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The effects of MCC-134 on the ATP-sensitive K^+ channels in pig urethra

Takakazu Yunoki[a,b,c], Noriyoshi Teramoto[a,*], Naruaki Takano[c], Narihito Seki[c], Kate E. Creed[d], Seiji Naito[c], Yushi Ito[a]

[a] *Faculty of Medicine, Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi Ward, Fukuoka 812-8582, Japan*
[b] *Japanese Society for the Promotion of Science, Tokyo 102-8471, Japan*
[c] *Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[d] *School of Veterinary Biology, Murdoch University, Murdoch, WA 6150, Australia*

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Abstract

We have investigated the effects of MCC-134 (1-[4-(1*H*-imidazol-1-yl)benzoyl]-*N*-methyl-cyclobutanecarbothioamide) on membrane currents and ATP-sensitive K^+ channel (K_{ATP} channel) opener-induced currents in pig urethra by use of patch-clamp techniques (conventional whole-cell configuration and nystatin perforated patch recordings). Tension measurement was also performed to study the effects of MCC-134 on the resting tone of pig urethral strips. MCC-134 reduced the resting tone of pig urethra in a concentration-dependent manner (EC_{50} = 6 μM). The MCC-134 (30 μM)-induced relaxation was suppressed by glibenclamide. In voltage-clamp experiments, MCC-134 produced a concentration-dependent inward K^+ current which was suppressed by application of glibenclamide at a holding potential of − 50 mV (symmetrical 140 mM K^+ conditions). Application of MCC-134 enhanced diazoxide-induced inward currents and inhibited pinacidil-induced inward currents in a concentration-dependent manner at − 50 mV. These results suggest that MCC-134 induces glibenclamide-sensitive K_{ATP} currents in pig urethra.

Keywords: K^+ channel, ATP-sensitive; MCC-134; Sulphonylurea receptor; Urethra; Smooth muscle

1. Introduction

Recent molecular cloning studies have revealed that ATP-sensitive K^+ channels (K_{ATP} channels) are heteromeric complexes composed of at least two subunits, inwardly rectifying K^+ channels (Kir6.x) and sulphonylurea receptors (SURs) (Aguilar-Bryan et al., 1995; Inagaki et al., 1995). The SURs are responsible not only for sulphonylurea sensitivity (such as glibenclamide, tolbutamide, etc.) but also for intracellular nucleoside diphosphate (NDP) sensitivity. K_{ATP} channel openers interact primarily with the SUR subunits, and not with the Kir subunits of the K_{ATP} channels (Schwanstecher et al., 1998).

In the field of urology, recent molecular identification of K_{ATP} channels has renewed interest in the development of detrusor-selective K_{ATP} channel openers for the treatment of detrusor instability. Various types of detrusor-selective K_{ATP} channel openers (YM-934 (2-(3,4-Dihydro-2,2-dimethyl-6-nitro-2*H*-1,4-benzoxazin-4-yl) pyridine *N*-oxide), Uchida et al., 1994; ZD6169 ((*S*)-*N*-(4-benzoylphenyl)-3,3,3-trifluro-2-hydroxy-2-methyl-propionamide), Trivedi et al., 1995; WAY-133537 ((*R*)-4-[3,4-dioxo-2-(1,2,2-trimethyl-propylamino)-cyclobut-1-enylamino]-3-ethyl-benzonitrile), Wojdan et al., 1999) have been newly synthesized, targeting K_{ATP} channels in urinary bladder. We believe that the significant differences in properties of K_{ATP} channels between urinary bladder and urethra hold out some hope for the development of tissue-selective K_{ATP} channel openers for urge urinary incontinence and that reduction in urethral smooth muscle tone and thus urethral pressure would be undesirable in a drug to treat urinary bladder instability (Teramoto et al., 1997). To achieve this aim, it is essential to investigate the effects of K_{ATP} channel openers on the membrane currents in detrusor as well as those in urethra.

Recently, it has been reported that MCC-134 (1-[4-(1*H*-imidazol-1-yl) benzoyl]-*N*-methyl-cyclobutanecarbothioa-

* Corresponding author. Tel.: +81-92-642-6077; fax: +81-92-642-6079.
E-mail address: noritera@linne.med.kyushu-u.ac.jp (N. Teramoto).

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mide), a novel vascular relaxing agent, possesses a selective action on the activity of recombinant K_{ATP} channels, depending on subtypes of SURs (such as SUR1, SUR2A and SUR2B; Shindo et al., 2000). Shindo et al. (2000) concluded further that MCC-134 is a useful pharmacological tool to determine which subtypes of SURs are functionally expressed in K_{ATP} channels. In the present experiments, we have investigated the effects of MCC-134 on resting tone and membrane currents in pig urethra. Furthermore, we have studied the interaction of MCC-134 on K_{ATP} channel opener (diazoxide and pinacidil)-induced currents, discussing functional subunits of the expressed SURs in pig urethral K_{ATP} channels.

2. Material and methods

2.1. Tension measurement

Fresh female pig urethras were obtained from a local slaughter house and transported in a cold saline solution (mM): Na^+ 137, K^+ 5.9, Mg^{2+} 0.5, Ca^{2+} 0.5, Cl^- 128.3, HCO_3^- 15.4, $H_2PO_4^-$ 1.2 and glucose 11.5 bubbled with 97% O_2 and 3% CO_2 (pH 7.25–7.3). A segment of proximal of urethra was excised from a region 1–2 cm from the bladder neck. Connective tissue and the mucosa were removed by dissection. For isometric tension recording, fine strips (2–3 mm length, 0.4–0.5 mm width, 0.3–0.4 mm thickness) were prepared as described previously (Teramoto and Ito, 1999). An initial tension equivalent to 1 g weight was applied to each strip, which was then allowed to equilibrate for approximately 1–1.5 h until the basal urethral tone became stable (37 °C). To prevent both noradrenaline outflow from sympathetic nerve terminals and β-adrenoceptor stimulation, 3 μM guanethidine and 0.3 μM propranolol were present throughout the experiments. Data were recorded on a Macintosh computer, through ‘‘MacLab 3.5.6’’ (ADInstruments, Castle Hill, Australia). The value of tension was expressed as g mg^{-1} of tissue.

