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Involvement of BK_{Ca} channels in the relaxation of detrusor muscle via β-adrenoceptors

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Abstract

Detrusor muscle relaxes upon activation of β-adrenoceptors on smooth muscle cells. However, the mechanism of relaxation following the stimulation of β-adrenoceptors remains unclear. In order to clarify the mechanism, we investigated the involvement of ion channels in bladder relaxation. In guinea-pig isolated bladder strips precontracted by high-K^+, isoproterenol caused concentration-dependent relaxation. The relaxation caused by isoproterenol (1 μM) was larger in 30 mM K^+ than in 120 mM K^+ (54.2 ± 8.0% and 18.2 ± 4.1% of papaverine-induced relaxation, respectively, $n = 4$). Iberiotoxin (100 nM) inhibited the isoproterenol-induced relaxation (vehicle 69.5 ± 8.0% vs. iberiotoxin 24.9 ± 6.2%, respectively, $n = 5$). Whole-cell patch-clamp recording revealed that isoproterenol as well as forskolin increased the iberiotoxin-sensitive K^+ currents, and this increase was abolished by protein kinase inhibitor. These results suggest that the isoproterenol-induced relaxation of guinea-pig bladder smooth muscle is mainly mediated by facilitation of BK_{Ca} channels subsequent to the activation of the cAMP/protein kinase A pathway.

Keywords: K^+ channel, Ca^{2+}-activated; Large conductance; Detrusor muscle; β-Adrenoceptor; Protein kinase A

1. Introduction

The detrusor muscle has been shown to be relaxed by the activation of β-adrenoceptors distributed on bladder smooth muscle in various species (Yamazaki et al., 1998) including guinea-pigs (Yamamoto et al., 1998). The relaxation of detrusor muscle via β-adrenoceptor activation dilates the bladder body and may play an important role in increasing urine storage during the collecting phase of bladder filling (Levin et al., 1980).

Large conductance, Ca^{2+}-activated K^+ channels (BK_{Ca} channels) are expressed abundantly in smooth muscle cells (Garcia-Calvo et al., 1994). Both membrane depolarization and an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) activate BK_{Ca} channels (Vergara et al., 1998). In smooth muscle cells, the opening of BK_{Ca} channels causes membrane hyperpolarization and relaxes smooth muscles by reducing the activity of voltage-dependent Ca^{2+} channels (Robitaille et al., 1993). BK_{Ca} channels have been shown to be a target for protein kinase A (Dworetzky et al., 1996) and protein kinase G (Alioua et al., 1998). The protein kinase A-mediated facilitation of BK_{Ca} channels has been shown to be involved in the relaxation mediated by G-protein coupled receptors such as the vasoactive intestinal peptide (VIP) receptor (Tanaka et al., 1999). As for the β-adrenoceptor, in rat aorta (Satake et al., 1996) and guinea-pig trachea (Jones et al., 1990), relaxation of smooth muscles by β-adrenoceptor stimulation has been shown to be mediated mainly by BK_{Ca} channels. However, in canine saphenous vein, ATP-sensitive K^+ channels play an important role in the hyperpolarization induced by isoproterenol (Nakashima and Vanhoutte, 1995). With regard to bladder detrusor muscles, the involvement of BK_{Ca} channels in β-adrenoceptor-mediated relaxation remains unclear. Thus, in the present study, we investigated the role of BK_{Ca} channels in the relaxation of urinary bladder detrusor muscles upon β-adrenoceptor stimulation.

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2. Methods

2.1. Measurement of tissue contraction

The experimental protocol complies with the guidelines for animal experiments approved by the University of Tokyo. Male Hartley guinea-pigs (250–350 g) were killed by exsanguination under pentobarbital (50 mg/kg, i.p.) anesthesia and the urinary bladder was isolated. The detrusor muscles of urinary bladders were immersed in Krebs-bicarbonate solution (in mM: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11 glucose, 25 $NaHCO_3$), and were cut into four longitudinal strips (5–7 mm length, 2–3 mm width). The strips were mounted in organ baths containing 10ml of Krebs solution maintained at 37°C and gassed with 95% O_2/5% CO_2. Preparations were stretched with an initial tension of 2 g, and changes in isometric tension were measured by force-displacement transducers (UL-20GR, Minebea Tokyo, Japan) connected to a carrier amplifier (AP-601G Nihon Koden, Tokyo, Japan). The bladders were precontracted by high-K^+ (30 or 120 mM) Krebs solution before the addition of drugs. When high-K^+ solutions were used to contract preparations, equimolar concentrations of Na^+ in the Krebs solution were replaced by K^+ to retain isotonicity. The tension developed by the tissue is expressed as a percentage of the maximum relaxation produced by 0.1 mM papaverine.

