Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 563 (2007) 203–208
www.elsevier.com/locate/ejphar

Acetic acid opens large-conductance Ca^{2+}-activated K^+ channels in guinea pig detrusor smooth muscle cells

Srinivas Ghatta [a,1], Irina Lozinskaya [b], Zuojun Lin [b], Earl Gordon [b], Robert N. Willette [b], David P. Brooks [b], Xiaoping Xu [b,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, North Dakota State University, Fargo ND 58105, USA
[b] Cardiovascular and Urogenital Center of Excellence in Drug Discovery, GlaxoSmithKline, King of Prussia, PA 19406, USA

Received 16 August 2006; received in revised form 15 February 2007; accepted 15 February 2007
Available online 27 February 2007

Abstract

Acetic acid was found to have actions on urinary bladder smooth muscle in our routine ion channel screening assays. Numerous studies have examined the mechanisms of bladder irritation by acetic acid; however, the direct effect of acetic acid on ion channels in detrusor smooth muscle cells has not been evaluated. We used whole-cell patch-clamp techniques to examine the effect of acetic acid on large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) from guinea pig detrusor smooth muscle cells and CHO cells expressing recombinant human $BK_{Ca}\alpha\beta_1$ (CHO $BK_{Ca}\alpha\beta_1$) and human $BK_{Ca}\alpha$ (CHO $BK_{Ca}\alpha$). Acetic acid activated BK_{Ca} currents in a concentration-dependent (0.01% to 0.05% v/v) manner in all the cell systems studied. Acetic acid (0.05%) increased BK_{Ca} current at +30 mV by 2764±918% (n=8) in guinea pig detrusor smooth muscle cells. Acetic acid (0.03%) shifted the $V_{1/2}$ of conductance–voltage curve by 64±14 (n=5), 128±14 (n=5), and 126±12 mV (n=4) in CHO $BK_{Ca}\alpha$, CHO $BK_{Ca}\alpha\beta_1$ and detrusor smooth muscle cells, respectively. This effect of acetic acid was found to be independent of pH and was also not produced by its salt form, sodium acetate. Automated patch-clamp experiments also showed similar activation of CHO $BK_{Ca}\alpha\beta_1$ by acetic acid. In conclusion, acetic acid directly activates BK_{Ca} channels in detrusor smooth muscle cells. This novel study necessitates caution while interpreting the results from acetic acid bladder irritation model.

Keywords: Acetic acid; Detrusor smooth muscle; Voltage-clamp; BK_{Ca} channels

1. Introduction

Urinary incontinence is characterized by the involuntary loss of urine and is frequently associated with hyperactivity of bladder detrusor muscle. Over 51 million Americans are reported to have these conditions. Both males and females are affected, with the highest prevalence observed in age groups >60 years. Recent therapeutic advances have improved treatment of this patient population; however, efficacy and side effect profiles of current therapeutics are far from ideal. As such, treatment of overactive bladder continues to represent a significant medical challenge.

Animal models can reveal the phenomena involved in the bladder that are related to the origin of incontinence. However, there is an unmet need of an ideal animal model for urinary incontinence. Chemical irritants are frequently used in animal models to simulate the signs of urinary incontinence. Direct intravesical instillation of acetic acid (up to 1.0%) has been widely used as a method to produce bladder irritation (Chang et al., 1998). Acetic acid is also used to prevent bladder infections from indwelling urinary catheters in humans.

Large-conductance, calcium-activated K^+ channels (BK_{Ca}) play critical roles in regulating human, rat, and guinea-pig detrusor smooth muscle reactivity (Herrera et al., 2000; Hashitani and Brading, 2003; Meredith et al., 2004). While examining the effects of NS 1619, a BK_{Ca} channel opener, on

* Corresponding author. Department of Investigative and Cardiac Biology, UW2511, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA. Tel.: +1 610 270 6858; fax: +1 610 270 6206.
E-mail address: Xiaoping.2.Xu@gsk.com (X. Xu).
[1] Current address. UW 2531, Respiratory and Inflammation—CEDD, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406.

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.02.037

bladder function in the acetic acid irritation model, we found, unexpectedly, that acetic acid activated BK_{Ca} channels of detrusor smooth muscle cells. These effects of acetic acid were also confirmed in the recombinant human BK_{Ca} channels expressed in CHO cells. Direct effects of the acetic acid on BK_{Ca} channels of detrusor smooth muscle cells would affect bladder function and complicate pharmacological manipulation in the acetic acid irritant models of overactive bladder. Precaution needs to be taken when interpreting results obtained from these models.