2.2. Cell dispersion

Fresh urethra from female pigs was collected from a local abattoir. Pig urethral myocytes were freshly isolated by the gentle tapping method after treatment with papain and collagenase as described previously (Teramoto and Brading, 1996). Relaxed spindle-shaped cells, with length varying between 400 and 500 μm, were isolated and stored at 4 °C. The dispersed cells were used within 2 h for experiments.

2.3. Recording procedure

Patch-clamp experiments were performed at room temperature (21–23 °C) as described previously (Teramoto et al., 2000, 2001). Junction potentials between bath and pipette solutions were measured with a 3 M KCl reference electrode and were <2 mV, so that correction for these potentials was not made. Capacitance noise was kept to a minimum by maintaining the test solution in the electrode as low as possible. At the beginning of each experiment, the series resistance was compensated.

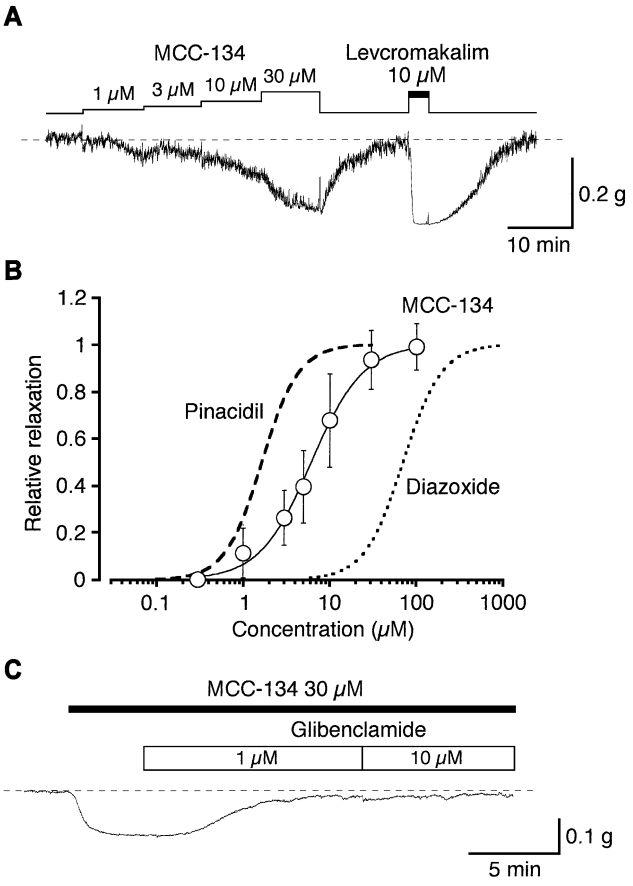

Fig. 1. The relaxing effects of MCC-134 on the resting tone of pig urethra. (A) The effects of cumulative addition of MCC-134 (1–30 μM) and after recovery, 10 μM levcromakalim. The dashed line indicates the mean resting urethral tone. (B) Relationships between the relative value of urethral relaxation and the concentration of K_{ATP} channel openers. The peak amplitude of the 10 μM levcromakalim-induced relaxation was taken as 1. The curves were drawn by fitting the equation using the least-squares method,

$$\text{Relative value} = 1/\{1 + (EC_{50}/D)^{n_H}\}$$

where EC_{50}, D and n_H are dissociation constant, concentration of K_{ATP} channel openers (μM) and Hill's coefficient, respectively. The following values were used for the curve fitting of MCC-134: $K = 6.2$ μM, $n_H = 1.5$ (1 μM, 10 observations; 3 μM, 15 observations; 5 μM, 9 observations; 10 μM, 17 observations; 30 μM, 17 observations; 100 μM, 6 observations; total of 17 different urethral strips). Each symbol indicates mean with S.D. shown by vertical lines. The urethral relaxing curves with the broken lines (pinacidil and diazoxide) are taken from Teramoto and Ito (1999) (pinacidil, $K = 1.6$ μM, $n_H = 2.1$; diazoxide, $K = 70.5$ μM, $n_H = 2.0$). (C) The 30 μM MCC-134-induced urethral relaxation was suppressed by application of glibenclamide.

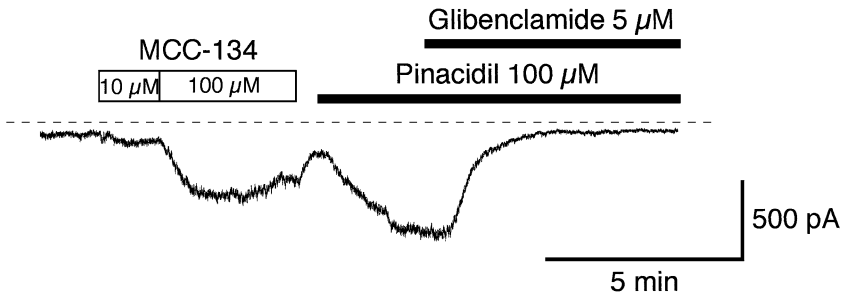

Fig. 2. Effects of MCC-134 (10–100 μM) on the membrane current at −50 mV from whole-cell recording. The bath solution was 140 mM K^+ PSS and the pipette solution was 140 mM KCl containing 5 mM EGTA (i.e., symmetrical 140 mM K^+ conditions). After MCC-134 was removed, the current recovered to the control level. Pinacidil induced an inward current which was suppressed by 5 μM glibenclamide. The dashed line indicates zero current.