2.2. Current recording

Single smooth muscle cells were enzymatically isolated from the urinary bladder of male guinea-pigs according to the method of Imaizumi et al. (1989) with minor modifications. Briefly, guinea-pigs were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Bladders were dissected and immersed in Ca^{2+}-free Krebs solution (in mM: 137 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 10 HEPES, 14 glucose, pH 7.4 with NaOH). Connective tissue and mucosal membranes were removed. The tissue was incubated at 37°C, first in Ca^{2+}-free Krebs solution for 60 min and then in Ca^{2+}-free Krebs solution containing 0.3% albumin, 0.3% collagenase, and 0.3% trypsin inhibitor for 30–60 min. Thereafter, the tissue was washed with Ca^{2+}-free Krebs solution and gently agitated with a pipette in order to dissociate cells. The freshly dissociated cells were stored at room temperature and were used within 8 h after isolation.

Currents were measured in the whole-cell configuration of the patch-clamp technique using Axopatch 200B amplifier (Axon instruments, Foster City, CA). The patch electrodes, made of borosilicate glass capillaries, were fire polished to have a resistance of 2.5–5 MΩ. The composition of the internal solution was as follows: (in mM) 145 KCl, 2 $MgCl_2$, 10 glucose, 2 ATP, 1 GTP, 10 HEPES, 5 EGTA, 3.3 $CaCl_2$ (calculated p(Ca) was 6.5), pH 7.2, with KOH for outward K^+ current recording (Song and Simard 1995), and 5 NaCl, 105 CsCl, 30 tetraethylammonium chloride, 10 HEPES, 5 Mg-ATP, 2 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), pH 7.2, with CsOH for Ca^{2+} current recording. Cells were perfused with Krebs solution containing 2mM $CaCl_2$.

Generation of voltage-clamp protocols and acquisition of data were carried out using pCLAMP software (version 6.0.4, Axon Instruments). The series resistance was electronically compensated through the amplifier. Current signals were filtered at 5 kHz before digitization. The linear passive leak currents were digitally subtracted using the P/4 method in which passive current components were