2. Methods

2.1. Single cell isolation

The studies were carried out in accordance with the US NIH Guide for the Care and Use of Laboratory Animals and the GlaxoSmithKline Animal Care and Use Committee. The urinary bladder was removed from adult guinea pigs euthanized by sodium pentobarbital overdose. The bladder was washed in cold, nominal Ca^{2+}-free solution containing (in mM): 137 NaCl, 5 KH_2PO_4, 1 $MgSO_4$, 10 glucose, 5 HEPES, 8 taurine and 1 mg/ml bovine serum albumin; pH 7.4. Fat, connective tissue, and urothelium were largely removed from the bladders. Bladder tissue was chopped into tiny pieces and incubated in nominal Ca^{2+}-free solution on ice for about 20 min. The tissue pieces were then transferred to an enzyme solution made by adding 50 μM $CaCl_2$, 1.5 mg/ml collagenase type II (Worthington Biochemical Corporation), 1 mg/ml protease XXIV (Sigma), and 0.25 mg/ml trypsin inhibitor (Sigma) to nominal Ca^{2+}-free solution. The tissues were either dissociated immediately or stored at 4 °C overnight prior to processing. Tissues were incubated in the enzyme solution at 37 °C and bubbled with 100% O_2. The supernatant was examined under the microscope for single detrusor smooth muscle cells. Cells were harvested in the supernatant by centrifugation and the tissue pieces were re-incubated in the fresh enzyme solution. Cell collection was repeated 2–3 times. The detrusor smooth muscle cells were stored at 4 °C in a Kraftbrühe medium composed of (in mM): 80 potassium glutamate, 20 K_2HPO_4, 20 KCl, 5 $MgCl_2$, 0.5 K_2EGTA, 2 Na_2ATP, 5 Na-pyruvate, 5 creatine, 20 taurine, 10 glycine, 10 glucose, and 5 HEPES. Patch-clamping was performed within 5-h of isolation.

2.2. Cell culture

Stable CHO cell lines expressing recombinant α subunit alone (CHO $BK_{Ca}\alpha$) and $\alpha+\beta_1$ subunits of human BK_{Ca} channel (CHO $BK_{Ca}\alpha\beta_1$) were generated by GlaxoSmithKline. CHO $BK_{Ca}\alpha$ was maintained in Dulbecco's modified Eagle's medium F-12 supplemented with 10% fetal bovine serum, 0.5 mg/ml geneticin (Invitrogen, Carlsbad, CA) in T-75 flasks at 37 °C and 5% CO_2. For CHO $BK_{Ca}\alpha\beta_1$, hygromycin B (0.1 mg/ml) was also added. Cell culture was performed in strict aseptic conditions in a class II biological hood. Cells were subcultured once every 3 days and were passed routinely when they became 60% confluent. For conventional patch-clamp experiments, 0.25% Trypsin with 0.1% EDTA was used to detach the cells from culture flasks. The cells were suspended in culture medium at room temperature and used within 5-h. For experiments using PatchXpress 7000A, the cells were detached using 0.05% Trypsin–EDTA (Gibco), suspended in HEPES buffered external solution and loaded in the $Sealchip_{16}$ within 5-min.

2.3. Patch-clamp protocols

Ionic currents were recorded using conventional patch-clamp techniques at room temperature (23 °C). Cells were placed in a small chamber (volume=0.7 ml) and continuously perfused with an external solution (3–4 ml/min). Electrodes were made from glass capillary tubes (TW150F-3, World Precision Instruments, Inc.) using a P-97 Micropipette Puller (Sutter Instrument Co.) and had a resistance of 2–4 MΩ when filled with internal solutions. An AXOPATCH 200B amplifier and pCLAMP software (version 8, Molecular Devices) were used for data acquisition. Cell membrane capacitance was compensated and the series resistance was compensated by about 70%. After stable control currents were recorded, the recording chamber was perfused with external solution containing drugs at 3–4 ml/min for at least 3-min. Currents were monitored continuously and the testing agent response was determined at steady-state. The BK_{Ca} current was induced by 200-ms depolarizing voltage steps from +10 to +80 mV or +20 to +90 mV for freshly isolated detrusor smooth muscle cells. Voltage steps were between +50 and +120 mV or +90 and +130 mV (10 mV increments) in CHO cells expressing recombinant human $BK_{Ca}\alpha\beta_1$. The holding potential was 0 mV and inter-pulse interval was 2-s. BK_{Ca} current amplitude was measured as the mean current during the last 100-ms of voltage steps and plotted against membrane voltages. % Change of current was calculated as $100*(I_d-I_c)/I_c$, where I_d was the current amplitude in the presence of a testing agent and I_c was the control current amplitude.