2.4. Drugs and solutions

For tension measurement, modified Krebs solution was used (mM): Na^+ 137, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 133.7, HCO_3^- 15.4, $H_2PO_4^-$ 1.2 and glucose 11.5 which was bubbled with 97% O_2 and 3% CO_2. For whole-cell recording, the following solutions were used: physiological salt solution (PSS) containing (mM): Na^+ 140, K^+ 5, Mg^{2+} 1.2, Ca^{2+} 2, glucose 5, Cl^- 151.4, HEPES 10, titrated to pH 7.35–7.40 with Tris base (sometimes 60 mM K^+ and 140 mM K^+ PSS were obtained by replacing 55 and 135 mM Na^+ with equimolar K^+); high potassium pipette

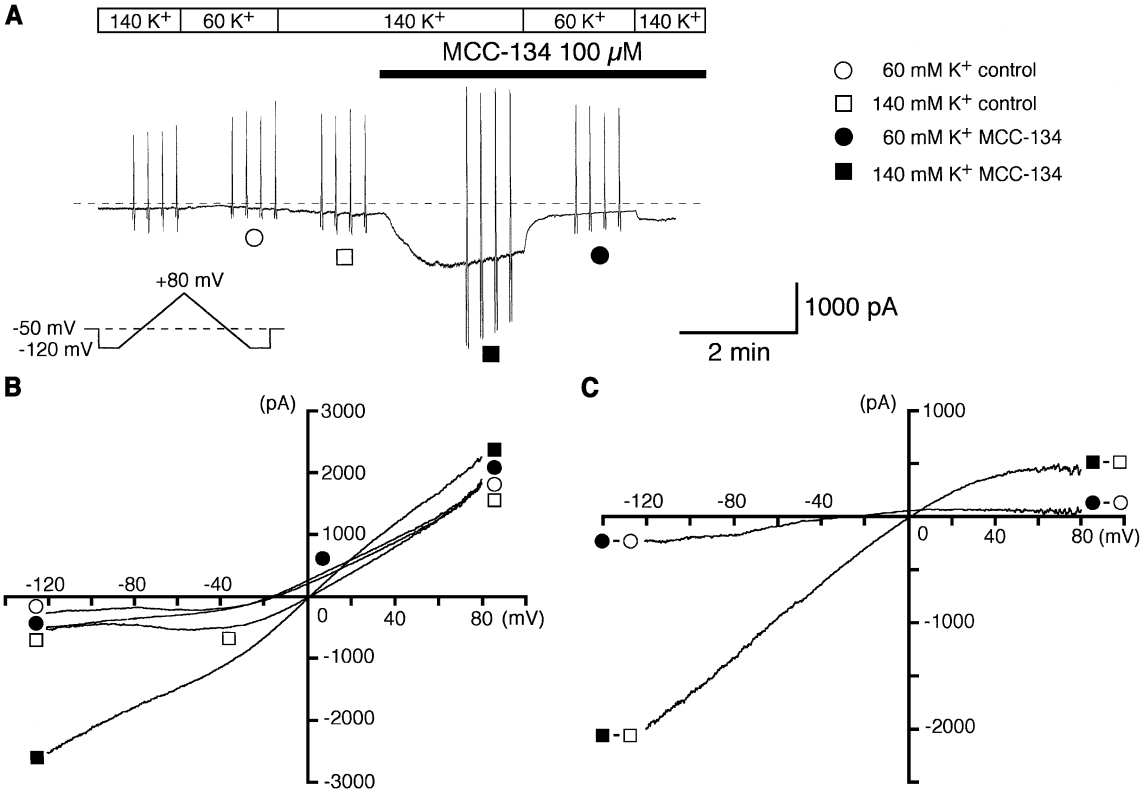

Fig. 3. Measurement of the MCC-134-induced membrane current in pig urethral myocytes. Holding potential was kept at −50 mV. Bath solution was initially 140 mM K^+ PSS, and $[K^+]_o$ was transiently reduced from 140 to 60 mM K^+. Pipette solution was 140 mM KCl containing 5 mM EGTA. Similar observations were obtained in five other cells. (A) Ramp currents induced by the four ramp potential pulses (see inset in A) applied every 15 s before and during application of 100 μM MCC-134. In the absence of MCC-134 (control), the ramp membrane currents were obtained in either the 140 or 60 mM K^+ solution. MCC-134 (100 μM) caused an inward current in symmetrical 140 mM K^+ conditions. When $[K^+]_o$ was reduced from 140 to 60 mM, the peak amplitude of the basal sustained MCC-134-induced current decreased at −50 mV. The vertical deflections indicate ramp currents. The dashed line indicates the zero-current level. (B) The mean ramp membrane currents (the falling phase of the ramp pulse) on an expanded time scale in several conditions. Each symbol is the same as in A. (C) Net membrane currents evoked by MCC-134 (100 μM) when $[K^+]_o$ was either 60 or 140 mM. Net membrane current was obtained by subtraction of the two ramp membrane currents (shown in B) recorded before and during application of 100 μM MCC-134 in each $[K^+]_o$ condition. The reversal potential of MCC-134-induced current in 140 mM K^+ was 1 mV. The reversal potential in 60 mM K^+ was −26 mV.