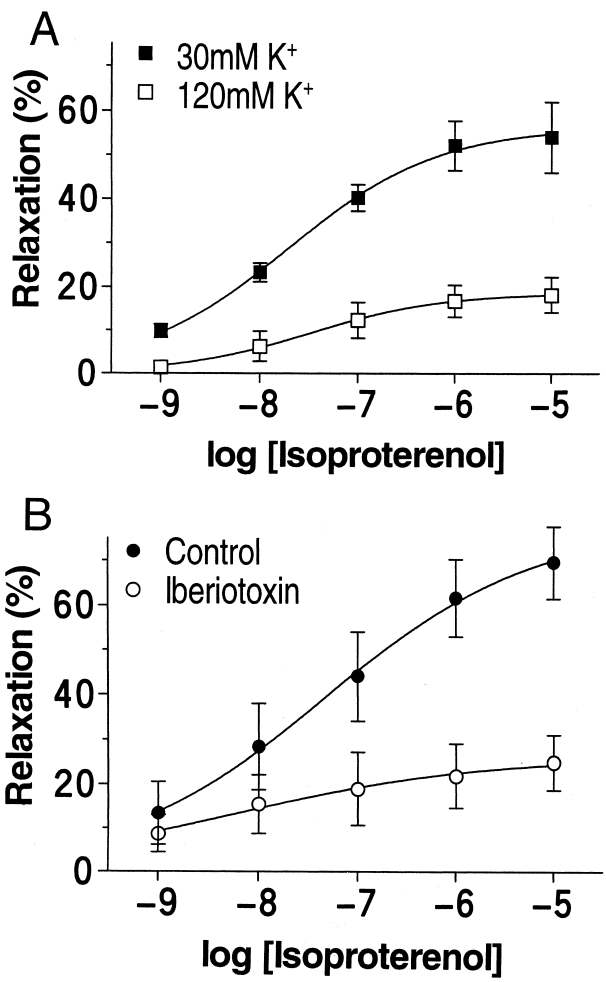

Fig. 1. Concentration–response curves of isoproterenol for relaxation in guinea-pig urinary bladder. (A) Preparations were precontracted by 30 mM K^+ (closed square) or 120 mM K^+ (open square). (B) Preparations were precontracted by 30 mM K^+ and exposed to vehicle (closed circle) or 100 nM iberiotoxin (open circle) before the addition of isoproterenol. Both treatments caused statistically significant effects ($P < 0.001$). Data represent means ± S.E.M., $n = 4$.

recorded by applying four sub-episodes of 1/4 of the test pulse amplitude. All the experiments were performed at room temperature.

2.3. Drugs

Collagenase was purchased from Yakult (Tokyo, Japan), DL-isoproterenol hydrochloride, protein kinase inhibitor (P-0300, Thr-Thr-Tyr-Ala-Asp-Phe-Ile-Ala-Ser-Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ala-Ile-His-Asp), forskolin, albumin, and trypsin inhibitor from Sigma (St. Louis, MO), and iberiotoxin from Peptide Institute (Osaka, Japan).

2.4. Statistics

The statistical significance of the drug effects was analyzed by comparing control and treated preparations using repeated measures two-way analysis of variance (ANOVA) at a significance level of 5%.

3. Results

First, we characterized the ion channels involved in the β-adrenoceptor-mediated relaxation of guinea-pig urinary bladder. Fig. 1 shows the concentration–response curve for isoproterenol-induced relaxation of bladder strips. Isoproterenol (1 nM–10 μM) relaxed preparations precontracted by high K^+ (30 or 120 mM) Krebs solution in a concentration-dependent manner (Fig. 1A). The magnitude of contraction was 1.7 ± 0.41 and 2.8 ± 0.93 g in 30 and 120 mM K^+-treated groups, respectively. In 30 mM K^+ solution, the maximal relaxation produced by isoproterenol was $54.2 \pm 8.0\%$ of the papaverine-induced maximal relaxation. However, in 120 mM K^+ solution, the maximal relaxation produced by isoproterenol was only $18.3 \pm 4.1\%$. Hence, these results suggest that the opening of K^+ channels was involved in the β-adrenoceptor-mediated relaxation. To identify the K^+ channels involved in the isoproterenol-induced relaxation of bladder strips, we examined the effect of K^+ channel inhibitors on β-adrenoceptor-mediated relaxation. We applied iberiotoxin, a selective BK_{Ca} channel inhibitor. The size of the contraction elicited by 30 mM K^+ Krebs solution were 1.7 ± 0.47 and 3.1 ± 0.87 g in control and iberiotoxin-treated preparations, respectively. Pretreatment of urinary bladder muscles by iberiotoxin (100 nM) inhibited the isoproterenol-induced relaxation in 30-mM K^+ solution (Fig. 1B). The maximal relaxation produced by isoproterenol was $69.5 \pm 8.0\%$ in control, but $24.9 \pm 6.2\%$ in the iberiotoxin-treated group, respectively. Glibenclamide, a specific K_{ATP} channel blocker, failed to attenuate the isoproterenol-induced relaxation at the concentration of 1 μM (data not shown). These results suggest that BK_{Ca} channels are involved in the relaxant effect of isoproterenol in guinea-pig urinary bladder.

