Low levels of $K_{ATP}$ channel activation decrease excitability and contractility of urinary bladder

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Received 2 November 2000; accepted in final form 11 January 2001

ABSTRACT

Low levels of $K_{ATP}$ channel activation decrease excitability and contractility of urinary bladder. Am J Physiol Regulatory Integrative Comp Physiol 280: R1427–R1433, 2001.—Activation of ATP-sensitive potassium ($K_{ATP}$) channels can regulate smooth muscle function through membrane potential hyperpolarization. A critical issue in understanding the role of $K_{ATP}$ channels is the relationship between channel activation and the effect on tissue function. Here, we explored this relationship in urinary bladder smooth muscle (UBSM) from the detrusor by activating $K_{ATP}$ channels with the synthetic compounds $N$-(4-benzoylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZD-6169) and levcromakalim. The effects of ZD-6169 and levcromakalim on $K_{ATP}$ channel currents in isolated UBSM cells, on action potentials, and on related phasic contractions in isolated UBSM strips were examined. ZD-6169 and levcromakalim at 1.02 and 2.63 μM, respectively, caused half-maximal activation ($K_{1/2}$) of $K_{ATP}$ currents in single UBSM cells (see Heppner TJ, Bonev A, Li JH, Kau ST, and Nelson MT. Pharmacology 53: 170–179, 1996). In contrast, much lower concentrations ($K_{1/2}$ = 47 nM for ZD-6169 and $K_{1/2}$ = 38 nM for levcromakalim) caused inhibition of action potentials and phasic contractions of UBSM. The results suggest that activation of <1% of $K_{ATP}$ channels is sufficient to inhibit significantly action potentials and the related phasic contractions. ZD-6169; levcromakalim; incontinence; electrophysiology; guinea pig; potassium channel openers

URINARY BLADDER SMOOTH MUSCLE (UBSM) from the detrusor exhibits spontaneous action potentials (3, 8), which are thought to underlie the nature of spontaneous phasic contractions in this tissue. Excitability in UBSM and the subsequent contractions are dependent on $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels (3, 17). The repolarization of the action potential depends in part on activation ofiberotoxin-sensitive, large-conductance, $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels (8), and the afterhyperpolarization is caused by activation of apamin-sensitive, small-conductance $K_{Ca}$ channels (4, 9, 14). Thus cell excitability, and therefore cytosolic $Ca^{2+}$ concentration, can be decreased through the activation of $K^+$ channels that act to hyperpolarize the membrane potential and decrease action potential frequency.

UBSM contains different types of $K^+$ channels (3, 8, 15), including ATP-sensitive $K^+$ ($K_{ATP}$) channels that can be activated by a variety of potassium channel openers (KCOs) (1, 5, 6, 12–14, 22, 25). In tonic smooth muscle, such as vascular smooth muscle, KCOs, including pinacidil, cromakalim, and levcromakalim activate $K_{ATP}$ channels with one-half activation of the currents in the 1–3 μM range (20, 21). However, KCOs cause vasodilation at lower concentrations than are required for one-half activation of whole cell $K_{ATP}$ currents. These compounds cause one-half relaxation of isolated vessels in the concentration range of 30–600 nM (20, 21, 26). This observation suggests that a small increase in $K_{ATP}$-channel conductance can have a large effect on the cell membrane potential and smooth muscle tone (18, 21). Unlike vascular smooth muscle, UBSM exhibits action potentials and phasic contractions. Therefore, we hypothesized that UBSM may be very sensitive to KCOs because small changes in $K_{ATP}$-channel conductance are likely to move the resting membrane potential away from the threshold of action potential activation, and thus have significant inhibitory effects on action potentials and related phasic contractions.

Trivedi et al. (24) and Howe et al. (11) described a new bladder-selective KCO, ZD-6169, the S-enantiomer of the racemic compound $N$-(4-benzoylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide, which opens $K_{ATP}$ channels in guinea pig detrusor (7, 16) and hyperpolarizes the membrane potential through $K_{ATP}$ channel activation (7, 16, 24). ZD-6169 and levcromakalim effectively inhibit $K^+$-induced contractions of guinea pig bladder (5, 16). ZD-6169 is also highly effective at inhibiting spontaneous contractions of rat bladder in vivo (25). However, the effects of KCOs, and ZD-6169 in particular, have not been studied on UBSM action potentials and phasic contractions in physiological external $K^+$ solution.