solution containing (mM): K^+ 140, Cl^- 140, glucose 5, EGTA 5, and HEPES 10/Tris (pH 7.35–7.40). The perforated-patch technique with nystatin was also occasionally used to record whole-cell currents (Teramoto and Brading, 1996). In short, nystatin was freshly dissolved in acidified methanol (1 N HCl to about pH 2) and the pH was adjusted to 7.4 with Tris base. This stock solution (10 mg ml^{-1}) was diluted in the pipette solution at a final concentration of 50 μg ml^{-1} just before use. Whole-cell recording was performed with a pipette which was first dipped in normal pipette solution (nystatin-free) and then back-filled with nystatin-containing pipette solution. This resulted in chemical perforation of the membrane. Cells were allowed to settle in the small experimental chamber (80 μl in volume). The bath solution was superfused by gravity throughout the experiments at a rate of 2 ml min^{-1}. All drugs were obtained from Sigma (Tokyo, Japan). MCC-134 (Tokyo Mitsubishi Pharmaceuticals, Tokyo, Japan), levcromakalim (SmithKline Beecham Pharmaceuticals, Harlow, UK) and glibenclamide were prepared daily as 100 mM stock solutions in dimethyl sulphoxide (DMSO). The maximum final concentration of DMSO in the bath solution was 0.03% for tension measurements and 0.3% for patch-clamp experiments. These concentrations were shown not to affect either the urethral resting tone or K^+ channels.

2.5. Data analysis

The whole-cell current data were low-pass filtered at 500 Hz by an eight-pole Bessel filter, sampled at 25 ms intervals and analysed on a computer (PowerMac G3, Tokyo, Japan) by the commercial software 'MacLab 3.5.6' (ADInstruments). Data points were fitted using the method of least-squares. Statistical analyses were performed with a two-paired *t*-test (two-factor with replication). Changes were considered significant at $P < 0.05$. Data are expressed as mean with the standard deviation (S.D.).

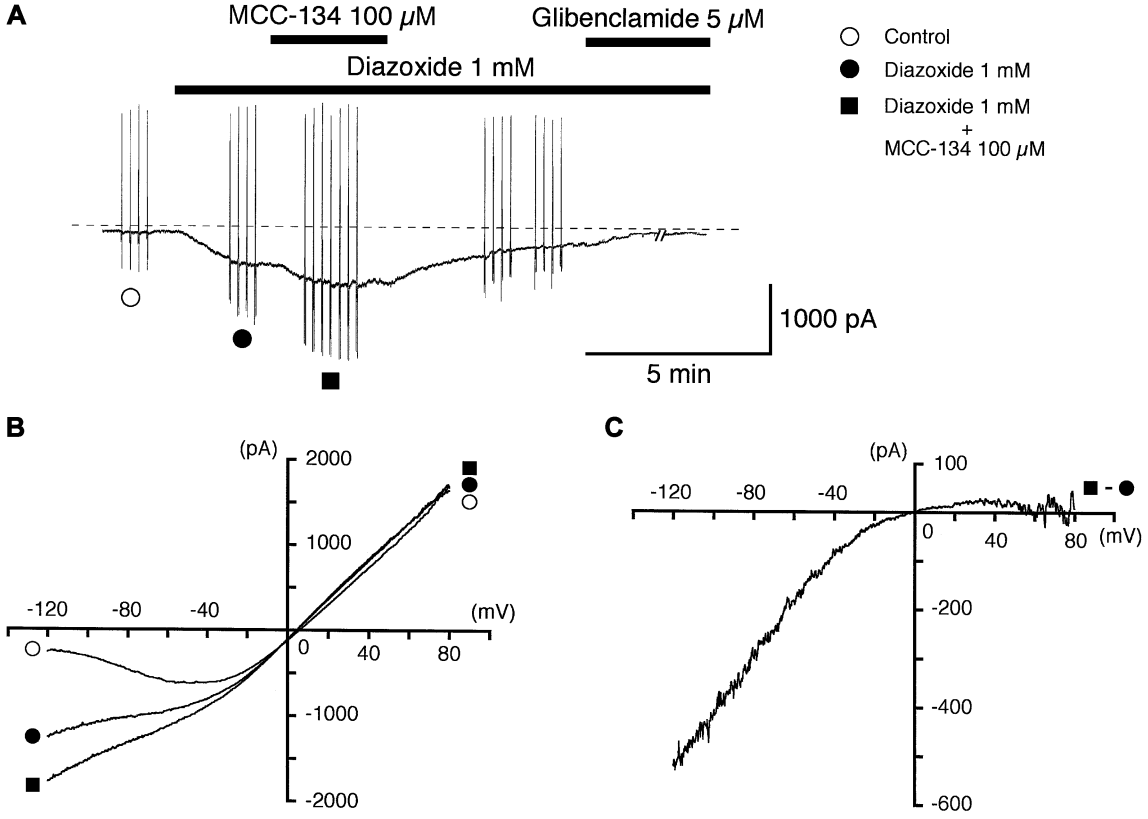

Fig. 4. The effects of MCC-134 (100 μM) on the 1 mM diazoxide-induced membrane current at a holding potential of −50 mV. Whole-cell recording, bath solution 140 mM K^+ PSS, pipette solution 140 mM K^+ containing 5 mM EGTA. (A) Current trace. The vertical lines are responses to triangular ramp potential pulses of 200 mV s^{-1} from −120 to +80 mV, applied after an initial 300 ms conditioning pulse to −120 mV (see inset in Fig. 3A). Diazoxide caused an inward membrane current (peak amplitude about 440 pA, measured from the current level in the presence of 5 μM glibenclamide). The current was enhanced by application of 100 μM MCC-134, gradually recovered to a steady-state amplitude after MCC-134 was removed, and was then suppressed by 5 μM glibenclamide. The gap in the presence of 5 μM glibenclamide was approximately 2 min. The dashed line indicates zero current. (B) Current–voltage curves measured from the falling phase of the ramp pulses. Each symbol is the same as in A. The lines are mean membrane currents from the four ramps in each condition. (C) The MCC-134-sensitive membrane current obtained by subtraction of the membrane current in the absence from that in the presence of 100 μM MCC-134 when 1 mM diazoxide was in the bath.

