two experimental periods were expressed as percentage change from the 30-minute baseline period before dosing. For plasma concentration determinations, the parent compound was extracted from heparinized plasma samples by means of liquid–liquid extraction and quantified by liquid chromatography-mass spectrometry.

RESULTS

General Characteristics of Obstructed Pigs

Eighty percent (24 of 30) of pigs attaining 17 weeks after obstruction were found to have involuntary bladder contractions by cystometry, with an average experimental lifetime of 92 ± 12 days (range, 14–184 days) after implantation of the telemetry devices. These animals typically retained from 2 to 5 liters of urine as determined by draining the bladder after experiment. At necropsy, empty bladders from obstructed animals were found to weigh 4–10 times age-matched controls (data not shown). Proper ring placement was confirmed at necropsy in all animals, and three of the six obstructed pigs that did not demonstrate involuntary detrusor contractions had low urine retention volumes and only mild bladder hypertrophy.

Characterization of Involuntary Contractions

Under isoflurane anesthesia, voiding contractions and micturition were inhibited, but low amplitude, rhythmic bladder contractions were observed. Representative traces showing changes in intravesical pressure (P_{ves}), abdominal pressure (P_{abd}), and MAP are presented in Figure 1. The involuntary bladder contraction patterns are of similar amplitude but of lower frequency and shorter duration than those observed by Foster et al. [1989] from conscious mini-pigs obstructed for 3 months. The contractions also appear qualitatively similar morphologically to those obtained in conscious obstructed pigs 4 months after obstruction as described by Mills et al. [2000].

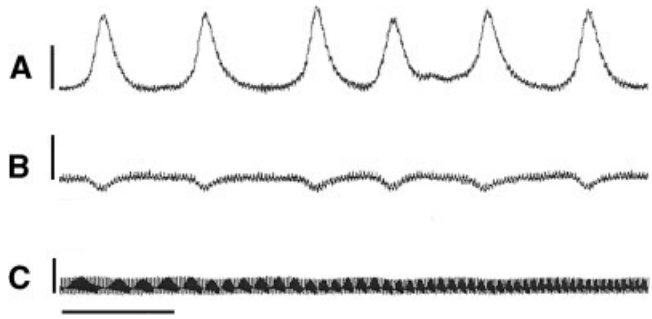

Fig. 1. Representative simultaneous traces obtained from an obstructed pig. **A:** Intravesical pressure; bar = 5 cm H_2O. **B:** Intra-abdominal pressure; bar: 5 cm H_2O. **C:** Arterial pressure; bar = 75 mm Hg. Horizontal bar = 1 minute.

As summarized in Table I, bladder contractions had a baseline frequency of 25 ± 2 per 30-minute period, amplitude of 16 ± 1 cm H_2O, duration of 35 ± 2 seconds, ICI of 43 ± 4 seconds, and a mean AUC of 4,348 ± 451 cm H_2O/sec. The baseline for P_{ves} was relatively stable during the course of the experiment, although in some instances, a gradual increase of 1–2 cm H_2O was noted. The P_{abd} was typically quiescent during the contractions. The contractions were not inhibited by the ganglionic blocker hexamethonium (12 mg/kg, i.v.; data not shown).

Control experiments where the hydroxypropyl-beta-cyclodextrin vehicle alone was dosed demonstrated that involuntary bladder contraction pattern and cardiovascular parameters remained stable over the 90-minute duration of the experiment. As shown in Table II, no statistically significant changes in number of contractions, amplitude, duration, and ICI or total area under the curve were noted during the first or second 30-minute periods after dosing. Similarly, mean arterial blood pressure and heart rate values were stable for the duration of the vehicle experiments (Table II).

Effects of (-)-Cromakalim and YM934

As shown in Figure 2, (-)-cromakalim evoked a dose-dependent suppression of involuntary contractions. At the 10 nmol/kg dose (corresponding to a plasma concentration of 4.2 ± 0.4 ng/mL 15 minutes after dosing), a small but significant decrease (20%) in the AUC was observed, which was largely derived from a decrease in the frequency of contractions. At the 30 nmol/kg dose (corresponding to 13.8 ± 1.2 ng/mL), a 61% reduction in AUC was noted, which was derived from significant reductions in the number of contractions (40%) and contraction amplitude (42%). No significant changes were noted in the contraction duration at either dose.