To determine the conductance–voltage (G–V) relationship, the tail current at returning to −60 mV following voltage steps (−120 to +200 mV in 20 mV increments from a holding potential of −60 mV, 100-ms duration for detrusor smooth muscle cells and CHO $BK_{Ca}\alpha\beta_1$ and 50-ms duration for CHO $BK_{Ca}\alpha$) was recorded. For each cell, tail currents were normalized by the maximal tail current following the +200 mV voltage step in the presence of 0.03% acetic acid and the data were fit to the Boltzmann equation, $G/G_{max}=1/(1+\exp[(V-V_{1/2})/k])$, to determine the half-activation voltage ($V_{1/2}$).

PatchXpress 7000A (Molecular Devices) and $Sealchip_{16}$ (AVIVA Biosciences) were used to test the effects of acetic acid and butyric acid on recombinant human $BK_{Ca}\alpha\beta_1$ channels expressed in CHO cells. One ml glass vials (BioTech Solutions) were used for containing the external control and acetic acid or butyric acid solutions. Cell membrane capacitance was compensated and access resistance was also compensated by 40%. Drug solutions (45 μl) were added to the recording well three times (1 min incubation per addition) using the "suction before adding" PatchXpress protocol option (residual volume

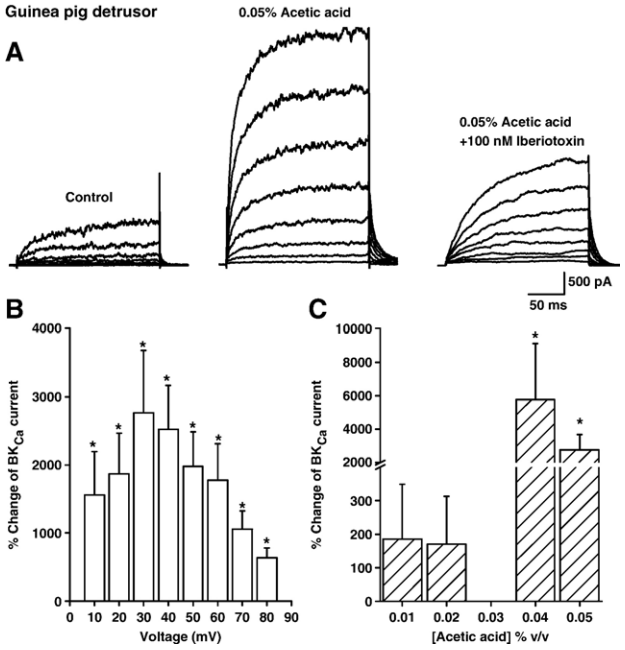

Fig. 1. Effects of acetic acid on BK_{Ca} current of guinea pig detrusor smooth muscle cells. A: BK_{Ca} current was activated by 0.05% acetic acid and its effect was partially reversed by 100 nM iberiotoxin. BK_{Ca} currents were evoked by test pulses (+10 mV to +80 mV in 10 mV increments) from a holding potential of 0 mV. B: % Increases of BK_{Ca} current at various membrane potentials induced by 0.05% acetic acid ($n=8$). C: Concentration-dependent % increase of BK_{Ca} current measured at +30 mV ($n=8$–10). *$P<0.05$, differs significantly from zero.

5 μl). Reversibility of acetic acid or butyric acid activity was examined by 3 min washing with the control external solution. BK_{Ca} current was induced by 200-ms depolarizing voltage steps between +80 and +120 mV in 10 mV increments at a holding potential of 0 mV and an inter-pulse interval of 2-s.

2.4. Solutions

Internal solution (in mM): 140 KCl, 5 EGTA, 1 $MgCl_2$, 5 MgATP, 5 HEPES, pH=7.2. $CaCl_2$ (0.2 mM) was added to achieve approximately 0.01 μM free Ca^{2+} concentration. HEPES buffered external solution consisted of (in mM): 140 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES; pH=7.4. For experiments to determine *G–V* relationships, $CaCl_2$ (2.1 mM for detrusor smooth muscle cells and 4 mM for CHO cells) was added to the above internal solution to yield free calcium concentrations of ~0.18 and 1 μM, respectively (Best et al., 1977) and external solution contained (in mM) 140 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, pH=7.4. Acetic acid and butyric acid were diluted in the HEPES buffered solution to the desired concentrations (percentage by volume) and used immediately.