3. Results

3.1. The effects of MCC-134 on the resting urethral tone

Fig. 1A shows that cumulative application of MCC-134 (1–30 μM) produced a concentration-dependent relaxation of the resting urethral tone. After washing out the drug, the urethral tone recovered to the control level. Subsequently, 10 μM levcromakalim was applied in order to obtain the maximum relaxation in the urethral strips. Fig. 1B shows the concentration–response curve of MCC-134, expressed relative to the maximum relaxation to 10 μM levcromakalim. The EC_{50} value for the MCC-134-induced relaxation was 6.2 μM. The pinacidil- and the diazoxide-induced urethral relaxing curves are taken from Teramoto and Ito (1999), respectively (pinacidil, $EC_{50} = 1.6$ μM; diazoxide, $EC_{50} = 70.5$ μM). The relaxation produced by MCC-134 (30 μM) was suppressed by application of 1 μM glibenclamide to the control level (Fig. 1C, $n = 12$). Further application of a higher concentration of glibenclamide (10 μM) did not change the urethral tone ($n = 12$).

3.2. Effects of MCC-134 on membrane currents in pig proximal urethra

When the pipette solution was filled with 140 mM KCl solution containing 5 mM EGTA and the bath was superfused with 140 mM K^+ PSS (symmetrical 140 mM K^+ conditions) at a holding potential of −50 mV in a conventional whole-cell configuration, cumulative application of MCC-134 caused a significant inward current in a concentration-dependent manner (Fig. 2). As shown in Fig. 2, the peak amplitude of the 100 μM MCC-134-induced inward current was much smaller than the 100 μM pinacidil-induced inward current, demonstrating less potency.

To make a rough estimation of the ion selectivity and the reversal potential of this current, voltage ramps were applied and the extracellular K^+ concentration ($[K^+]_o$) was changed by iso-osmotic substitution of Na^+. Fig. 3A shows the experimental protocol (conventional whole-cell recording). In the absence of MCC-134 (control), current–voltage relationships were obtained by the application of four ramp pulses in solutions containing 140 mM K^+ PSS followed by 60 mM K^+ PSS and then back to 140 mM K^+ PSS. MCC-134 (100 μM) was then applied to the bath solution, causing a sustained inward current in 140 mM K^+ PSS and then the same voltage protocol was performed. When $[K^+]_o$ was decreased from 140 to 60 mM, the MCC-134-induced membrane current at −50 mV was markedly decreased. When $[K^+]_o$ was returned to 140 mM again, the amplitude of the MCC-134-induced current became larger, however it did not reach the original level. Fig. 3B shows the average of the four ramp currents before and during application of 100 μM MCC-134 for the cell shown in Fig. 3A. In each $[K^+]_o$ condition, the net membrane current activated by 100 μM MCC-134 was obtained by subtracting the averaged control current from the mean MCC-134-induced current. The current demonstrated an inwardly rectifying property. The reversal potential of the MCC-134-induced membrane current in this cell was 1 mV in 140 mM K^+ solution and −26 mV in 60 mM K^+ solution (Fig. 3C). Mean values from five cells were 0 ± 3 mV ($n = 5$) for 140 mM K^+ solution and -22 ± 5 mV ($n = 5$) for 60 mM K^+ solution. These values were close to the theoretical potassium equilibrium potential (E_K) in each $[K^+]_o$ condition (140 mM K^+; $E_K = 0$ mV, 60 mM K^+; $E_K = -21$ mV). The inward currents induced by MCC-134 in each $[K^+]_o$ was inhibited by additional application of 5 μM glibenclamide (data not shown). These results suggest that in pig urethral myocytes, the MCC-134-induced membrane currents are carried mainly by K^+ through channels that are sensitive to glibenclamide.

3.3. Effects of 100 μm MCC-134 on the diazoxide-induced glibenclamide-sensitive membrane currents

Diazoxide (1 mM) also caused an inward current (Fig. 4A,B). Application of MCC-134 (100 μM) reversibly enhanced the diazoxide-induced current at −50 mV ($1.51 \pm$