The goal of the present study was to determine the degree of $K_{ATP}$ channel activation required to inhibit

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action potentials and related phasic contractions of UBSM using ZD-6169 and levcromakalim as KCOs. We found that ZD-6169 and levcromakalim at concentrations >10 nM inhibited action potentials and contractions. These findings suggest a direct action of ZD-6169 and levcromakalim on KATP channels in UBSM to decrease membrane excitability and contractility. The results support the idea that low levels of KATP-channel activation have substantial effects on UBSM excitability and contractility.

METHODS

Tissue preparation and organ bath experiments (contractility studies). Guinea pigs (250–350 g) were euthanized by halothane overdose followed by exsanguination. This procedure was reviewed and approved by the Office of Animal Care Management at the University of Vermont. The entire urinary bladder was removed and placed in ice-cold physiologically saline solution (PSS, see below for composition). The bladder was pinned to the bottom of a Petri dish containing nominally Ca2+-free dissection solution (see below).

Small strips (100- to 300-μm wide and 3- to 5-mm long) from the detrusor muscle were cut free from the bladder wall and transferred to a Petri dish containing dissection solution. Miniature aluminum clips were placed at each end of the muscle strip to allow mounting of the strip in a tissue bath. Individual strips were placed in thermostatically controlled (37°C) tissue baths (2 ml volume). One end of the strip was attached to a stationary metal hook while the other end was connected to a force-displacement transducer (model BG-10G; Kulite Semiconductor Products) for isometric tension recording. The force generation by the muscle strips was recorded on a computer-based data-acquisition system (Axon Instruments, Foster City, CA) and a chart pen recorder. The strips were suspended under a 1-mN tension. There was a 60- to 90-min equilibration period.

Measurements of membrane potential. For intracellular recordings, several thin strips of UBSM were removed from the serosal surface and pinned to the bottom of a recording chamber. Oxygenated solution was superfused over the tissue (1 ml/min), and the temperature was maintained at 37°C. To facilitate intracellular recordings, tissue movement was reduced by increasing the osmolarity of the superfusing PSS solution with the addition of 350 mM sucrose (8, 12). Microelectrodes were pulled on a Flaming/Brown gas puller (Sutter Instrument, Novato, CA) and had a resistance of 40–50 MΩ when filled with 3 M KCl. The transmembrane potential was measured using a Dagan 8500 intracellular amplifier (Dagan, Minneapolis, MN), and the electrical signals were acquired using the Axotape program (Axon Instruments) and stored on magnetic tape using a modified Sony 75ES DAT recorder.

Cell isolation and whole cell KATP current recordings. To dissociate UBSM cells, three or four tissue strips were placed in 1.5 ml of dissociation solution, which contained 1.0 mg/ml collagenase. The tissue was incubated at 37°C in a rotating water bath for 60 min. After the incubation, the digested tissue was washed several times in dissociation media. The digested tissue was then dispersed with gentle trituration. Several drops of the solution containing the dissociated cells were then placed in a recording chamber. Most cells were elongated and had a bright, shiny appearance when examined using phase-contrast microscopy.

Whole cell currents through KATP channels were measured using the conventional whole cell configuration of the patch clamp technique, as previously described (1, 2, 7). The bathing solution contained (in mM) 82 NaCl, 60 KCl, 0.1 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES, pH 7.4. The pipette solution contained (in mM) 102 KCl, 1 CaCl2, (free Ca2+ 20 nM) 1 MgCl2, 10 EGTA, 10 HEPES, 38 KOH, 0.2 GTP, 0.1 ADP, and 0.1 ATP (free ATP = 0.0053), pH 7.2. Under these conditions and a holding potential of −70 mV, the KATP currents were inward (1, 2). KATP currents were low-pass filtered at 2 Hz with an eight-pole Bessel filter and digitized at 10 Hz. All experiments were conducted at 22°C.