To clarify the mechanism, we next examined the effect of β-adrenoceptor stimulation on BK_{Ca} channels of single urinary bladder smooth muscle cells using the patch-clamp technique. Fig. 2 shows the current–voltage relationship of the outward current in isolated bladder smooth muscle cells. Isoproterenol enhanced outward K^+ currents. In the

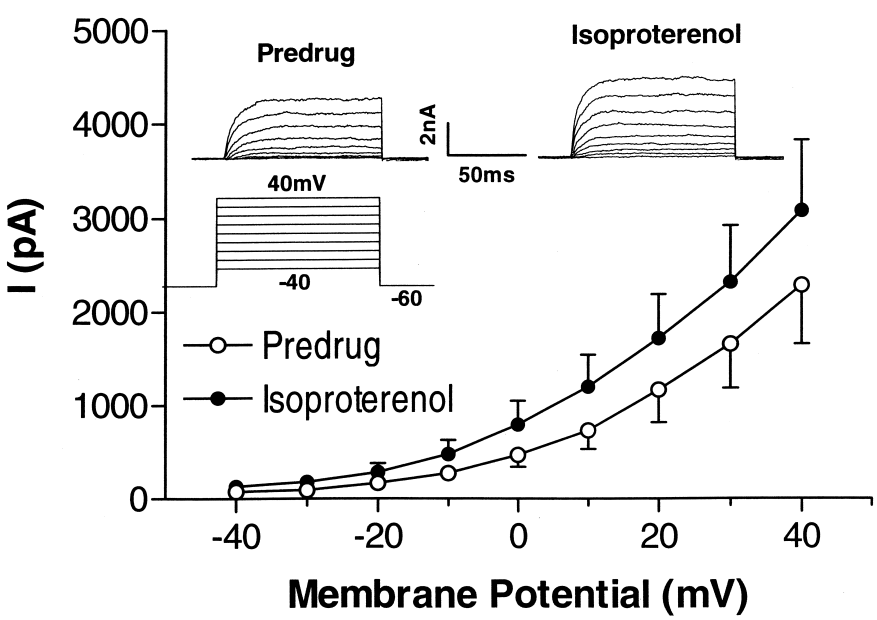

Fig. 2. Effect of isoproterenol (1 μM) on outward K^+ channel currents in whole-cell patch-clamped guinea-pig detrusor muscle cells. Traces of K^+ currents were elicited by test pulses from V_h of −60 mV in the absence (top left) and the presence (top right) of isoproterenol from the same cell. Current traces are shown after leak subtraction. Voltage dependence of the magnitude of K^+ currents is shown in the absence (open circle) and the presence (closed circle) of isoproterenol. The effect of isoproterenol was statistically significant ($P < 0.05$). Each point represents the mean ± S.E.M., $n = 5$.