2.5. Data analysis

We used pClamp software Version 8 (Molecular Devices) for data analysis, Origin Version 7 (OriginLab) for curve fitting and Prism Version 4 (GraphPad) for statistics. All results were presented as mean±S.E.M. (*n*). % Changes were compared with 0 using one-population *t*-test or one-way ANOVA followed by

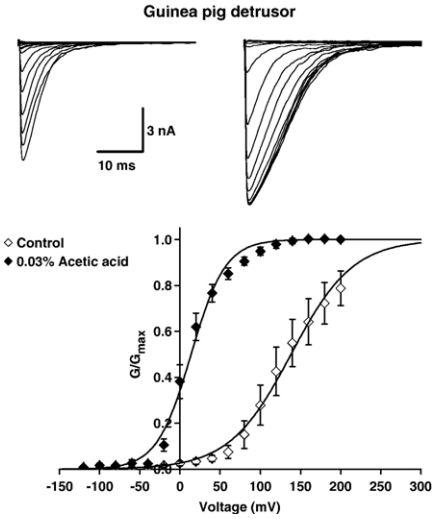

Fig. 2. Steady-state activation curves of BK_{Ca} channels in guinea pig detrusor smooth muscle cells. Top panel: Example tail current traces in control and in 0.03% acetic acid. Bottom panel: Normalized conductance–voltage relationships ($n=4$). Smooth curves are the Boltzmann fits to the averaged data.

Tukey test. Treatment and control results were compared using paired *t*-test. *P* value <0.05 was considered statistically significant.

3. Results

3.1. Effect of acetic acid on BK_{Ca} current in detrusor smooth muscle cells

Acetic acid activated BK_{Ca} current in freshly isolated guinea pig detrusor smooth muscle cells (Fig. 1). The selective BK_{Ca}

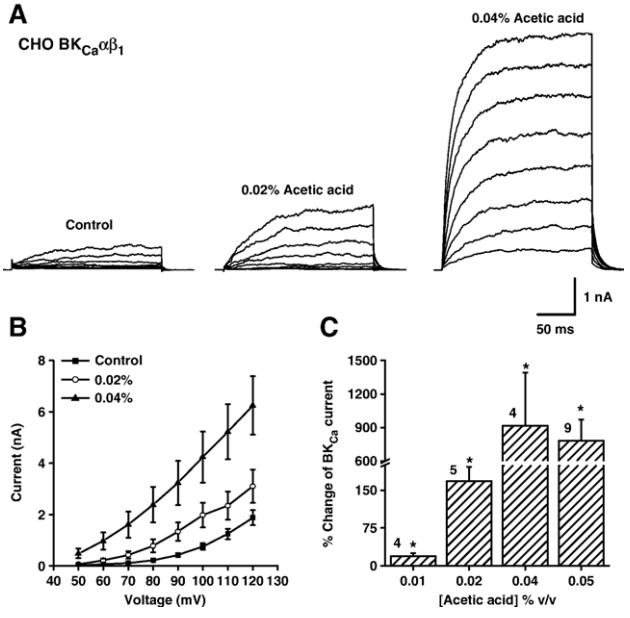

Fig. 3. Concentration-dependent activation of CHO $BK_{Ca}\alpha\beta_1$ by acetic acid. A: Example BK_{Ca} current traces recorded from a CHO cell in control, 0.02% and 0.04% acetic acid. B: The current/voltage relations for the recordings ($n=4$–9). C: Averaged % change of BK_{Ca} current measured at +120 mV induced by various concentrations of acetic acid. Number of cells tested at each concentration was indicated. *$P<0.05$, differs significantly from zero.

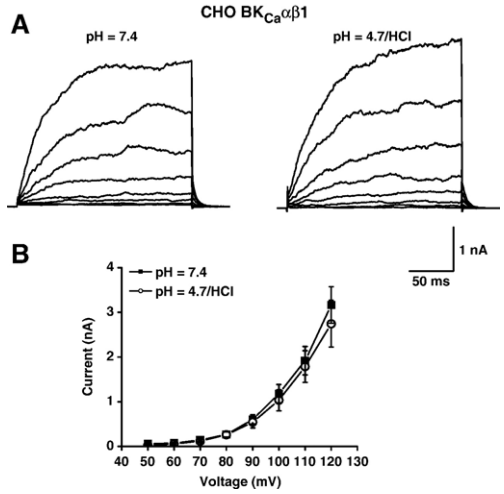

Fig. 4. Activation of BK_{Ca} current by acetic acid is not due to acidification. A: BK_{Ca} currents obtained from a CHO cell in control (pH=7.4) and acidified external solution (pH=4.7/HCl). B: current/voltage relations for the BK_{Ca} currents (n=4).

channel blocker, iberiotoxin (100 nM), partially reversed the activation induced by 0.05% acetic acid on BK_{Ca} current (Fig. 1A). The % increase of BK_{Ca} current induced by 0.05% acetic acid at different membrane potentials was bell shaped, with a maximal response (2764±918%, n=8) at +30 mV (Fig. 1B). The concentration-dependent responses at +30 mV are illustrated in Fig. 1C.