Solutions and drugs. PSS was made daily and contained (in mM) 119 NaCl, 4.7 KCl, 24 NaHCO3, 1.2 KH2PO4, 2.5 CaCl2, 1.2 MgSO4, and 11 glucose. The solution was aerated with 95% O2–5% CO2 to obtain pH 7.4. Dissection/dissociation solution contained (in mM) 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 HEPES, and 2 MgCl2, with pH adjusted to 7.3 with NaOH. ZD-6169 was obtained from AstraZeneca Pharmaceuticals. Atropine, phentolamine, propranolol, suramin, and tetrodotoxin were purchased from Sigma. ZD-6169 and levcromakalim were dissolved in dimethyl sulfoxide and further diluted in PSS. The maximum concentration of dimethyl sulfoxide in the tissue baths was 0.01%. ZD-6169 and levcromakalim were administered directly into the tissue baths as cumulative concentrations.

Statistics. Summary data are presented as means ± SE for n, the number of separate preparations, isolated from different animals. Isolated bladder strips contracted with irregular frequency and amplitude, therefore, a 5-min period of the contraction curve was used to assess contractility. Force integral was calculated by integrating the area under the force-time curve for a period of 5 min. Contractile frequencies, amplitudes, and force integrals are expressed relative to control (absence of pharmacological intervention) values. The last 5 min before drug application (for the control) and the period from the 10th to 15th min for each applied concentration of the drugs were taken as the analysis periods. Statistical analysis of drug effects and the difference between treatment groups were determined using ANOVA where Tukey-Kramer multiple comparisons test was used for multiple comparison. A P value <0.05 was considered significant. The percent KATP channel activation was calculated from the concentration-response curves.

RESULTS

Activation of KATP current by KATP channel openers ZD-6169 and levcromakalim. The effects of ZD-6169 and levcromakalim on the KATP currents were investigated at a holding potential of −70 mV, and the intracellular calcium solution was buffered at 20 nM to minimize activation of voltage-dependent K+ channels and large conductance Ca2+-activated K+ channels (see Refs. 1, 2, 7). Figure 1 illustrates that ZD-6169 (10 μM) and levcromakalim (10 μM) activate an inward KATP current, which was reversed by the KATP-channel inhibitor glibenclamide (10 μM). We previously demonstrated that ZD-6169 and levcromakalim activate
K<sub>ATP</sub> currents in UBSM with a one-half activation constant of 1.02 μM for ZD-6169 and 2.63 μM for levcromakalim and a Hill coefficient of 1.46 for ZD-6169 and 1.62 for levcromakalim, respectively (7).

Inhibition of UBSM action potentials by low concentrations of ZD-6169. The average resting membrane potential recorded in intact bundles of detrusor UBSM was −40.3 ± 2.4 mV, which is similar to previous reports (8, 22). Most of the muscle preparations showed spontaneous electrical activity, exhibited in the form of bursts of action potentials (Fig. 2A). ZD-6169, applied at a concentration of 100 nM, which causes ~3% activation of K<sub>ATP</sub> current (see Fig. 4 and DISCUSSION), caused a small hyperpolarization and inhibition of action potentials (n = 3; Fig. 2A). Washout of ZD-6169 resulted in a fast recovery of the spontaneous action potentials to the control level (Fig. 2A). Small hyperpolarization and inhibition of action potentials could be observed at a concentration of ZD-6169 as low as 10 nM (Fig. 2B), which corresponds to <0.2% activation of K<sub>ATP</sub> currents (see Fig. 4A and DISCUSSION). Higher concentrations of ZD-6169 (≥100 nM) further hyperpolarized the membrane and inhibited the smooth muscle action potentials (Fig. 2B). ZD-6169 at 10 μM caused ~6 mV hyperpolarization (7, 16). Levcromakalim also inhibited action potentials (not illustrated).

The membrane potential measurements indicate that the activation of K<sub>ATP</sub> channels by KCOs is associated with membrane hyperpolarization and inhibition of the action potentials. To understand further how ZD-6169- and levcromakalim-induced changes in K<sub>ATP</sub> currents and spontaneous electrical activity affect tissue function, we investigated the effect of these K<sub>ATP</sub> channel openers on spontaneous phasic contractions.