TABLE I. Summary of Baseline Parameters[a]

Total AUC (cm H_2O . sec)	Frequency (contractions per 30 min)	Pressure amplitude (cm H_2O)	Duration (sec)	Intercontraction interval (sec)	Mean arterial pressure (mm Hg)	Heart rate (beats/min)	Duration of Obstruction (days)	n
4348 ± 451	25 ± 2	16 ± 1	35 ± 2	43 ± 4	95 ± 2	93 ± 4	210 ± 13	25

[a]Baseline period data was acquired over 30 min from anesthetized, partially obstructed pigs. Shown are means ± SEM from 25 separate experiments with 15 different pigs.

Analysis of (-)-cromakalim-evoked changes in total AUC yielded an $ED_{35\%}$ value of 14.9 nmol/kg. A plasma concentration−response curve was generated by plotting the concentration of parent compound detected in plasma 15 minutes after dosing vs. the percentage inhibition of bladder AUC or percentage decrease in MAP. For example, by using this analysis, the plasma concentration of (-)-cromakalim corresponding to the AUC $ED_{35\%}$ was 6.4 ng/mL.

YM934 also inhibited involuntary contractions in a dose-dependent manner (Fig. 3A). No substantial changes in AUC or other parameters were noted at the 1 nmol/kg dose (corresponding to 0.5 ± 0.1 ng/mL). At the 3 nmol/kg dose (2.0 ± 0.4 ng/mL) and 10 nmol/kg dose (5.8 ± 1.0 ng/mL), the total AUC was reduced by 37% and 65%, respectively, which was again, derived from significant reductions in both frequency and amplitude of contractions (Table II). The AUC $ED_{35\%}$ value of YM934 was calculated to be 3.6 nmol/kg, i.v., with a corresponding plasma concentration of 2.2 ng/mL.

Bladder vs. Cardiovascular Selectivity

The changes in mean arterial blood pressure and heart rate evoked by both (-)-cromakalim and YM934 are summarized in Table II. With (-)-cromakalim, no significant change in MAP or heart rate was noted at 10 nmol/kg. At 30 nmol/kg, an acute drop of approximately 40% was routinely observed immediately after dosing. However, this effect moderated over time, resulting in an average reduction of 24% in MAP over the 30-minute observation period. No significant changes in heart rate were noted at either dose. YM934 also evoked dose-related decreases in MAP. Although no significant effects on MAP were noted at 1 and 3 nmol/kg doses, a significant 34% reduction in mean arterial pressure was noted after YM934 at the 10 nmol/kg dose.

To compare relative in vivo selectivity of (-)-cromakalim and YM934, a ratio of the effective dose to decrease MAP by 10% was compared with that required to suppress involuntary contraction AUC by 35% (Table III; Fig. 4). Ratios greater than unity would indicate bladder selectivity. By this comparison, the MAP/AUC selectivity ratios of (-)-cromakalim and YM934 were 0.83 and 0.85, respectively. Thus, neither compound demonstrated significant suppression of involuntary bladder contractions without a concomitant reduction in MAP.

Effect of Tolterodine

Tolterodine was dosed at 1, 10, and 100 nmol/kg, which corresponded to respective plasma drug concentrations of

TABLE II. Effects of KCOs and Tolterodine on Involuntary Contractions and Cardiovascular Parameters in Partial Outlet Obstructed Pigs[a]