To understand the mechanism of acetic acid activation of BK_{Ca} channels, we examined the effects of acetic acid (0.03%) on the conductance–voltage (G–V) relationships of guinea pig detrusor smooth muscle cells. Acetic acid (0.03%) shifted the

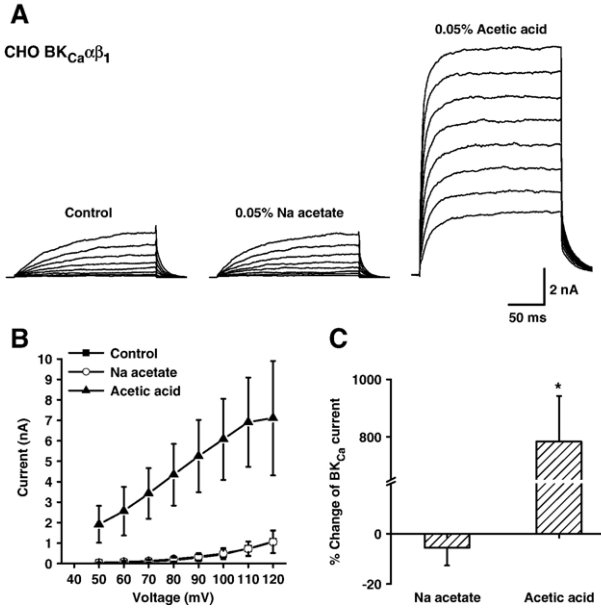

Fig. 5. Acetic acid but not sodium acetate activates CHO $BK_{Ca}\alpha\beta_1$. A: Representative BK currents in control, 0.05% sodium acetate and 0.05% acetic acid recorded from the same cell. B: The current/voltage relations for the BK_{Ca} currents (n=4). C: Averaged % change of BK_{Ca} current measured at +120 mV in 0.05% sodium acetate and 0.05% acetic acid (n=4). *P<0.05, differs significantly from zero.

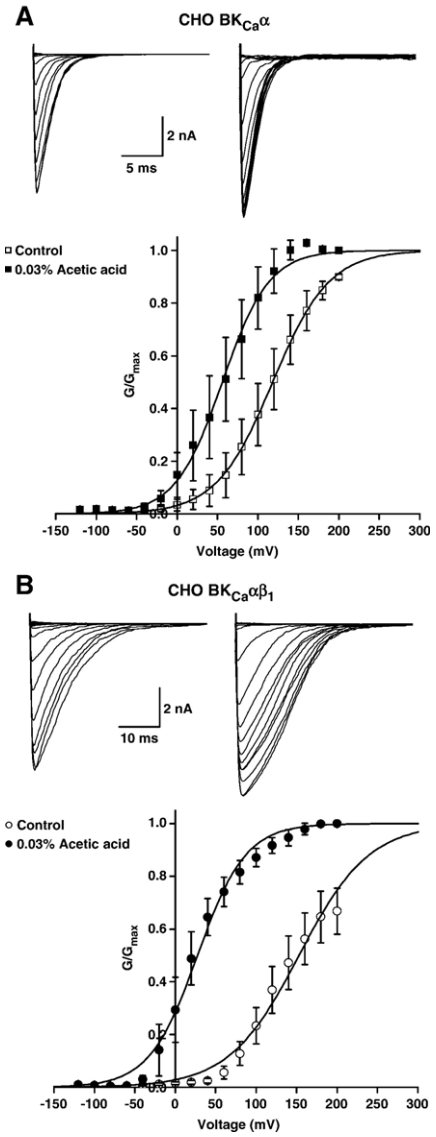

Fig. 6. Shift of voltage-dependent activation of recombinant BK_{Ca} channels by acetic acid. A: CHO $BK_{Ca}\alpha$. B: CHO $BK_{Ca}\alpha\beta_1$. Top panel: Example tail current traces in control and in 0.03% acetic acid. Bottom panel: Normalized conductance–voltage relationships (n=5). Smooth curves are the Boltzmann fits to the averaged data.

$V_{1/2}$ in guinea pig detrusor smooth muscle cells by 126±12 mV (n=4) (Fig. 2).

3.2. Effect of acetic acid on recombinant human $BK_{Ca}\alpha\beta_1$ channels

BK_{Ca} channels in detrusor smooth muscle cells from a variety of species are composed of a regulatory β_1-subunit along with a pore forming α-subunit (Petkov et al., 2001). We examined the effects of acetic acid on recombinant human $BK_{Ca}\alpha\beta_1$ channels expressed in CHO cells. The addition of acetic acid activated the $BK_{Ca}\alpha\beta_1$ channels in a concentration-dependent manner (Fig. 3). The amplitude of the BK_{Ca} current measured at +120 mV was increased by 784±189% (n=9, P<0.05) at 0.05%. The addition of acetic acid (0.05% v/v) reduced the pH of the HEPES buffered external solution from