Inhibition of spontaneous phasic contractions by low concentrations of ZD-6169 and levcromakalim. In UBSM, a phasic contraction reflects an elevation of Ca<sup>2+</sup> entry through voltage-dependent Ca<sup>2+</sup> channels caused by a burst of action potentials (8, 10). The amplitude of a phasic contraction depends on the increase in Ca<sup>2+</sup> entry caused by membrane depolarization during an action potential, whereas the duration of a phasic contraction depends on the duration of a burst of action potentials. The frequency of phasic contractions should reflect mechanisms that temporarily cause action potentials to cease, such as an increase in K<sup>+</sup> conductance.

Activation of K<sub>ATP</sub> channels by ZD-6169 and levcromakalim caused a concentration-dependent decrease in phasic contractions. The predominant effect of both ZD-6169 and levcromakalim was to decrease contraction frequency and muscle force integral, but not amplitude or duration (Fig. 3).

Fig. 2. Intracellular recordings from intact strips of smooth muscle from guinea pig urinary bladder. A: inhibitory effect of ZD-6169 (100 nM) on spontaneous action potentials. Action potential re- sumed after washout of ZD-6169. B: concentration dependence of the effect of ZD-6169 (1 nM-1 μM) on the resting membrane potential and action potentials. Note the decrease in action potential frequency and membrane hyperpolarization with increasing concentrations of ZD-6169.
Fig. 3. Effects of ZD-6169 and levromakalim on the spontaneous phasic contractions of guinea pig urinary bladder smooth muscle (UBSM).

A: original records illustrating inhibitory effect of ZD-6169 and levromakalim (10–300 nM) on the spontaneous phasic contractions. Complete inhibition of contractions occurred with 300 nM ZD-6169 and levromakalim. The contractions reoccurred after washout of the drugs.

B-E: summary data showing the effects of ZD-6169 and levromakalim on contraction frequency (B), amplitude (C), duration (D), and force integral (E). Data were obtained from 6 strips isolated from 6 different animals. *P < 0.05, **P < 0.01, ***P < 0.001.
A significant inhibition of contraction frequency and muscle force was observed at 30 nM ZD-6169 and levcromakalim. At a concentration of 300 nM ZD-6169 and levcromakalim, the contractions were completely inhibited in five of six preparations. Washout of ZD-6169 and levcromakalim restored the spontaneous contractile activity (Fig. 3A). Figure 3, B-E, summarizes the data for the effects of ZD-6169 and levcromakalim on contraction frequency, amplitude, duration, and force integral. ZD-6169 at a concentration of 47 nM and levcromakalim at a concentration of 38 nM were required for one-half activation of KATP currents (7), indicating a 22- and 69-fold difference in sensitivity, respectively (Fig. 4). The KATP-channel inhibitor glibenclamide completely reversed the levcromakalim- and ZD-6169-induced inhibition of spontaneous phasic contractions (Fig. 5).

**DISCUSSION**

The activity of KATP channels in smooth muscle has been shown to be important in regulating membrane potential and muscle tone (21). Activation of KATP channels causes membrane potential hyperpolarization, thereby decreasing smooth muscle tone by reduction in steady-state Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels (18). UBSM, unlike arterial smooth muscle, exhibits action potentials and phasic contractions. Theoretically, very small changes in K\textsuperscript{+} conductance should be able to decrease UBSM excitability by moving the membrane potential away from the action potential threshold (1). Therefore, we tested the hypothesis that a small degree of activation of KATP channels would inhibit action potentials and phasic contractions in UBSM.

Previously, ZD-6169 was found to be effective in inhibiting 15 mM K\textsuperscript{+}-induced phasic contractions of detrusor in micromolar concentrations, with an IC\textsubscript{50} of 1.6 μM (16, 25). Levcromakalim has also been reported to inhibit 20 mM K\textsuperscript{+}-induced UBSM contractions with an IC\textsubscript{50} of 0.86 μM (5). Therefore, under conditions of elevated K\textsuperscript{+} (15–20 mM), significant KATP channel activation is required to inhibit phasic contractions. This is not surprising because elevation of K\textsuperscript{+} to 15–20 mM would depolarize the membrane potential and affect the potassium equilibrium potential. In contrast, our results indicate that low percent activation of KATP channels by ZD-6169 and levcromakalim significantly inhibits action potentials and phasic contractions in UBSM.