			Involuntary bladder contraction parameters					Cardiovascular effects			
Compound	Dose (nmol/kg)	[Parent] @ 15 min. (ng/ml)	Frequency (% change)	Total AUC (% change)	Amplitude (% change)	Duration (% change)	ICI (% change)	MAP (% change)	Heart rate (% change)	Duration of obstruction (days)	n
	10	4.2 ± 0.4	−13 ± 4*	−20 ± 8*	−7 ± 6	−8 ± 5	151 ± 123	−7 ± 4	−6 ± 2	241 ± 61	4
(-)-Cromakalim	30	13.8 ± 1.2	−40 ± 10*	−61 ± 11*	−42 ± 9*	−5.3 ± 11	637 ± 580	−24 ± 6*	−5 ± 8	241 ± 61	4
	1	0.5 ± 0.1	−7 ± 5	−2 ± 10	1 ± 4	−1 ± 3	13 ± 11	1 ± 3	−4 ± 5	224 ± 22	4
	3	2.0 ± 0.4	−24 ± 6*	−37 ± 6*	−31 ± 10*	−6 ± 3	61 ± 22	−7 ± 4	5 ± 8	230 ± 27	4
YM934	10	5.8 ± 1.0	−51 ± 21*	−65 ± 14*	−31 ± 18	−9 ± 8	1402 ± 1752	−34 ± 4*	3 ± 32	242 ± 26	4
	1	0.32 ± 0.04	−10 ± 7	−5 ± 9	6 ± 7	10 ± 6	12 ± 14	−2 ± 3	0 ± 7	170 ± 17	3
	10	4.1 ± 1.7	−3 ± 10	−4 ± 10	−1 ± 7	3 ± 3	15 ± 21	−1 ± 2	−7 ± 4	218 ± 38	4
Tolterodine	100	42.1 ± 11.3	−11 ± 4	−23 ± 10	−17 ± 14	7 ± 5	12 ± 12	−1 ± 3	−9 ± 2	205 ± 45	4
	Period 1	–	−8 ± 10	−8 ± 4	−12 ± 7	6 ± 7	4 ± 27	−2 ± 2	−3 ± 2	231 ± 22	4
Vehicle	Period 2	–	−7 ± 14	−14 ± 6	−14 ± 11	1 ± 8	22 ± 40	−4 ± 2	−7 ± 3	231 ± 22	4

[a]Data are expressed as percentage change versus baseline period obtained from anesthetized partial urethral outlet obstructed pigs dosed with various doses of KCOs ((-)-cromakalim, YM934) and muscarinic receptor antagonist, tolterodine. Asterisks denote significant difference from baseline values ($P < 0.05$).

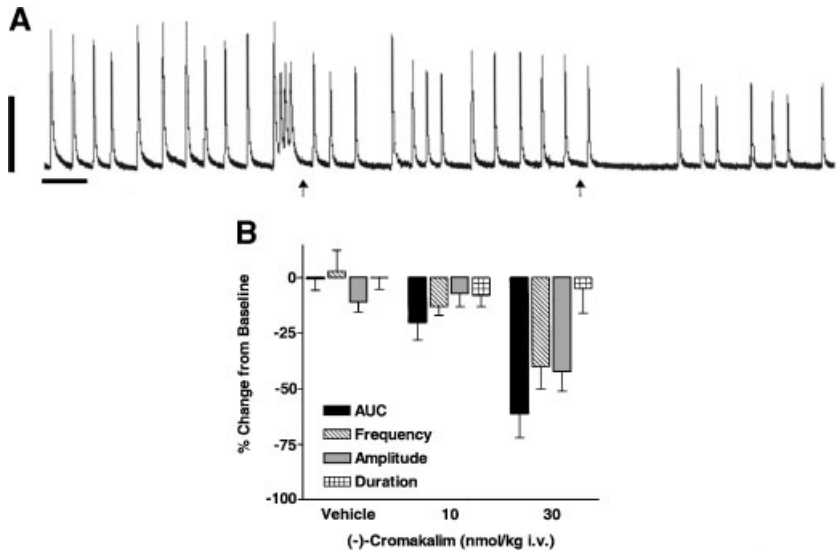

Fig. 2. Dose-dependent inhibition of involuntary contractions by (-)-cromakalim. **A:** Typical intravesical pressure trace showing baseline acquisition period, 30-minute period after 10 nmol/kg (-)-cromakalim, and 30-minute period after 30 nmol/kg (-)-cromakalim (doses indicated by arrows). Vertical bar = 10 cm H_2O; horizontal bar = 5 minutes. **B:** Changes in total area under the curve (AUC), frequency, amplitude, and duration of contractions after 10 and 30 nmol/kg (-)-cromakalim. The Y-axis represents percentage change from baseline values. The baseline values of animals subsequently dosed with (-)-cromakalim were AUC = 5,951 ± 2,262 cm H_2O/sec, frequency = 19 ± 3 contractions/30 minutes, amplitude = 21 ± 1 cm H_2O, duration = 39 ± 6 seconds, and intercontraction interval = 48 ± 20 seconds. Data shown are means ± SEM (n = 4). Statistical significance and the corresponding plasma levels of (-)-cromakalim are summarized in Table III.

0.3 ± 0.04, 4.1 ± 1.7, and 42 ± 11 ng/mL assessed 15 minutes after dosing. No significant effects were observed on bladder contraction AUC or any of the other parameters examined (Fig. 5). At the highest dose tested, tolterodine was unable to inhibit contractions by more than 23% compared with the baseline period.