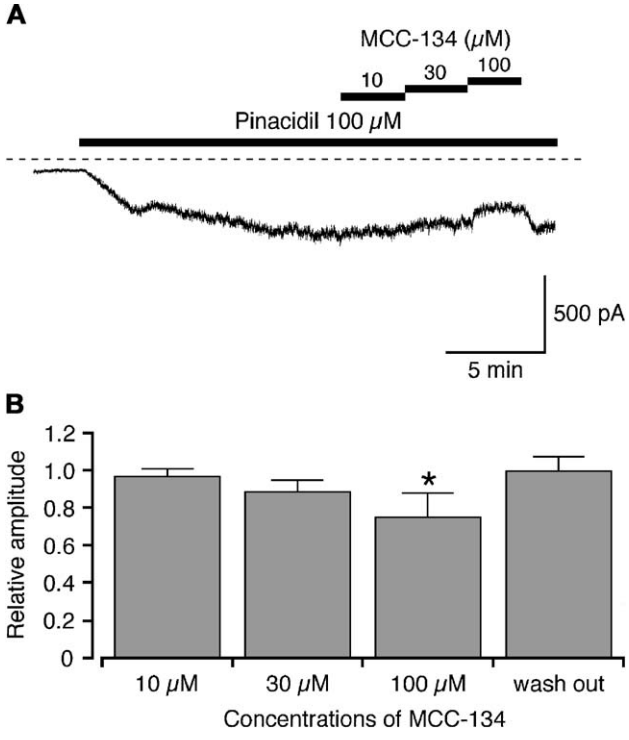

Fig. 5. Effects of MCC-134 (10–100 μM) on the 100 μM pinacidil-induced inward membrane current at −50 mV in symmetrical 140 mM K^+ solutions by use of nystatin-perforated patch configuration. (A) Current trace. The dashed line indicates zero current. (B) Relationship between relative value of the peak amplitude of the pinacidil-induced current and the concentration of MCC-134. The mean amplitude of the pinacidil-induced current at the holding potential of −50 mV just before application of MCC-134 (30 s duration) was normalized as 1. Each column indicates the mean of three to eight observations with S.D. shown by vertical lines. Asterisk indicates a statistically significant difference, demonstrated using a paired *t*-test (*$P < 0.05$).

0.09, $n=3$). The net membrane current obtained by subtraction of the two mean ramp currents recorded before and during application of MCC-134 in the presence of 1 mM diazoxide demonstrated an inward rectification at positive potentials (Fig. 4C).

3.4. Effects of MCC-134 on the pinacidil-induced glibenclamide-sensitive membrane currents

After the nystatin-perforated patch had become established, an inward current was evoked by application of pinacidil (100 μM) in symmetrical 140 mM K^+ conditions (Fig. 5A). When applied in a cumulative manner, MCC-134 reversibly caused a small but significant inhibition of the basal amplitude of the pinacidil-induced current at −50 mV ($n=3–8$, Fig. 5B). As shown in Fig. 6A, pinacidil (100 μM) caused an inward current in symmetrical 140 mM K^+ conditions. Application of MCC-134 (100 μM) caused a small but significant inhibition of the basal amplitude of the pinacidil-induced current at −50 mV (0.73 ± 0.18, $n=5$). The net membrane current obtained by subtraction of the two mean ramp currents recorded before and during application of MCC-134 in the presence of 100 μM pinacidil demonstrated a weak inward rectification at positive potentials (Fig. 6C).

4. Discussion

4.1. The rank order of potency of MCC-134 in comparison to those of other K_{ATP} channel openers

Although MCC-134 was originally designed to be synthesized as a vasorelaxing agent, no study in vascular smooth muscle has been currently reported yet using tension measurement. In the present experiments, we have been able to evaluate the potency of MCC-134 (EC_{50} value; 6.2 μM) on fresh smooth muscle in the absence of either excess $[K^+]_o$ (high KCl test protocol) or agonists (such as acetylcholine, serotonin etc.), in comparison with levcromakalim, one of the most potent K_{ATP} channel openers. The rank order of potency in K_{ATP} channel openers was clearly found to be levcromakalim>pinacidil>MCC-134>diazoxide (dissociation constant, levcromakalim, 0.3 μM; pinacidil, 1.6 μM;