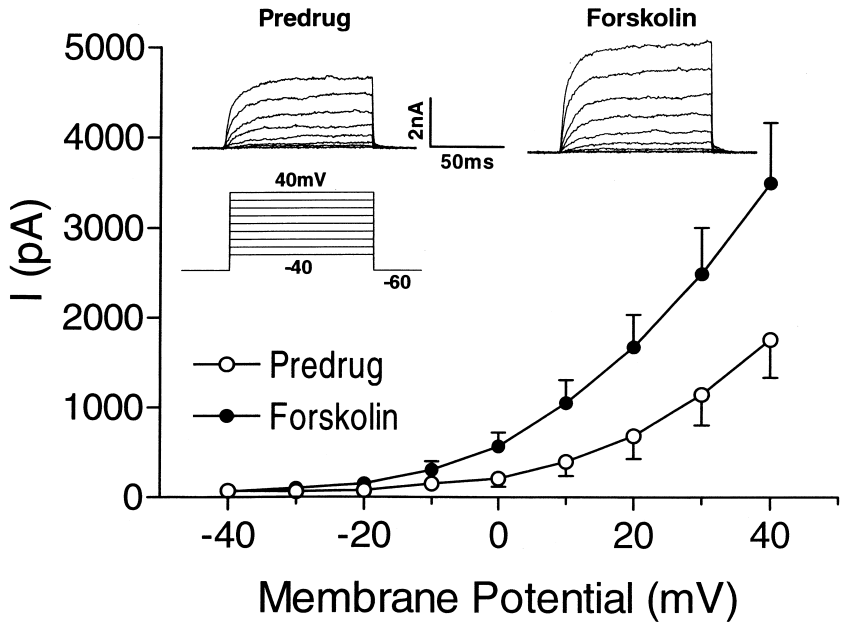

Fig. 3. Effect of forskolin (3 μM) on K^+ currents in whole-cell patch-clamped guinea-pig detrusor muscle cells. Traces of K^+ currents were elicited by test pulses from V_h of −60 mV in the absence (top left) and the presence (top right) of forskolin from the same cell. Current traces are shown after leak subtraction. Voltage dependence of the magnitude of K^+ currents is shown before (open circle) and after (closed circle) the application of forskolin. The effect of forskolin was statistically significant ($P < 0.05$). Each point represents the mean ± S.E.M., $n = 6$.

presence of isoproterenol, the outward K^+ channel current equivalent to the predrug (control) level was activated at a more negative potential than control, by approximately 10 mV. However, it was not clear whether the increase in outward current was accounted for by the leftward shift of the *I–V* curve or by the change in the maximum current, because the current did not reach a maximal level at + 40 mV in the presence or absence of isoproterenol. The activation of K^+ currents was faster in the presence of isoproterenol ($\tau = 10.2 \pm 1.2$ and 7.4 ± 1.0 ms in the absence and the presence of isoproterenol, respectively, $n = 5$).

In order to clarify whether the cAMP/protein kinase A pathway mediates the increase in K^+ current produced by

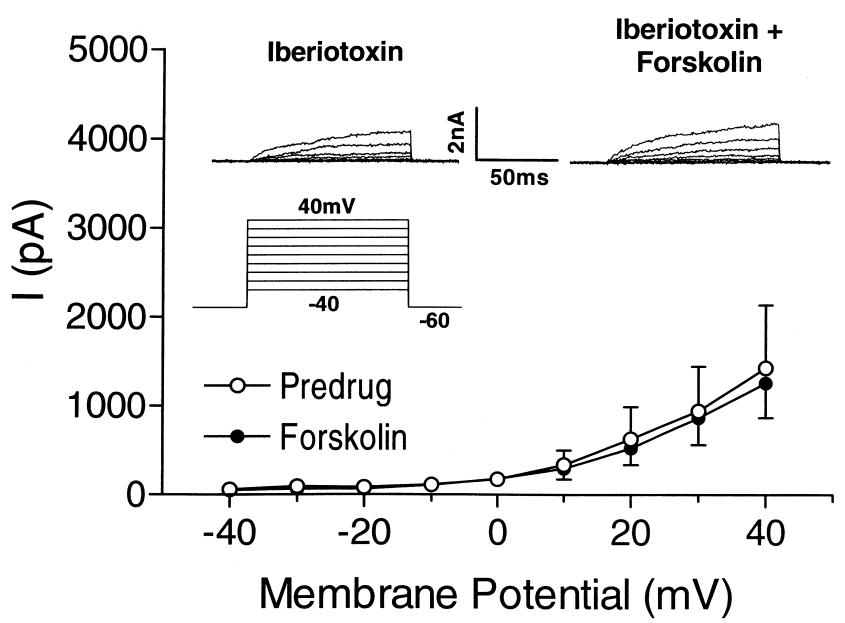

Fig. 4. Effect of forskolin (3 μM) on K^+ currents in whole-cell patch-clamped guinea-pig detrusor muscle cells in the presence of iberiotoxin. Traces of K^+ currents were elicited by test pulses from V_h of −60 mV in the absence (top left) and the presence (top right) of forskolin. Current traces are shown after leak subtraction. Voltage dependence of the magnitude of K^+ currents is shown in the absence (open circle) and the presence (closed circle) of forskolin. The effect of forskolin was not significant ($P = 0.6305$). Each point represents the mean ± S.E.M., $n = 5$.