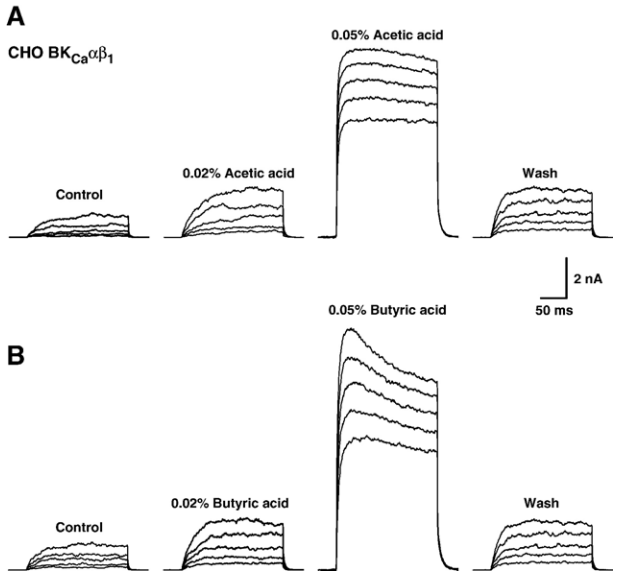

Fig. 7. Reversible activation of CHO $BK_{Ca}\alpha\beta_1$ by acetic acid and butyric acid. A: BK_{Ca} current recorded using automated patch-clamp technique (PatchXpress 7000A) in control, 0.02% and 0.05% acetic acid and after wash. Holding voltage was 0 mV and test potentials was +80 to +120 mV in 10-mV increments. B: Results for butyric acid.

7.4 to 4.7. To find out whether acidification of external solution contributes to BK_{Ca} current increases induced by acetic acid, we adjusted the pH of HEPES buffered external solution to 4.7 using hydrochloric acid (HCl). We found that reducing the pH of HEPES buffered external solution to 4.7 with HCl did not activate $BK_{Ca}\alpha\beta_1$ current (Fig. 4). Following the addition of HCl, the average % change of BK_{Ca} current was 1±5% (n=4) at +120 mV. Interestingly, the addition of sodium acetate (0.05%) had no effect on the $BK_{Ca}\alpha\beta_1$ current despite profound activation by 0.05% acetic acid in the same cell (Fig. 5). At a membrane potential of +120 mV, the change in $BK_{Ca}\alpha\beta_1$ current for 0.05% sodium acetate and 0.05% acetic acid was −6±7% (n=4) and 782±160% (n=4), respectively (P<0.001).

To determine whether the β_1 subunit is necessary for the acetic acid activation of BK_{Ca} channels, we examined the effects of acetic acid (0.03%) on the conductance–voltage (G–V) relationships of both CHO $BK_{Ca}\alpha$ and CHO $BK_{Ca}\alpha\beta_1$. Acetic acid (0.03%) shifted the $V_{1/2}$ of CHO $BK_{Ca}\alpha$ by 64± 14 mV (n=5). The shift of $V_{1/2}$ was 128±14 mV (n=5) for CHO $BK_{Ca}\alpha\beta_1$, significantly larger than that of CHO $BK_{Ca}\alpha$ (Fig. 6).

In automated patch-clamp studies (PatchXpress 7000A), acetic acid induced concentration-dependent activation of CHO $BK_{Ca}\alpha\beta_1$ (Fig. 7A), analogous to the results obtained above using the traditional patch-clamp method. Similar to acetic acid, butyric acid (0.02% and 0.05% v/v) also activated the CHO $BK_{Ca}\alpha\beta_1$ current (Fig. 7B). The effects of both acetic acid and butyric acid were largely reversible upon wash out.

4. Discussion

In the present study we have demonstrated that acetic acid, commonly used as an irritant to simulate overactive bladder in animal models, activated BK_{Ca} channels in detrusor smooth muscle cells. The effects of acetic acid were observed in native guinea pig (frequently used for *in vivo* and *in vitro* bladder studies) detrusor smooth muscle cells and CHO cells expressing recombinant human BK_{Ca} channels. These data suggest that acetic acid and related derivatives (*e.g.*, butyric acid) have complex actions in the bladder by increasing bladder reactivity through known irritant effects and unknown detrusor function by activating BK_{Ca} channels in smooth muscle cells. Our findings are especially important when considering results obtained from overactive bladder models employing acetic acid (Mitsui et al., 2002, 2003; Ishida et al., 2003; Thor et al., 2002; Kiss et al., 2001; Yu and de Groat, 1998).