DISCUSSION

The results of the present study confirm and extend previous observations [Foster et al., 1989; Turner, 1997] that involuntary bladder contractions of obstructed pig are sensitive to suppression by K_{ATP} channel openers such as (-)-cromakalim, but not by muscarinic receptor antagonists or ganglionic blockade. The simultaneous assessment of effects of KCOs on both bladder and MAP provides a valuable model system to compare in vivo selectivity of these agents in a species for which baseline urodynamic parameters closely resemble humans. By this comparison, it is observed that (-)-cromakalim and YM934 at doses that suppress involuntary contractions also substantially decrease MAP, consistent with the lack of selectivity of the first-generation KCOs reported in other preclinical models of bladder dysfunction [Foster et al., 1989; Edwards et al., 1991; Howe et al., 1995].

Spontaneous bladder contractions were not observed in all obstructed pigs and when present, only after several weeks of obstruction. Although the present study did not include nonobstructed control pigs, Sibley [1985] did not detect spontaneous contractions in four of four sham operated, nonobstructed control pigs during filling cystometry, nor did Mills et al. [2000] in 14 conscious, unobstructed, telemetry-implanted pigs. Therefore, it appears these spontaneous nonvoiding contractions are secondary to the obstruction, not related to the telemetry implantation per se and are not a normal finding during routine filling cystometry as has been reported in humans [Salvatore et al., 2001].

The involuntary bladder contraction patterns described in the present anesthetized study are of similar amplitude but of lower frequency and shorter duration than those observed by Foster et al. [1989] from conscious mini-pigs obstructed for 3 months. The contractions also appear qualitatively similar morphologically to those obtained in conscious obstructed pigs 4 months after obstruction as described by Mills et al. [2000].

The KCOs (-)-cromakalim and YM934 elicited dose-dependent reductions in the AUC, which were derived from decreases in both frequency and amplitude of involuntary contractions (Figs. 2 and 3; Table III). Our studies show that YM934 was more potent than (-)-cromakalim on both a dose (4.1-fold) and plasma concentration (2.9-fold) basis. These data are in agreement with previous reports suggesting a 2.7-fold potency difference in rat portal vein [Uchida et al., 1994] and a 2-fold difference in human detrusor strips [Martin et al., 1997; Chess-Williams et al., 1999].

Foster et al. [1989] previously reported that cromakalim at 0.3 mg/kg, i.v. completely abolished unstable bladder

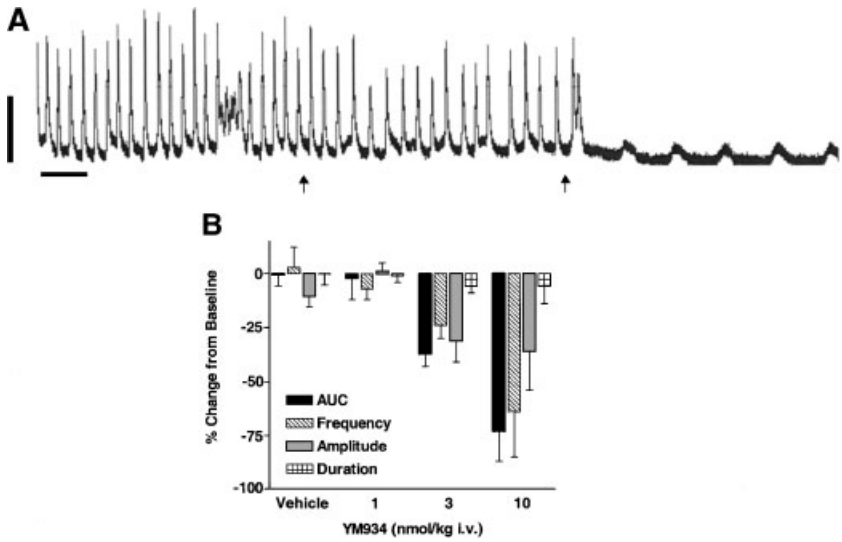

Fig. 3. Dose-dependent inhibition of involuntary contractions by YM934. **A:** Typical intravesical pressure trace showing baseline acquisition period, 30-minute period after 3 nmol/kg YM934, and 30-minute period after 10 nmol/kg YM934 (doses indicated by arrows). Vertical bar = 8 cm H_2O; horizontal bar = 5 minutes. **B:** Changes in total area under the curve (AUC), frequency, amplitude, and duration of contractions after 1, 3, and 10 nmol/kg YM934. The Y-axis represents percentage change from baseline values. The baseline values of animals subsequently dosed with YM934 were AUC = 4,010 ± 445 cm H_2O/sec, frequency = 24 ± 2 contractions/30 minutes, amplitude = 17 ± 4, duration = 36 ± 2 seconds, and inter-contraction interval = 44 ± 5 seconds. Data shown are means ± SEM (n = 8). Statistical significance and the corresponding plasma levels of YM934 are summarized in Table III.