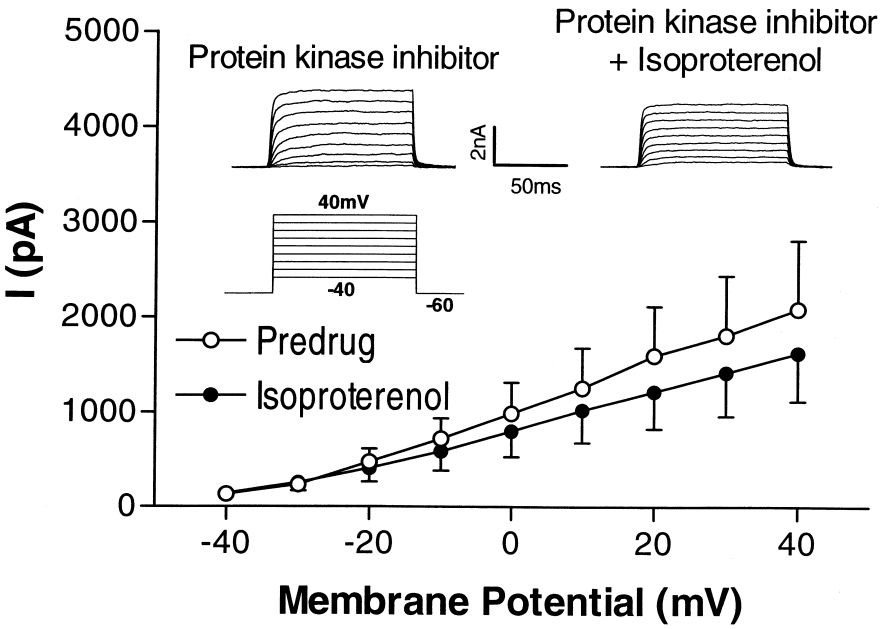

Fig. 5. Effect of isoproterenol (1 μM) on K^+ currents in whole-cell patch-clamped guinea-pig detrusor muscle cells in the presence of protein kinase inhibitor (20 μM). Voltage dependence of the magnitude of K^+ currents is shown in the absence (open circle) and the presence (closed circle) of isoproterenol. Protein kinase inhibitor was applied through patch pipettes. Traces of K^+ currents were elicited by test pulses from V_h of −60 mV in the absence (top left) and the presence (top right) of isoproterenol from the same cell. Current traces are shown after leak subtraction. The effect of isoproterenol was not significant ($P = 0.3342$). Each point represents the mean ± S.E.M., $n = 5$.

stimulation of adrenoceptors, the effect of forskolin was examined. Forskolin (3 μM) increased the outward K^+ current (Fig. 3) in detrusor smooth muscle cells. The voltage required to elicit the outward currents was shifted by 20 mV toward the hyperpolarized direction. The activation of BK_{Ca} channels was faster in the presence of forskolin ($\tau = 20.1 \pm 6.0$ and 8.9 ± 1.8 ms in the absence and the presence of forskolin, respectively, $n = 5$).

The forskolin-activated current turned out to be the BK_{Ca} channel current. The effect of iberiotoxin on the