We used CHO cell lines expressing recombinant human $BK_{Ca}\alpha$ and $BK_{Ca}\alpha\beta_1$ channels to characterize the effects of acetic acid on BK_{Ca} channels in detail (Zhou et al., 1998). Acute application of acetic acid activated the recombinant BK_{Ca} channels. As expected, the increase in BK_{Ca} current was significantly attenuated, but not blocked, by iberiotoxin. This may be due to insurmountable activation of the channel by acetic acid. An important mechanism by which acetic acid increases BK_{Ca} current is a significant shift of the G–V curves toward more hyperpolarized voltages. From G–V curves, it was found that acetic acid can act on the α subunit alone. However, the β_1 subunit of $BK_{Ca}\alpha\beta_1$ significantly potentiates the activating effects of acetic acid. Acetic acid shifted the $V_{1/2}$ of BK_{Ca} channel activation in detrusor smooth muscle cells to the same extent as in CHO $BK_{Ca}\alpha\beta_1$. This is not surprising due to the fact that the BK_{Ca} channels in smooth muscle cells are composed of both α and β_1 subunits. Many other studies also implicated β_1 subunit for its role in biophysical properties of BK_{Ca} channels (Hagen and Sanders, 2006).

Reducing the pH of the extracellular solution to 4.7 (to match the pH observed with 0.05% v/v acetic acid) had no effect on the BK_{Ca} current in recombinant CHO $BK_{Ca}\alpha\beta_1$ cell line. It is also unlikely that intracellular acidification could account for the BK_{Ca} activation induced by acetic acid since intracellular acidification has been reported either to have no effect (Hayabuchi et al., 1998) or to inhibit BK_{Ca} current (Raingo et al., 2005). Thus, activation of the BK_{Ca} current by acetic acid appears to be independent of extracellular or intracellular acidification.

The ability of acetic acid to activate BK_{Ca} channels was not mimicked by the acetate ion, as sodium acetate failed to evoke currents in the recombinant CHO cells. The extent of dissociation of sodium acetate to acetic acid is less. This was indirectly evident by no change in pH of the 0.05% sodium acetate solution. Thus, the BK_{Ca} activation effect is likely mediated by acetic acid but not sodium acetate. The fact that butyric acid similarly activated the BK_{Ca} channels suggests that the carboxyl moiety is important for activity. This hypothesis is supported by the fact that various BK_{Ca} openers of natural and chemical origin, (such as arachidonic acid, pimaric acid, dehydroabeitic acid, niflumic acid, and DHS-1), all possess a carboxyl group. In addition, structure activity relationships confirmed that carboxyl groups are essential for BK_{Ca} channel opening activity (Ohwada et al., 2003).

In urinary bladder smooth muscle cells, BK_{Ca} channels mediate action potential repolarization and limit contractility by

reducing the activity of Ca^{2+} channels (Hashitani and Brading, 2003; Herrera et al., 2005). Recent knock-out studies support the role of BK_{Ca} channels in bladder function (myogenic contractions and urination frequency) and suggest phenotypic similarities with overactive bladder. Specifically, deletion of the BK_{Ca} channel pore-forming α subunit results in increased bladder pressure, incontinence, and decreased bladder capacity (Thorneloe et al., 2005). The acetic acid effects described in this study, activation of BK_{Ca} currents, are expected to reduce detrusor muscle excitability. Paradoxically, *in vivo* intravescical infusion of acetic acid unequivocally elicits bladder contractions (Mitsui et al., 2001). This phenomenon is believed to be mediated by activation of neuronal A-δ and C-afferent fibers. Overall, neuronal mediated contraction overrides BK_{Ca} channel activation. However, additional direct effects of acetic acid on detrusor smooth muscle cells cannot be ruled out.

The present study provides the first direct evidence that acetic acid has BK_{Ca} channel-opening effects. The concentrations of acetic acid used in this study (0.01–0.05% v/v in the HEPES buffered external solution) were well below the concentrations normally infused in models of overactive bladder *in vivo* (up to 1%). Thus, it is reasonable to assume that direct effects of acetic acid on detrusor smooth muscle cells are fully engaged when acetic acid is used in models of overactive bladder (Chang et al., 1998; Chen et al., 2001). The present findings are particularly important when interpreting results obtained from acetic acid models of overactive bladder.

Acknowledgement

The authors are grateful to their colleagues at Tanabe Pharmaceutical Company, Japan for their help.

References

Best, P.M., Donaldson, S.K., Kerrick, W.G., 1977. Tension in mechanically disrupted mammalian cardiac cells: effects of magnesium adenosine triphosphate. J. Physiol. 265, 1–17.

Chang, C.J., Huang, S.T., Hsu, K., Lin, A., Stoller, M.L., Lue, T.F., 1998. Electroacupuncture decreases c-*fos* expression in the spinal cord induced by noxious stimulation of the rat bladder. J. Urol. 160, 2274–2279.