A wide variety of synthetic compounds, including ZD-6169 and levcromakalim, activates KATP channels in smooth muscle (7, 19–21, 23). ZD-6169 and levcromakalim increased KATP currents with an apparent one-half activation constant of 1.02 and 2.63 μM, respectively, and Hill coefficient of 1.46 and 1.62, respec-

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Fig. 4. Effect of ZD-6169 (A) and levcromakalim (B) on UBSM force (force integral) and KATP currents obtained from 6 isolated strips and 7 or 8 isolated single cells, respectively. The data were normalized and fitted with the following equation: \( \% \text{response} = 100\% / [1 + (k / \text{[concentration]}^n)] \), where \( k \) is the concentration for half-maximal effect and \( n \) is the Hill coefficient. ZD-6169 \( k \) and \( n \) values were 1.02 and 1.46 μM for KATP currents and 0.047 and 1.26 μM for force integral, respectively. Levcromakalim \( k \) and \( n \) values were 2.63 and 1.62 μM for KATP currents and 0.038 and 1.24 μM for force integral, respectively. The data for concentration-response curves for the effects of ZD-6169 and levcromakalim on KATP currents were taken from a previous study (7). ■, Current; ▼, muscle force.

Fig. 5. After inhibition of the spontaneous phasic contractions by ZD-6169 or levcromakalim, the contractions resumed after the addition of the KATP-channel inhibitor Glib.
tively (Fig. 4; see also Ref. 7). We observed statistically significant inhibition of phasic contractions at 30 nM ZD-6169 and levcromakalim (Fig. 3), which would correspond to activation of <1% of the \( K_{\text{ATP}} \) currents (Fig. 4). ZD-6169 at 300 nM caused complete inhibition of action potentials and phasic contractions (Figs. 2 and 3), corresponding to activation of <15% of the \( K_{\text{ATP}} \) currents (Fig. 4A). At the same concentration, levcromakalim also caused complete inhibition of spontaneous phasic contractions (Fig. 3) corresponding to activation of only ~3% of the \( K_{\text{ATP}} \) currents (Fig. 4B). The fraction of \( K_{\text{ATP}} \) channels that affects UBSM function is likely to be less than these values. The relationship between ZD-6169 and levcromakalim concentration and \( K_{\text{ATP}} \) currents was determined in single UBSM cells dialyzed with low intracellular ATP concentration (Fig. 4; see also Ref. 7). Elevating ATP concentration decreases \( K_{\text{ATP}} \) channel open probability to one, which is unlikely (1). Therefore, under physiological conditions, activation of <1% of the \( K_{\text{ATP}} \) channels decreases UBSM excitability and phasic contractions. These results support the general concept that regulation of \( K_{\text{ATP}} \) channels is a potent mechanism to regulate UBSM function.

Perspectives

Urinary incontinence is associated with abnormal detrusor contractions and the involuntary leakage of urine. This urinary bladder dysfunction significantly impairs the lifestyle of millions of people. One type of urinary incontinence is known as unstable bladder or hyperactive bladder. The cause of unstable bladder is thought to lie within the UBSM. Current treatments for unstable bladder are not very effective and have unwanted side effects. In recent years, much effort has been devoted to increasing our understanding of ion channels, such as \( K_{\text{ATP}} \) channels, that are believed to play a significant role in regulating UBSM excitability. It is hoped that activation of these ion channels would decrease the excitability of UBSM and be useful in the treatment of unstable bladder. To develop ion channel therapeutics that are effective in controlling incontinence, the relationship between ion channel activation and functional effects must be clearly understood. In this study, we used a multifaceted approach to study the effects of \( K_{\text{ATP}} \) channel openers on single isolated UBSM cells and correlated these findings with functional studies. Together with previous findings (Refs. 1, 2, 7, 16), the present results point to a key role for \( K_{\text{ATP}} \) channels in the control of membrane potential, action potential generation, and related phasic contractions of UBSM. Our findings suggest that very low concentrations of \( K_{\text{ATP}} \)-channel openers are sufficient to inhibit UBSM contractions. Thus low-dose applications of \( K_{\text{ATP}} \) channel openers may cause minimal side effects and be an effective therapeutic for the treatment of certain types of urinary incontinence.

This work was supported by National Institute of Health Grant DK-53832 to M. T. Nelson. G. M. Herrera is a National Science Foundation Graduate Research Fellow.

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