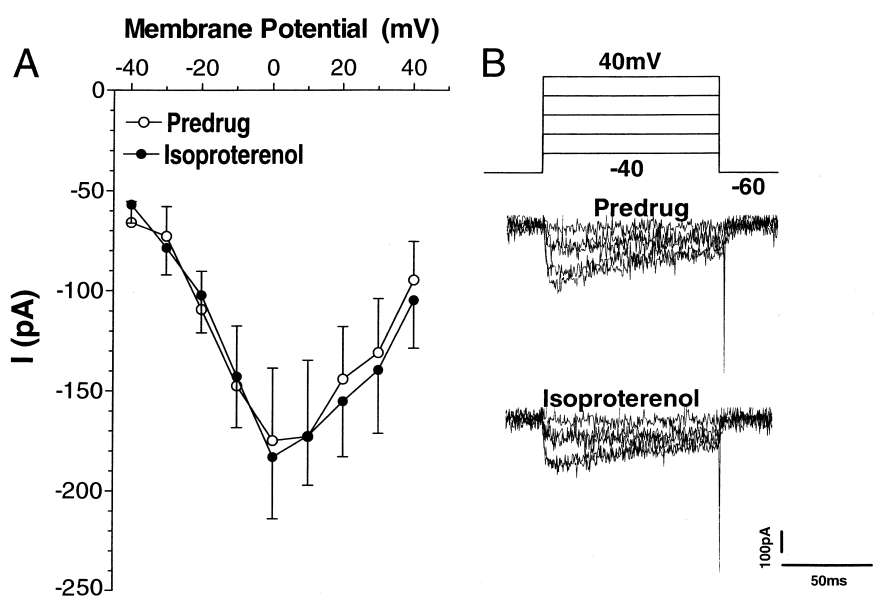

Fig. 6. Effect of isoproterenol (1 μM) on I_{Ca} in whole-cell patch-clamped guinea pig detrusor muscle cells. Traces of I_{Ca} were elicited by test pulses from V_h of −60 mV (B, top) before (B, middle) and after (B, bottom) application of isoproterenol. Voltage dependence of the peak amplitude of I_{Ca} is shown in the absence (open circle) and the presence (closed circle) of isoproterenol. Current traces are shown after leak subtraction. The effect of isoproterenol was not significant ($P = 0.7205$). Each point represents the mean ± S.E.M., $n = 6$.

forskolin-induced facilitation of the BK_{Ca} channel current is shown in Fig. 4. Iberiotoxin suppressed the outward K^+ channel current, and the residual outward currents exhibited slower kinetics than the currents recorded before application of iberiotoxin. Replacement of the extracellular Ca^{2+} by Ba^{2+} also reduced the outward K^+ current by approximately 85%. The iberiotoxin-resistant K^+ current was completely blocked by 10 mM tetraethylammonium chloride (data not shown). Pretreatment of urinary bladder smooth muscle cells by 30 nM iberiotoxin almost completely abolished the effect of forskolin (Fig. 4). In the presence of iberiotoxin, the activation kinetics of outward K^+ currents were no longer affected by treatment with forskolin ($\tau = 37.6 \pm 12.5$ and 35.7 ± 11.7 ms in the absence and the presence of forskolin, respectively, $n = 5$).

The addition of protein kinase inhibitor (20 μM) in the pipette abolished the facilitation of the K^+ channel current by isoproterenol, suggesting that the effect of isoproterenol was mediated by cAMP-dependent phosphorylation (Fig. 5).

The intracellular application of protein kinase inhibitor modified the kinetics of the K^+ channel current. However, the mechanism for this direct effect of protein kinase inhibitor is currently unknown. H-89 (10 μM) also abolished the stimulating effect of isoproterenol on BK_{Ca} channel currents (data not shown).

Because the intracellular calcium concentration and/or calcium influx influences the activity of BK_{Ca} channels, the changes in the BK_{Ca} channel current could be the result of the enhancement of voltage-dependent L-type Ca^{2+} channel currents. However, isoproterenol did not change the current–voltage relationships of I_{Ca} (Fig. 6B).

4. Discussion

In the present study, we investigated the possibility that the activation of BK_{Ca} channels underlies the relaxation of detrusor smooth muscle caused by β-adrenoceptor stimulation.

Isoproterenol caused concentration-dependent relaxation in urinary bladder muscle strips precontracted by 30 mM K^+. However, the relaxant effect of isoproterenol was much weaker with a higher (120 mM) K^+ concentration (Fig. 1A). High concentrations of extracellular K^+ raise the equilibrium potential for K^+ and have been shown to inhibit the relaxant effects of K^+ channel activators in organ bath studies (Yanagisawa et al., 1990; Trivedi et al., 1995). Thus, our results suggest that the opening of K^+ channels is involved in the isoproterenol-induced relaxation. Even in 120 mM K^+ solution, a slight relaxant effect of isoproterenol was observed. Hence, the existence of a K^+ channel-independent mechanism in detrusor relaxation cannot be excluded. Iberiotoxin, a specific BK_{Ca} channel blocker, inhibited the effect of isoproterenol, suggesting that the BK_{Ca} channel is mainly responsible for the β-adrenoceptor-mediated relaxation of guinea-pig detrusor muscle.