contractions in the conscious, obstructed mini-pig and reduced blood pressure by 30 mm Hg. As shown in Figure 2 and Table II, (-)-cromakalim at a 35-fold lower dose (corresponding to 8.6 μg/kg, i.v.) reduced the bladder AUC by 61% and MAP by 22% (21 mm Hg). Higher doses of (-)-cromakalim were not administered due to a large acute drop in MAP immediately after dosing. The present studies were carried out under isoflurane anesthesia that alone has been shown to have vasodilatory properties [Sakai et al., 2000]. We have observed that baseline MAP values are approximately 16 mm Hg higher in conscious vs. isoflurane-anesthetized obstructed pigs (111 ± 4 mm Hg vs. 95 ± 2 mm Hg; unpublished observations). The known vasodilatory effects of isoflurane may account, in part, for the observation of greater hypotensive potency of (-)-cromakalim in the present study.

As indicated in Table II, heart rate remained stable, despite significant reductions in MAP by (-)-cromakalim and YM934. The blunting of reflex tachycardia by isoflurane in response to (-)-cromakalim-induced hypotension has been described in dogs [Fujiwara and Murray, 1999; Sakai et al., 2000]. A 34 μmol/kg dose of (-)-cromakalim in the conscious dog caused a 10% drop in MAP and 60% elevation in heart rate, yet under isoflurane anesthesia, MAP was reduced by 17% with only a 14% elevation in heart rate [Sakai et al., 2000]. In conscious animals, the drop in mean arterial pressure due to vasorelaxation can be compensated to some extent by an increase in heart rate, and hence, overt hypotension may not be seen until higher doses [Sakai et al., 2000]. Data from this study indicating that reflex tachycardia in response to KCO-induced hypotension may be less pronounced suggest that the isoflurane-anesthetized pig may be a more sensitive model for evaluating the vasodilatory properties of KCOs.

Previous clinical efficacy data for K_{ATP} openers in the treatment of OAB is limited. In a study with (-)-cromakalim [Nurse et al., 1991], 35% patients reported improvements in objective and subjective symptoms. However, the absence of a control group, lack of information on the dose administered, and the small size of the trial makes assessment of the efficacy or selectivity profile difficult. When (-)-cromakalim was dosed

TABLE III. Summary of the Relative Potency and Selectivity of (-)-Cromakalim and YM934 for Inhibition of Involuntary Bladder Contractions vs. Effects on Mean arterial Pressure

	Inhibition of involuntary bladder contraction AUC $ED_{35\%}$ (nmoles/kg, i.v.)	Decrease in mean arterial pressure $ED_{10\%}$ (nmoles/kg, i.v.)	Estimated bladder selectivity ratio
(-)-Cromakalim	14.9	12.6	0.85
YM934	3.6	3.0	0.83

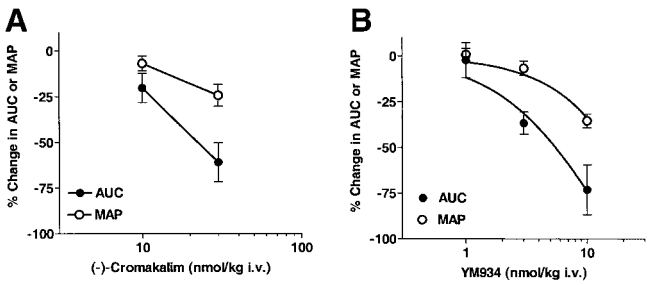

Fig. 4. Dose–response curves illustrating the effects of (**A**) (-)-cromakalim and (**B**) YM934 on involuntary contraction area under the curve (AUC) and mean arterial pressure (MAP) in anesthetized partially obstructed swine. Data are expressed as percentage change vs. mean values from baseline period.

at 1 mg to normotensive volunteers, no change in MAP was noted; however, an increase in heart rate of 18 beats/min (24%) was observed at 6 hours after dosing [Donnelley et al., 1990]. In separate studies, the C_{max} for (-)-cromakalim at this dose has been reported to be 8.7 ng/mL [Davies et al., 1987] to 9.4 ng/mL [Carey et al., 1989]. These plasma levels are comparable to the estimated concentration (5.3 ng/mL) corresponding to the hypotensive $ED_{10\%}$ in the pig model.