Chen, Z., Anderson, D.L., Faison, W.L., Baer, P.G., 2001. Biphasic urethral sphincter responses to acetic acid infusion into the lower urinary tract in anesthetized cats. J. Urol. 166, 1539–1548.

Hagen, B.M., Sanders, K.M., 2006. Deglycosylation of the beta1-subunit of the BK channel changes its biophysical properties. Am. J. Physiol., Cell Physiol. 291, C750–C756.

Hashitani, H., Brading, A.F., 2003. Electrical properties of detrusor smooth muscles from the pig and human urinary bladder. Br. J. Pharmacol. 140, 146–158.

Hayabuchi, Y., Nakaya, Y., Matsuoka, S., Kuroda, Y., 1998. Effect of acidosis on Ca^{2+}-activated K^+ channels in cultured porcine coronary artery smooth muscle cells. Pflugers Arch. 436, 509–514.

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.

Herrera, G.M., Etherton, B., Nausch, B., Nelson, M.T., 2005. Negative feedback regulation of nerve-mediated contractions by K_{Ca} channels in mouse urinary bladder smooth muscle. Am. J. Physiol. 289, R402–R409.

Ishida, F., Sato, T., Imaizumi, M., Funayama, N., Ikegami, K., Nimura, T., Kawatani, M., 2003. MK-801 inhibits the micturition reflex in chronic bladder irritation caused by crystalluria in the rat. Auton. Neurosci. 105, 1–7.

Kiss, S., Yoshiyama, M., Cao, Y.Q., Basbaum, A.I., de Groat, W.C., Lecci, A., Maggi, C.A., Birder, L.A., 2001. Impaired response to chemical irritation of the urinary tract in mice with disruption of the preprotachykinin gene. Neurosci. Lett. 313, 57–60.

Meredith, A.L., Thorneloe, K.S., Werner, M.E., Nelson, M.T., Aldrich, R.W., 2004. Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+}-activated K^+ channel. J. Biol. Chem. 279, 36746–36752.

Mitsui, T., Kakizaki, H., Matsuura, S., Ameda, K., Yoshioka, M., Koyanagi, T., 2001. Afferent fibers of the hypogastric nerves are involved in the facilitating effects of chemical bladder irritation in rats. J. Neurophysiol. 86, 2276–2284.

Mitsui, T., Kakizaki, H., Matsuura, S., Tanaka, H., Ameda, K., Yoshioka, M., Koyanagi, T., 2002. NK(1) receptor and its interaction with NMDA receptor in spinal c-*fos* expression after lower urinary tract irritation. Am. J. Physiol. 283, R576–R582.

Mitsui, T., Kakizaki, H., Matsuura, S., Tanaka, H., Yoshioka, M., Koyanagi, T., 2003. Chemical bladder irritation provokes c-*fos* expression in the midbrain periaqueductal gray matter of the rat. Brain Res. 967, 81–88.

Ohwada, T., Nonomura, T., Maki, K., Sakamoto, K., Ohya, S., Muraki, K., Imaizumi, Y., 2003. Dehydroabietic acid derivatives as a novel scaffold for large-conductance calcium-activated K^+ channel openers. Bioorg. Med. Chem. Lett. 13, 3971–3974.

Petkov, G.V., Bonev, A.D., Heppner, T.J., Brenner, R., Aldrich, R.W., Nelson, M.T., 2001. Beta1-subunit of the Ca2+-activated K+ channel regulates contractile activity of mouse urinary bladder smooth muscle. J. Physiol. 537, 443–452.

Raingo, J., Rebolledo, A., Grassi de Gende, A.O., Sanz, N., Tommasi, J., Milesi, V., 2005. pH effects on high conductance Ca(2+)-activated K(+) channels (BK(Ca)) in human internal mammary artery smooth muscle cells. Life Sci. 77, 1993–2003.

Thor, K.B., Katofiasc, M.A., Danuser, H., Springer, J., Schaus, J.M., 2002. The role of 5-HT(1A) receptors in control of lower urinary tract function in cats. Brain Res. 946, 290–297.

Thorneloe, K.S., Meredith, A.L., Knorn, A.M., Aldrich, R.W., Nelson, M.T., 2005. Urodynamic properties and neurotransmitter dependence of urinary bladder contractility in the BK channel deletion model of overactive bladder. Am. J. Physiol. 289, F604–F610.

Yu, Y., de Groat, W.C., 1998. Effects of ZD6169, a KATP channel opener, on bladder hyperactivity and spinal c-fos expression evoked by bladder irritation in rats. Brain Res. 807, 11–18.

Zhou, X.B., Schlossmann, J., Hofmann, F., Ruth, P., Korth, M., 1998. Regulation of stably expressed and native BK channels from human myometrium by cGMP- and cAMP-dependent protein kinase. Pflugers Arch. 436, 725–734.