In patch-clamp recordings, isoproterenol enhanced the outward K^+ current in isolated smooth muscle cells of guinea-pig bladder (Fig. 2), and protein kinase inhibitor (Fig. 5) and iberiotoxin (data not shown) abolished the facilitation of the K^+ current by isoproterenol (Fig. 5). Forskolin also increased the outward K^+ current (Fig. 3), and this facilitating effect was completely blocked by iberiotoxin (Fig. 4). Both isoproterenol and forskolin accelerated the activation of K^+ currents, and such effects were blocked by iberiotoxin. Thus, this facilitation of K^+ currents by isoproterenol and forskolin appears to be the result of an increase in BK_{Ca} currents, which activate faster than the delayed rectifier type K^+ currents. These results suggest that the production of cAMP and activation of protein kinase A, subsequent to β-adrenoceptor stimulation, activate BK_{Ca} channels and play an important role in detrusor relaxation. These results agree with the report that the stimulation of β_2-adrenoceptors increases the BK_{Ca} channel current via phosphorylation of Ser^{869} of BK_{Ca} channel α-subunit by protein kinase A in Xenopus oocytes (Nara et al., 1998). However, the consequence of activation of protein kinase A seems to differ between organs and species. Besides our results, the activation of protein kinase A has been found to facilitate BK_{Ca} channel opening in rabbit trachea (Kume et al., 1989) and guinea-pig basilar artery (Song and Simard, 1995), but to inhibit BK_{Ca} channels in AtT20 mouse pituitary cells (Shipston et al., 1996). These differences may be due to the tissue-specific distribution of the BK_{Ca} channel β-subunit (hSloβ) or to the existence of other subtypes of β-subunit (Wallner et al. 1999; Wanner et al., 1999), because hSloβ is known to drastically change the Ca^{2+} sensitivity and responsiveness to protein kinase A of BK_{Ca} channels (Dworetzky et al., 1996). Further investigations should be carried out to explain this difference.

In the present study, the slowly developing outward K^+ current component persisted after treatment with iberiotoxin. The iberiotoxin-resistant current developed significantly more slowly than the iberiotoxin-sensitive current (compare Figs. 3 and 4). In urinary bladder smooth muscle cells of guinea-pigs, a delayed rectifier type K^+ current is activated on depolarization as well as the BK_{Ca} channel current (Hirano et al., 1998). In the present study, the fast activating component of the K^+ current was suppressed by iberiotoxin, and the remaining K^+ current was completely blocked by tetraethylammonium, thus suggesting that the iberiotoxin-resistant current mainly consists of the delayed rectifier type K^+ current. Although the delayed rectifier K^+ current has been reported to be enhanced by protein kinase A phosphorylation in rabbit portal vein (Aiello et al., 1998) and canine colon (Koh et al., 1996), the lack of an effect of forskolin in iberiotoxin-pretreated cells suggests that the iberiotoxin-resistant K^+ current is not af-

fected by the activation of the cAMP/protein kinase A pathway in guinea-pig detrusor smooth muscle. Various properties of delayed rectifier currents have been reported in different tissues or species. However, in detrusor smooth muscle, the properties of delayed rectifier currents are relatively unclear and require further investigation.

In airway smooth muscle, it is reported that both protein kinase A-dependent and protein kinase A-independent pathways mediate the facilitation of BK_{Ca} channel activity after stimulation of β-adrenoceptors (Kume et al., 1994). In the present study, in the presence of protein kinase inhibitor, the K^+ channel currents were slightly decreased by isoproterenol (Fig. 5), which may reflect a protein kinase A-independent action of isoproterenol in the guinea-pig detrusor. Detailed mechanisms need to be examined in future studies. These results show that protein kinase A-dependent phosphorylation appears to play a major role in the isoproterenol-induced activation of BK_{Ca} channels in urinary bladder smooth muscle cells. In accordance with our study, the contractile response of guinea-pig bladder has been reported to be modified by an increase in cAMP levels (Longhurst et al., 1997).

In the present study, isoproterenol showed no facilitation or inhibition of Ca^{2+} channel currents (Fig. 6), which suggests that the increase in the BK_{Ca} current and subsequent relaxation of smooth muscle by isoproterenol is not due to the change in Ca^{2+} influx on depolarization.

In conclusion, the present study demonstrated for the first time that the stimulation of β-adrenoceptors relaxes guinea-pig detrusor by opening BK_{Ca} channels through protein kinase A activation.

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