Although no clinical data addressing the efficacy of YM934 in overactive or unstable bladder has been published, tolerability after oral administration has been investigated in male volunteers [Burggraaf et al., 1998]. In that study, plasma concentrations exceeding 4 ng/mL were associated with headache in the absence of major changes in routine laboratory or vital signs. It is interesting to note that the plasma concentration of YM934 corresponding to the MAP $ED_{10\%}$ in the obstructed pig (2.6 ng/mL) is similar to the plasma concentration associated with headache in normal human volunteers. Therefore, the plasma concentrations of (-)-cromakalim and YM934 that correspond to a 10% reduction in MAP in the pig model appear comparable to concentrations that elicit increases in heart rate and the incidence of headache, respectively, in humans.

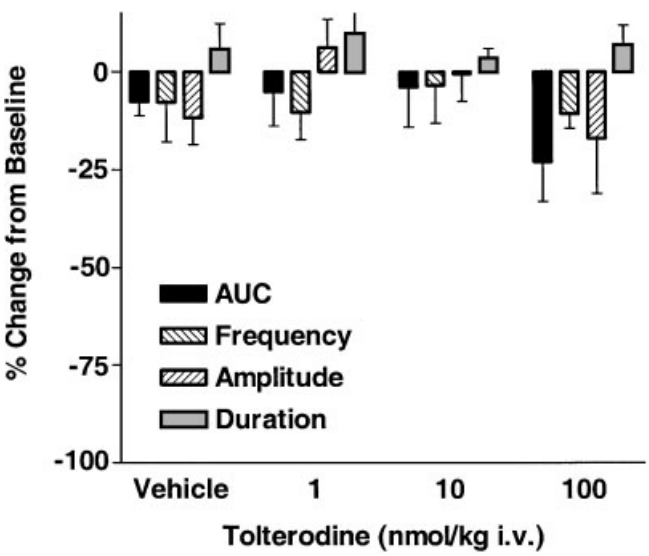

Fig. 5. Effect of tolterodine on involuntary contractions in anesthetized partially obstructed pigs. Shown are changes in total area under the curve (AUC), frequency, amplitude, and duration of contractions after 1, 10, and 100 nmol/kg tolterodine. The Y-axis represents changes from baseline values. The baseline period values of animals subsequently dosed with tolterodine were AUC = 4,137 ± 406 cm H_2O/sec, frequency = 24 ± 2 contractions/30 minutes, amplitude = 15 ± 2, duration = 33 ± 2 seconds, and intercontraction interval = 45 ± 8 seconds. Data shown are means ± SEM (n = 9). Changes in AUC, frequency, amplitude, and duration were not significantly different from baseline values.

A critical goal in the development of KCOs for the treatment of OAB is to achieve clinically meaningful effects on bladder function without reductions in arterial pressure. Because muscarinic antagonists currently used to treat OAB lack efficacy to inhibit urodynamic instability, it is difficult to designate a level of efficacy in this animal model as clinically relevant. When (-)-cromakalim and YM934 were assessed at doses causing complete inhibition of involuntary bladder contraction activity, significant reductions in blood pressure also occurred. However, it is possible that even modest reductions in the frequency, amplitude, or AUC of unstable or involuntary contractions may have clinical relevance. In the absence of a well-defined clinical benchmark, a 35% level of inhibition of involuntary bladder contraction AUC (AUC $ED_{35\%}$) was chosen for comparative purposes, because this level is clearly above the background "noise" of the assay and consistently shows robust statistical significance. Considering a 35% reduction in involuntary bladder contraction AUC as a significant level of efficacy, and a 10% reduction in MAP as a significant liability, a rough index of bladder selectivity of these agents may be determined. By this analysis, the bladder $ED_{35\%}$ for (-)-cromakalim was 14.9 nmol/kg, whereas the MAP $ED_{10\%}$ was 12.6 nmol/kg, yielding a ratio of 0.85. The corresponding AUC $ED_{35\%}$ and MAP $ED_{10\%}$ values for YM934 were 3.6 and 3.0 nmol/kg, i.v., respectively, yielding a ratio of 0.83. These results suggest no inherent bladder vs. vascular selectivity for these KCOs. The potency and bladder selectivity of (-)-cromakalim in this study was comparable to that previously reported in a urethane-anesthetized obstructed rat model (AUC $ED_{35\%} = 30$ nmol/kg, i.v.; selectivity ratio MAP $ED_{10\%}$/AUC $ED_{35\%} = 1$) [Fabiyi et al., 2003]. Chess-Williams et al. [1999] reported minimal (less than threefold) bladder–vascular selectivity of both (-)-cromakalim and YM934, based on an assessment of relative potencies to relax human detrusor and mesenteric artery tissue samples in vitro.