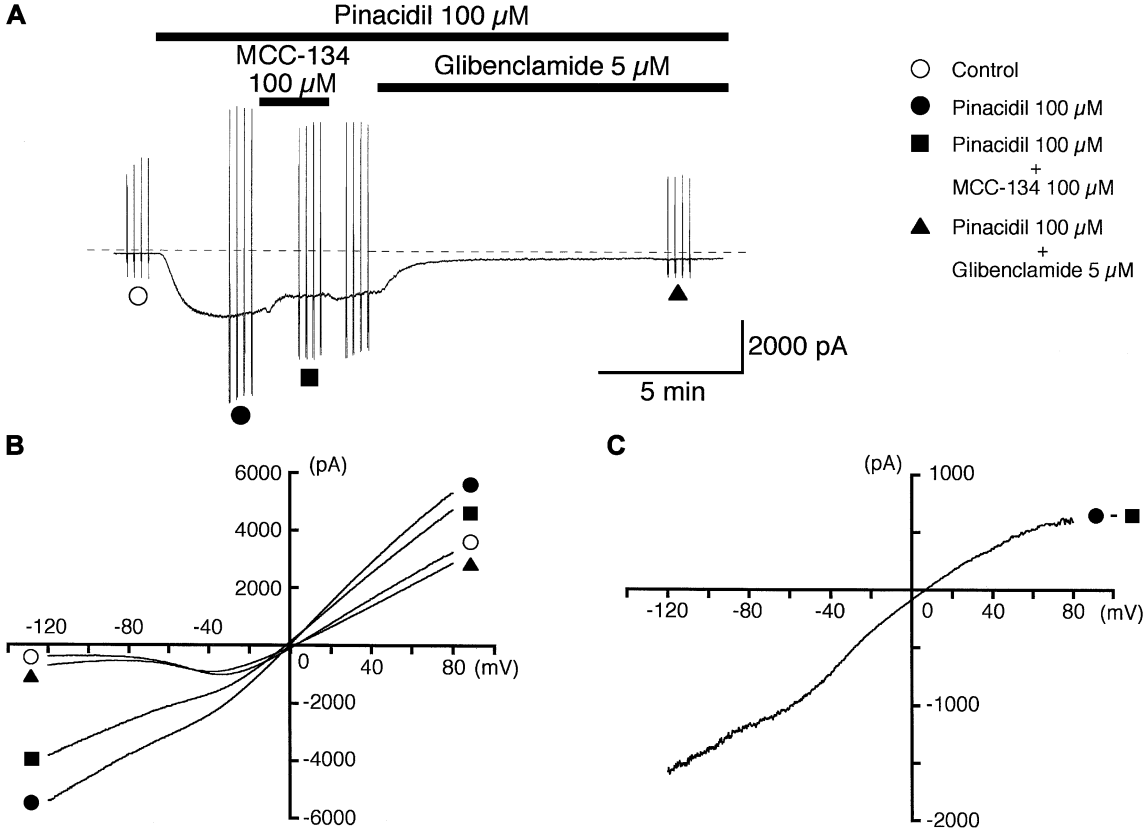

Fig. 6. Inhibitory effects of MCC-134 (100 μM) on the pinacidil-induced glibenclamide-sensitive inward membrane current at −50 mV in symmetrical 140 mM K^+ solution. Holding potential was kept at −50 mV. (A) Current trace. The vertical lines are responses to triangular ramp potential pulses (see inset in Fig. 3A). Pinacidil (100 μM) caused an inward membrane current (peak amplitude approximately 2.3 nA, measured from the current level in the presence of 5 μM glibenclamide). MCC-134 (100 μM) reversibly inhibited the pinacidil-induced current. The dashed line indicates zero current. (B) Current–voltage relationship curves measured from the negative-going limb of the ramp pulse. Each symbol is the same as in the current trace (A). The lines are mean membrane currents from the four ramps in each condition. (C) Net membrane currents. The MCC-134-sensitive membrane current was obtained by subtraction of the mean ramp current recorded before from that during application of MCC-134 in the presence of 100 μM pinacidil.

MCC-134, 6.2 μM; diazoxide, 70.5 μM, Teramoto and Ito, 1999). The MCC-134-induced relaxation of the urethral resting tone was suppressed by application of glibenclamide (1 μM). These results strongly suggest that the MCC-134-induced relaxation may be due to the activation of the glibenclamide-sensitive mechanisms in pig urethra.

4.2. Pharmacological properties of SURs of pig urethral K_{ATP} channels

From functional expression experiments, pharmacological and electrophysiological studies have indicated that SUR1/Kir6.2 represents the pancreatic β-cell K_{ATP} channel, that SUR2A/Kir6.2 is thought to represent the cardiac K_{ATP} channel, whereas SUR2B/Kir6.1 may represent the smooth muscle-type K_{ATP} channel (reviewed by Babenko et al., 1998). Recently, Shindo et al. (2000), using functional recombinant K_{ATP} channel studies, have reported that MCC-134 is a full agonist for the SUR2B/Kir6.2 channel and a partial agonist for the SUR2A/Kir6.2 channel but it inhibits the SUR1/Kir6.2, concluding that MCC-134 may be a prototype of new drugs acting selectively on distinct types of SURs. In the present experiments, (1) MCC-134 caused a glibenclamide-sensitive urethral relaxation, (2) MCC-134 induced glibenclamide-sensitive K^+ currents in pig urethral myocytes and (3) the threshold concentration of MCC-134 to induce these currents was around 10 μM. These results are similar to those which were observed in SUR2A/Kir6.2 or SUR2B/Kir6.2 (Shindo et al., 2000). However, it is clearly demonstrated that SUR2A/Kir6.2 channel is not activated by diazoxide (Babenko et al., 1998). Given this, it is tempting to speculate that K_{ATP} channels in pig urethra may be solely composed of SUR2B subunits.

However, we have also demonstrated the different effects of MCC-134 on K_{ATP} channel currents between the native urethral K_{ATP} channels and the recombinant K_{ATP} channels. (1) We have shown that 100 μM MCC-134 reversibly enhanced the diazoxide-induced K^+ currents although the same concentration had no significant effect on the 300 μM diazoxide-induced SUR2B/Kir6.2 channel current in recombinant K_{ATP} channel studies (Shindo et al., 2000). (2) In pig urethra, the peak amplitude of the 100 μM MCC-134-induced K^+ currents at -50 mV was much smaller than that induced by 100 μM pinacidil in the same dispersed cells, demonstrating that MCC-134 is less potent than pinacidil. Similarly, the same rank order of the relaxing potency of K_{ATP} channel openers was observed in tension measurements (MCC-134, EC_{50}, 6.2 μM; pinacidil, EC_{50}, 1.6 μM; Teramoto and Ito, 1999). The peak amplitude of the MCC-134 (100 μM)-induced channel current of the recombinant SUR2B/Kir6.2 channels was larger than that of the 100 μM pinacidil-induced K^+ current (Shindo et al., 2000). (3) MCC-134 (100 μM) had no effect on the pinacidil-induced current in the recombinant channels (Shindo et al., 2000), whereas the pinacidil-induced K^+ current was reversibly inhibited by MCC-134 in a concentration-dependent manner in pig urethra. We are not certain why these significant discrepancies exist between the native urethral K_{ATP} channels and the recombinant K_{ATP} channels regarding the effects of MCC-134. Thus, it is difficult to speculate that K_{ATP} channels in pig urethra are solely composed of SUR2B subunits.