As previously noted, the muscarinic receptor antagonist tolterodine was dosed at 1 nmol/kg (0.08 mg per 150 kg of pig) to 100 nmol/kg (8 mg/pig) yielding plasma levels ranging from 0.3 to 42 ng/mL. In humans, the recommended dose to treat overactive bladder is 1–2 mg b.i.d., with efficacious plasma concentrations averaging between 0.5 and 8 ng/mL for the 2-mg dose [PDR, 2000]. Despite administering doses in which pig plasma levels spanned and exceeded human therapeutic blood levels, tolterodine was unable to

inhibit frequency, amplitude, or AUC of spontaneous bladder contractions by more than 23% at the highest dose tested (Table II). This finding is consistent with the predominantly myogenic nature of the involuntary contractions in the obstructed condition [Igawa et al., 1992; Turner, 1997; Geloso and Levin, 1998] and is consistent with in vitro studies where atropine and tolterodine had no substantial effect on spontaneous contractile activity, although these agents suppressed carbachol and electrical field–stimulated contractions [Turner, 1997; Buckner et al., 2003].

The protocol used in the present study has several advantages that suggest its utility in the evaluation of potential therapeutic agents for the treatment of OAB. The ability to maintain a stable involuntary contraction period for 90 minutes and the inhibition of reflex micturition allow compound effects on involuntary contractions to be studied in isolation. Of course, any extrapolation from inhibition of spontaneous contractions in anesthetized pigs to relief of OAB symptoms in humans must be done with caution. Although evidence suggests that the spontaneous contractions in this model have a primarily myogenic origin, clinical OAB symptoms have multiple potential underlying causes, including increased afferent nerve activity that is not modeled in this system. Also, although using more docile, slower growing female pigs offers practical model development advantages and allows a direct comparison to the previous work of others such as Mills et al. [2000], observations made using ring-obstructed female pigs with high residual volumes should be extrapolated carefully to improvement of irritative symptoms in men suggestive of benign prostatic obstruction. This model perhaps has more current utility as a means to compare pharmacologically the potency and bladder–vascular selectivity of agents than to absolutely predict their therapeutic utility. Therefore, to what degree efficacy in this preclinical model translates to clinical efficacy and OAB symptom improvement remains to be elucidated.

CONCLUSIONS

We report a refinement of the partial outlet obstruction model in swine that uses indwelling telemetry and bladder access catheters and analytical cystometry software to characterize involuntary bladder contractions in the isoflurane anesthetized pig. The model was found to be sensitive and reproducible in comparing the ability of (-)-cromakalim, YM934, and tolterodine to inhibit involuntary bladder contractions while simultaneously monitoring any changes in mean arterial blood pressure. (-)-Cromakalim and YM934 were found to inhibit the frequency and AUC of involuntary bladder contractions in a dose-dependent manner, whereas tolterodine, at doses up to 100 μmol/kg, was ineffective in inhibiting involuntary bladder contractions in this model of bladder instability. These data support the notion that a smooth muscle relaxation by KCOs may result in superior efficacy to inhibit involuntary contractions compared muscarinic antagonists. However, (-)-cromakalim and YM934 both decreased arterial pressure at bladder effective doses consistent with previous studies suggesting a lack of functional bladder selectivity for these compounds. The use of anesthesia, although greatly simplifying the execution of the model, does introduce factors that complicate the correlation of experimental findings in the model to the clinical situation. However, as an experimental system able to mimic at least some aspects of obstructive bladder instability, the model described in this study could serve as a useful tool for the preclinical evaluation of the efficacy and bladder vs. vascular selectivity of potential therapeutic agents for the treatment of OAB.

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