Using reverse transcriptase-polymerase chain reaction (RT-PCR) studies, we have reported the presence of SUR1 as well as SUR2B in pig urethral smooth muscle although these studies have addressed only the expression of mRNA transcript (Yunoki et al., 2003). Both SUR1 and SUR2B are widely expressed in urinary bladder as well as vascular smooth muscle (guinea-pig detrusor, Gopalakrishnan et al., 1999; pig and human detrusor, Buckner et al., 2000; rat pulmonary artery, Cui et al., 2002) as determined by RT-PCR analysis. It seems conceivable that MCC-134 binds to the SUR1 subunit and may act as an antagonist in urethral K_{ATP} channels, causing inhibitory effects on the pinacidil-induced current.

Similarly, MCC-134 may bind to both SUR1 (antagonistic action) and SUR2B (agonistic action) in urethral K_{ATP} channels, since the peak amplitude of the MCC-134-induced current was much smaller than the pinacidil-induced current. However, since approximately 25% of the pinacidil-induced inward current was inhibited by MCC-134, we suggest that SUR subunits in pig urethral K_{ATP} channels may be composed mainly of SUR2B and that SUR1 may play a minor but significant role in regulating channel activity of urethral K_{ATP} channels.

In antisense studies, Yokoshiki et al. (1999) have recently reported that a small population of K_{ATP} channels in neonatal rat ventricular myocytes is constructed by a combination of SUR1 and SUR2A, suggesting the existence of a heterotetrameric combination. However, the stoichiometry of this SUR complex still remains uncertain, although it has been

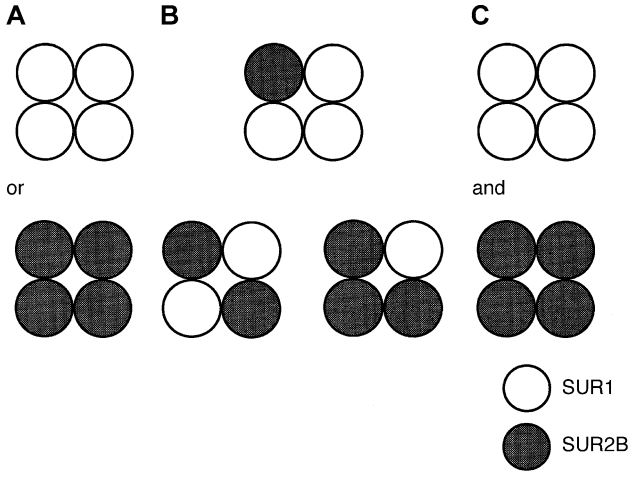

Fig. 7. Schematic diagrams illustrating possible combination of SUR subunits proposed in the present study. (A) The presence of either SUR1 or SUR2B homotetrameric subunits. (B) Heterotetrameric composition of SUR1 and SUR2B. (C) Coexistence of SUR1 homotetrameric subunits and SUR2B homotetrameric subunits.

reported that SURs in smooth muscle are composed of homotetrameric subunits of SUR2B (Isomoto et al., 1996). In Fig. 7, we show possible combinations of SURs. We suggest that the SUR subunits in pig urethral K_{ATP} channels are not homologously composed of SUR1 or SUR2B alone (Fig. 7A). We further suggest that the SUR subunits may be constructed with a heterocombination of SUR1 and SUR2B (Fig. 7B) or that both SUR1 and SUR2B may coexist as SUR homotetrameric subunits (Fig. 7C). Recently, Giblin et al. (2002) have demonstrated biochemical and electrophysiological evidence that SUR1 and SUR2 do not interact to form heteromultimers in K_{ATP} channels. However, the stoichiometry of the SUR complex still remains uncertain. Since we are not able to detect interaction between MCC-134 and SURs (such as precise binding site(s) of MCC-134) and mechanisms by which these interactions regulate K_{ATP} channel activity, it will be necessary to confirm the molecular identity of SUR subunits constituting functional K_{ATP} channels in smooth muscle.

4.3. Clinical implication of different SURs in the lower urinary tract

In lower urinary tract smooth muscle cells, two distinct types of K_{ATP} channels have been identified as target K^+ channels for levcromakalim by use of single-channel recordings (guinea-pig urinary bladder, K_{ATP} channel, Bonev and Nelson, 1993; pig urethra, K_{ATP} channel, Teramoto and Brading, 1996). It has been demonstrated clearly that these two K_{ATP} channels possess different nucleoside diphosphate (NDP) sensitivity and glibenclamide sensitivity, suggesting different types of SURs. Since K_{ATP} channel openers interact primarily with the SUR subunits, the significant differences in property of K_{ATP} channels between urinary bladder and urethra hold out some hope for the development of tissue-selective K_{ATP} channel openers for lower urinary tract dysfunction (Teramoto et al., 1997). In urinary bladder, it is generally believed that SUR2B was solely expressed as a regulatory protein of K_{ATP} channel (guinea-pig, Gopalakrishnan et al., 1999; pig and human, Buckner et al., 2000). On the other hand, in pig urethra, SUR2B as well as SUR1 are likely to play a functional role in regulating the activity of K_{ATP} channels (Yunoki et al., 2003). Although MCC-134 fully activates both urinary bladder K_{ATP} channels and pig urethral K_{ATP} channels through SUR2B, MCC-134 partially inhibits the activity of pig urethral K_{ATP} channels through SUR1. Thus, MCC-134 is thought to possess a weak detrusor selectivity in lower urinary tract smooth muscle.

In conclusion, MCC-134 caused a significant urethral relaxation through the glibenclamide-sensitive K_{ATP} currents in pig urethra. Furthermore, SUR2B as well as SUR1 are thought to play physiological roles in the regulation of the muscle tone in pig urethra according to the sensitivity of MCC-134.

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